ENVIRONMENTAL RESEARCH LABORATORY
GULF BREEZE

COLLECTED REPRINTS
1973-1974

U. S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH & DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORY
SABINE ISLAND
GULF BREEZE, FLORIDA 32561

ENVIRONMENTAL RESEARCH LABORATORY
GULF BREEZE

COLLECTED REPRINTS
1973--1974

U. S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH & DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORY
SABINE ISLAND
GULF BREEZE, FLORIDA 32561

TABLE OF CONTENTS

Contribution
No.

165. A. W. BOURQUIN
Estuarine microbes and organochlorine pesticides (a brief review).

167. MARLIN E. TAGATZ
A larval tarpon, Megalops atlanticus, from Pensacola, Florida.

168. P. W. BORTHWICK, G. H. COOK, and J. M. PATRICK, JR.
Mirex residues in selected estuaries of South Carolina - June 1972.

169. DAVID L. COPPAGE and EDWARD MATTHEWS
Short-term effects of organophosphate pesticides on cholinesterase of estuarine fishes and pink shrimp.

170. D. R. NIMMO, J. FORESTER, P. T. HEITMULLER, and G. H. COOK
Accumulations of Aroclor[R] 1254 in grass shrimp (Palaemonetes pugio) in laboratory and field exposures.

172. D. J. HANSEN, P. R. PARRISH, and J. FORESTER
Aroclor[R] 1016: Toxicity to and uptake by estuarine animals

174. PATRICK R. PARRISH
Aroclor[R] 1254, DDT, and DDD, and Dieldrin: Accumulation and loss by American oyster, (Crassostrea virginica) exposed continuously for 56 weeks.

175. S. C. SCHIMMEL, D. J. HANSEN, and J. FORESTER
Effects of Aroclor[R] 1254 on laboratory-reared embryos and fry sheepshead minnows (Cyprinodon variegatus).

176. PHILIP A. BUTLER
Trends in pesticide residues in shellfish.

177. D. J. HANSEN, S. C. SCHIMMEL, and JERROLD FORESTER
Aroclor[R] 1254 in eggs of sheepshead minnows: Effect on fertilization success and survival of embryos and fry.

178. P. R. PARRISH, JOHN A. COUCH, J. FORESTER, J. M. PATRICK, JR., and G. H. COOK
Dieldrin: Effects on several estuarine organisms.

179. M. E. TAGATZ, P. W. BORTHWICK, G. H. COOK, and D. L. COPPAGE
Studies of ground applications of malathion on salt-marsh environments in northwest Florida.

Contribution
No.

180. THOMAS W. DUKE
Criteria for determining importance and effects of pesticides on the marine environment: A brief overview.

181. D. J. HANSEN, S. C. SCHIMMEL, and E. MATTHEWS
Avoidance of Aroclor^{R} 1254 by shrimp and fishes.

183. A. L. JENSEN
Leslie matrix models for fisheries studies.

184. A. L. JENSEN
Predator-prey and competition models with state variables: biomass, number of individuals, and average individual weight.

190. WILHELM P. SCHOOR
Accumulation of Mirex-^{14}C in the adult blue crab (Callinectes sapidus).

191. PHILIP A. BUTLER
Biological problems in estuarine monitoring.

192. NELSON R. COOLEY
Occurrence of snook on the north shore of the Gulf of Mexico.

193. GERALD E. WALSH, TERRENCE A. HOLLISTER, and JERROLD FORESTER
Translocation of four organochlorine compounds by red mangrove, (Rhizophora mangle L.) seedlings.

195. THOMAS W. DUKE, and DAVID P. DUMAS
Implications of pesticide residues in the coastal environment.

196. PATRICK R. PARRISH, DAVID J. HANSEN, JOHN A. COUCH, JAMES M. PATRICK, JR., and GARY H. COOK
Effects of the polychlorinated biphenyl, Aroclor^{R} 1016, on estuarine animals.

198. D. R. NIMMO, and L. H. BAHNER
Some physiological consequences of polychlorinated biphenyl and salinity-stress in penaeid shrimp.

200. L. H. BAHNER
A salinity controller for flowing-water bioassays.

Contribution
No.

201. AL W. BOURQUIN, and GARY H. COOK
Degradation of malathion by estuarine microbes.

202. NELSON R. COOLEY
Effects of pesticides on protozoa.

203. A. W. BOURQUIN and GARY H. COOK
Impact of microbial seed cultures on the aquatic environment.

204. ALFRED J. WILSON, JR., and JERROLD FORESTER
Methods and problems in analysis of pesticides in the estuarine environment.

207. STEVEN C. SCHIMMEL, DAVID J. HANSEN, and JERROLD FORESTER
Effects of Aroclor^{R} 1254 on laboratory-reared embryos and fry, Cyprinodon variegatus (Pisces: Cyprinodontidae)

208. W. PETER SCHOOR
Theoretical model and solubility characteristics of Aroclor^{R} 1254 in water: Problems associated with low-solubility compounds in aquatic toxicity tests.

209. L. KEIFER, H. JANNASCH, K. NEALSON, and A. BOURQUIN
Observations of luminescent bacteria in continuous culture.

210. AL BOURQUIN, L. KEIFER, and S. CASSIDY
Microbial response to malathion treatments in salt marsh microcosms.

211. JOHN A. COUCH
Pathological effects of Urosporidium (Haplosporida) infection in microphallid metacercariae.

213. JOHN A. COUCH
Free and occluded virus, similar to Baculovirus, in hepatopancreas of pink shrimp

215. JOHN A. COUCH
An enzootic nuclear polyhedrosis virus of pink shrimp: Ultrastructure, prevalence, and enhancement.

216. JOHN A. COUCH, and DELWAYNE R. NIMMO
Ultrastructural studies by shrimp exposed to the pollutant chemical, polychlorinated biphenyl (Aroclor^{R} 1254).

219. J. A. COUCH, and D. R. NIMMO
Detection of interaction between natural pathogens and pollutant chemicals in aquatic animals.

Contribution
No.

220. JOHN A. COUCH
Ultrastructural and protargol studies of Lagenophrys callinectes (Ciliophora: Peritrichida).

238. P. W. BORTHWICK
A clinical centrifuge tube for small blood samples.

242. JOHN A. COUCH, and DELWAYNE R. NIMMO
(Abstract). Cytopathology, ultrastructure, and virus infection in pink shrimp exposed to the PCB, Aroclor® 1254.

245. PHILIP A. BUTLER
Estuaries.

247. JAMES W. GILLETTE, JAMES HILL, IV., ALFRED W. JARVINEN, and W. PETER SCHOOR
A conceptual model for the movement of pesticides through the environment.

250. J. COUCH, G. GARDNER, J. HARSHBARGER, M. TRIPP, and P. YEVICH
Histological and physiological evaluations in some marine fauna.

251. JELLE ATEMA, CHARLES C. COUTANT, PATRICIA De COURSEY, DAVID HANSEN, JAMES S. KITTREDGE, JOHN J. MAGNUSON, DON MILLER and MARK J. SCHNEIDER
Behavioral measures of environmental stress.

EXTRAMURAL RESEARCH PUBLICATIONS

EPA-660.3-75-015 Effects of Mirex and Methoxychlor on striped mullet, Mugil cephalus L. May, 1975. Lee, Jong H., Colin E. Nash and Joseph R. Sylvester. (Oceanic Foundation, Makapuu Point, Waimanalo, Hawaii 96795). Grant No. R 802348, Program Element No. 1EA077, ROAP/Task No. 10 AKC/040. David J. Hansen, Project Officer.

EPA-660/3-75-024 The effect of Mirex and Carbofuran on estuarine microorganisms. June, 1975. Brown, Lewis R., Earl G. Alley and David W. Cook Performing organization: Mississippi State University, Mississippi State, Miss. 39762 Contract No. 68-03-0288, Program Element No. 1-EA077. ROAP/Task No. 10 AKC/33. Al W. Bourquin, Project Officer.

AUTHOR INDEX

CONTRIBUTION NO. 126

Composition of *Thalassia testudinum* and *Ruppia maritima*

GERALD E. WALSH AND THOMAS E. GROW

LITTLE is known at present about the nutritive value of aquatic plants, especially in relation to annual variations in their chemical constituents. Turtle grass (*Thalassia testudinum*) and widgeon grass (*Ruppia maritima*) are common in the inshore waters of Florida (Phillips, 1960). They are important constituents of estuarine nursery grounds for marine animals and many forms of plant and animal life are associated with them (Hudson et al., 1970). The seagrasses are eaten by fishes, turtles, and other aquatic animals (Randall, 1965), and birds (Olney, 1968). Detritus derived from seagrasses is eaten by small marine animals (Menzies and Rowe, 1969; Fenchel, 1970. Also, *T. testudinum* and its epiphytes are important in biogeochemical cycles in estuarine areas (Parker, 1966).

Both *T. testudinum* and *R. maritima* have been used successfully in preliminary experiments as fertilizers for tomatoes (van Breedveld, 1966) and as feed supplements for Sheep (Bauersfeld et al., 1969).

Because of the importance of *T. testudinum* and *R. maritima* to estuarine ecosystems, we investigated seasonal distributions of protein, carbohydrate, trace elements, and energy content of their leaves and rhizomes. Also, the potential nutritive value of the seagrasses was evaluated.

METHODS

Thalassia testudinum and *R. maritima* were collected between 6 June 1969 and 27 May 1970 from a mixed bed at the western edge of Sabine Island in Santa Rosa Sound near Gulf Breeze, Florida. They were taken in the morning to avoid possible diel variation in the factors measured. Abundance of *R. maritima* was greatly reduced in February 1970 and enough plant material could not be collected for all tests.

After collection, plants were taken immediately to the laboratory, where the epiphyton was removed. The leaves were separated from the rhizomes and all were washed quickly in a stream of distilled

water. Leaves and roots were dried to constant weight in an oven at 100 C and ground in a Wiley mill to pass the 40-mesh sieve. The pulverized material was stored *in vacuo* over anhydrous calcium carbonate until tested.

Ash content was determined by combustion in a muffle furnace at 55 C for five hours.

Total protein was measured by the method of Strickland and Parsons (1965) using acetonylacetone (2,5-hexanedione) reagent and the procedure was standardized against the Kjeldahl-Nessler method. We report protein content as percentage of dry weight and of ash-free dry weight.

Total carbohydrate was measured by a variation of the anthrone method for particulate carbohydrate (Strickland and Parsons, 1965). Fifty mg of each sample were suspended in 50 ml of 0.2 N H_2SO_4 in a 125-ml Erlenmeyer flash. The sample was hydrolysed at 100 C for 90 min. with mixing every 15 min. The hydrolysate was passed through a glass-fiber filter of 0.45μ porosity and 0.2 ml of the filtrate was pipetted into a test tube. To this was added 10.8 ml of anthrone reagent (0.2 g anthrone, 8.0 ml 95 per cent alcohol, 30.0 ml distilled water, and 100 ml concentrated H_2SO_4). The solution was heated at 100 C for five min. and brought quickly to room temperature in an ice-water bath. After 15 min. the extinction was measured against distilled water at 6200 A in a one-cm glass cell in a Beckman DU spectrophotometer. Glucose was used in preparation of standard carbohydrate solutions. The data are expressed as percentage carbohydrate in dry weight and in ash-free dry weight.

Concentrations of sodium, potassium, magnesium, iron, manganese, and zinc in leaves and rhizomes were measured by atomic absorption spectroscopy, using a modification of the method of David (1958). Approximately 0.01 g of dried plant material was placed in a 30 ml Kjeldahl digestion flask with two ml of a 1:7 sulphuric acid-perchloric acid mixture and 10 to 12 ml of nitric acid. Digestion was continued until organic matter was completely destroyed. Four glass beads were added to each flash to prevent bumping.

After digestion, the flash was cooled to room temperature and three ml of distilled water added. After gentle shaking, the contents were transferred to a 25-ml volumetric flask. This washing procedure was repeated twice with five ml of distilled water and the

hydrolysate taken to 25 ml with distilled water. The hydrolysate was analyzed on a Beckman Model 1301 atomic absorption unit equipped with a Beckman DB-G spectrophotometer. Concentrations of the elements are reported as parts per thousand (ppt) of dry weight.

Caloric contents were determined on a Parr Series 1200 adiabatic calorimeter. Fuse wire and acid corrections were made for each determination and results are expressed as kilocalories per gram of ash-free dry weight.

RESULTS AND DISCUSSION

Ash. Annual mean values and ranges of values for ash, protein, carbohydrate, and energy are given in Table 1. Annual variation in ash content was not found and analysis of variance indicated that all mean values were significantly different at the 0.05 level. The

TABLE 1

Annual means for ash, protein, carbohydrate, and energy contents of *Thalassia testudinum* and *Ruppia maritima* between June 1969 and May 1970.

Component	Annual mean	Range
Ash, % dry weight		
T. testudinum leaves	24.5	20.6-26.9
T. testudinum rhizomes	23.8	21.4-25.4
R. maritima leaves	18.8	15.8-23.8
R. maritima rhizomes	22.4	18.6-24.8
Protein, % ash-free dry weight		
T. testudinum leaves	25.7	13.6-37.1
T. testudinum rhizomes	11.0	7.7-14.7
R. maritima leaves	23.2	13.5-32.6
R. maritima rhizomes	20.0	14.1-26.9
Carbohydrate, % ash-free dry weight		
T. testudinum leaves	23.6	18.3-35.8
T. testudinum rhizomes	72.1	54.5-80.3
R. maritima leaves	27.0	24.3-34.3
R. maritima rhizomes	63.6	52.0-73.3
Energy, Kcal/g ash-free dry weight		
T. testudinum leaves	4.66	4.47-4.79
T. testudinum rhizomes	4.88	4.76-5.16
R. maritima leaves	4.44	4.28-4.69
R. maritima rhizomes	4.25	4.09-4.38

values obtained for ash contents were similar to those for most other aquatic plants (Westlake, 1965) and for leaves of *T. testudinum* (Burkholder et al., 1959).

Protein. There was considerable annual variation in the amount of protein in ash-free dry weight of leaves. The highest value found for *T. testudinum* leaves was 2.7 times that of the lowest, while that for *R. maritima* leaves was 2.4 times greater. Annual variation in

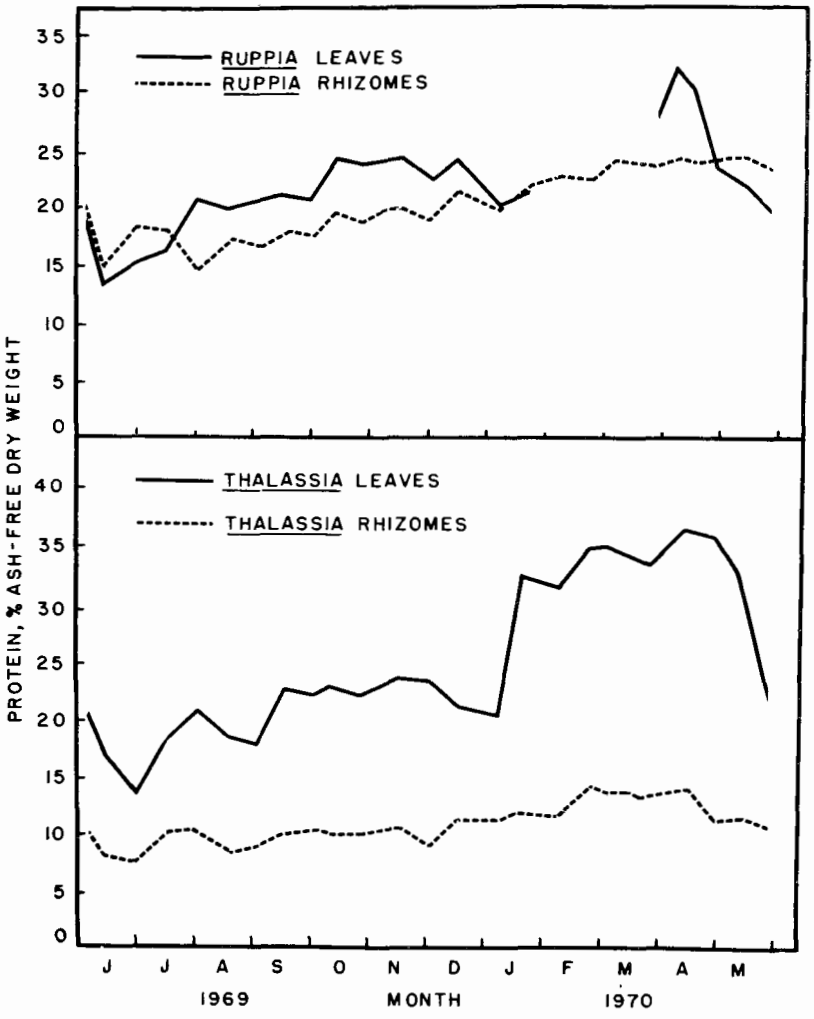


Fig. 1. Annual variation of protein in *Thalassia testudinum* and *Ruppia maritima*.

protein in underground parts was less. In both *T. testudinum* and *R. maritima,* the highest concentration in the rhizomes was 1.9 times that of the lowest.

During the annual cycle, the protein content of the leaves of *T. testudinum* was always greater than that of the rhizomes (Figure 1), the annual mean concentration in leaves being over two times larger (Table 1). Concentration of protein in the leaves of *T. testudinum* increased in the late winter and spring between 9 January and 16 April. Concentrations decreased rapidly thereafter, and were lowest in summer on 2 July 1969. In the rhizomes, however, concentrations of protein increased only slightly between 26 February and 16 April 1970, and fell less precipitously than did those of the leaves.

The annual mean concentration of protein in the leaves of *R. maritima* was slightly greater than that in the rhizomes, but concentration was greater in the rhizomes in the summer months of May, June, and July. Concentrations in the leaves reached a peak on 8 April 1970, and fell rapidly thereafter. Lowest concentration was found in the summer on 4 August 1969. Concentration of protein in the rhizomes of *R. maritima* rose slowly in the nine-month period between 4 August and 14 May, with lowest concentrations occuring in summer in June and early August.

The above findings are related to the functional aspects of leaves and rhizomes. Leaves generally have a greater amount of protein than rhizomes because they are largely concerned with biosynthesis and, consequently, contain large amounts of enzymes and many membranes. However, rhizomes are storage organs and contain relatively large amounts of carbohydrate, as will be shown later. Leaf protein is greatest in spring when biosynthesis is rapid, whereas concentrations of carbohydrate in rhizomes are greatest in fall and winter.

Bauersfeld et al. (1969) suggested that *T. testudinum* may be of value as a feed additive for domestic animals. They reported that the leaves of *T. testudinum,* after a single washing with distilled water, contained between 9.0 and 14.1 per cent protein on a dry weight basis, whereas the rhizomes contained 15 per cent. Burkholder et al. (1959) reported that the dried leaves of *T. testudinum* contained 13.1 per cent protein. Neither study, however, reported the dates on which samples were taken.

On a percentage dry weight basis, the protein contents of our samples were: *T. testudinum* leaves, 10.3-29.7; rhizomes, 5.8-12.2; *R. maritima* leaves, 10.9-28.5; rhizomes, 10.4-18.1. These values are, in general, higher than those for many other plants. Among the

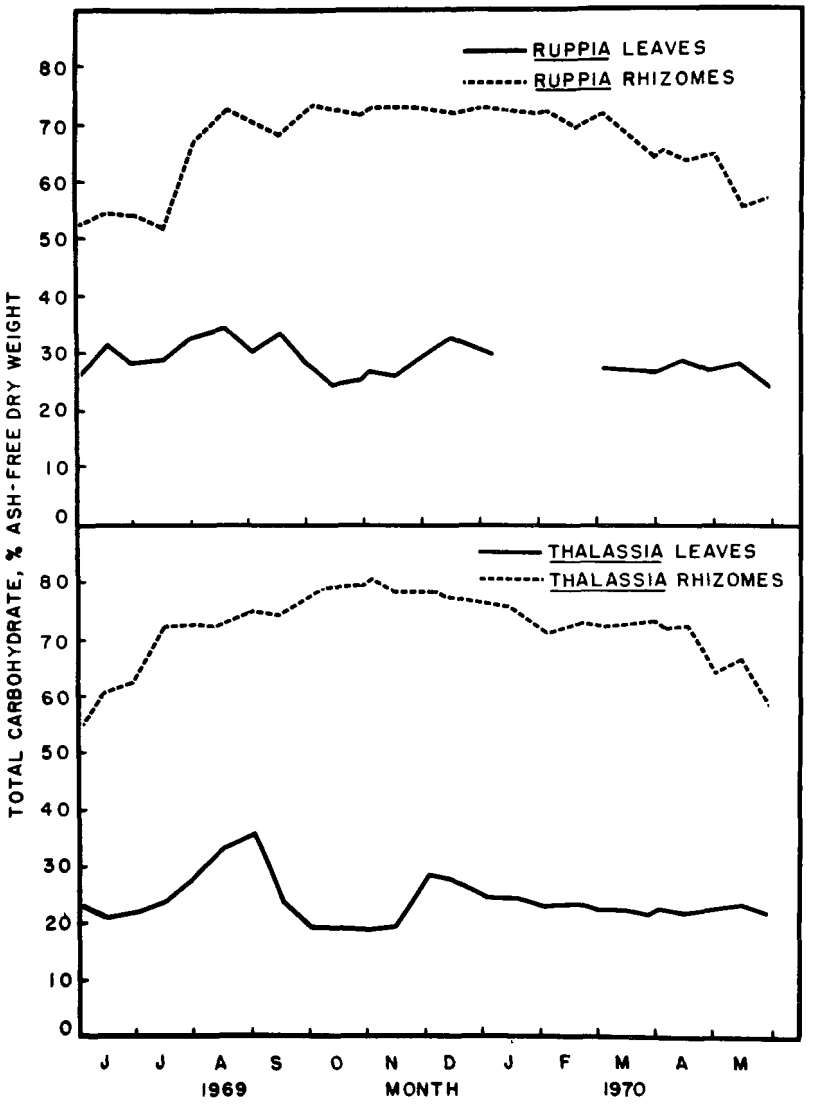


Fig. 2. Annual variation of carbohydrate in *Thalassia testudinum* and *Ruppia maritima*.

aquatic plants, *Myriophyllum* sp. contained approximately 7.8 per cent protein in dry weight (Oelshlegel, 1969) and *Spartina alterniflora* 8.9 per cent (Hall et al., 1970). Boyd (1970), in a study of the protein content of 11 freshwater species, reported a range of from 4.0 per cent (*Typha latifolia*) to 21.6 per cent (*Nuphar advena*). Yee (1970) reported 17.5 per cent protein in *Hydrilla* sp. and 30.5 per cent in *Pistia stratoides*. Among foodstuffs, 114 lines of corn contained 9.8-16.3 per cent protein (Davis et al., 1970), 49 varieties of grain sorghum contained 8.6-16.5 per cent (Virupaksha and Sastry, 1968) and wheat grain between 8.3-12.4 per cent (Chrominski, 1967).

Though high in protein, it is doubtful that these seagrasses could be used directly as food by humans. The unanimous consensus of a taste panel at the Gulf Breeze laboratory was that dried leaves and rhizomes are gritty, and have a strong, unpleasant odor and flavor.

Carbohydrate. In contrast to protein contents, carbohydrate contents of rhizomes were greater than those of leaves (Table 1) because rhizomes are storage organs for starch. Fig. 2 shows that the carbohydrate contents of rhizomes, as percentage ash-free dry weight, began to rise in July due to production and storage in summer, and attained peak concentrations in October and November. Decrease in spring was probably due to utilization of stored carbohydrate for biosynthesis and respiration.

The carbohydrate contents of the seagrasses tested were similar to those of other plants. As percentage dry weight, *T. testudinum* leaves contained between 12.5 and 25.5 per cent carbohydrate, whereas the rhizomes contained between 41.5 and 62.9 per cent. Leaves of *R. maritima* contained between 20.0 and 27.2 per cent and rhizomes between 35.8 and 55.1 per cent carbohydrate. Reported values for other plants, as percentage dry weight, are: alfalfa, 13-25 (Raguse and Smith, 1965, 1966; Grotelueschen and Smith, 1967); red clover, 14-21 (Raguse and Smith, 1966), and timothy, 48 (Grotelueschen and Smith, 1967). Most of the values for seagrasses were within these ranges.

Energy. The energy contents (Table 1) of all samples were very similar to those reported for most other plants (Cummings, 1967) and no annual trends were observed.

Elements. Annual variations in concentrations of sodium, potas-

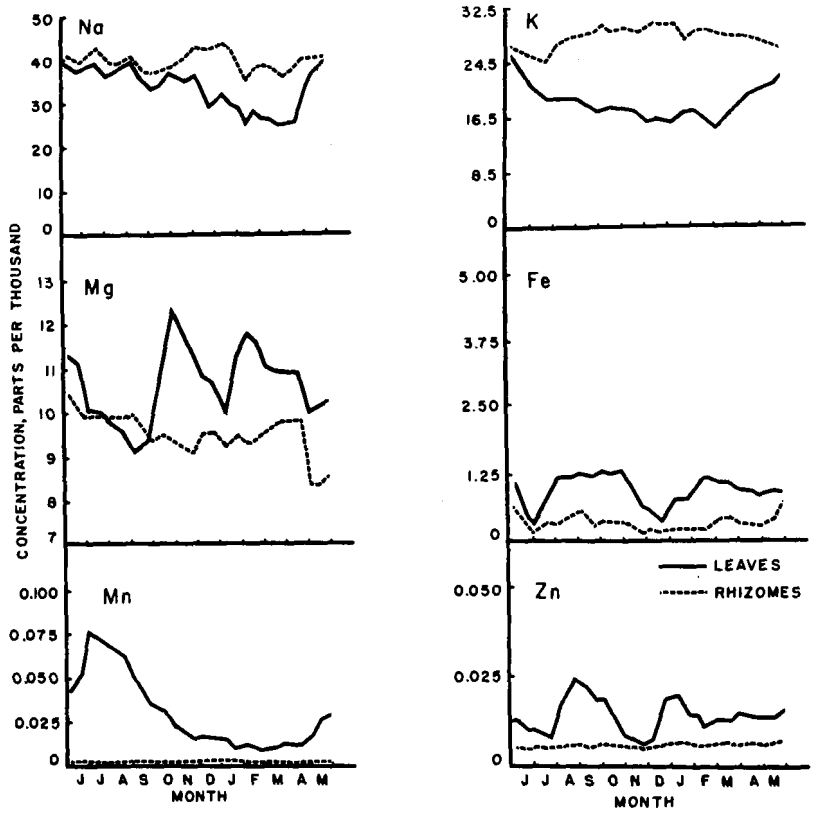


Fig. 3. Annual variations of some elements in *Thalassia testudinum.*

sium, magnesium, iron, manganese, and zinc are shown in Figures 3 and 4. The variations appear to be associated with age and functional aspects of the materials analyzed.

In several aquatic macrophytes, concentrations of some elements decline with age. For example, concentrations of nitrogen, phosphorus, sulfur, calcium, and potassium decline with age in *Typha latifolia* and in the bulrush *Scripus americanus* (Boyd, 1970). Concentrations of zinc, manganese and iron are lowest in mature *Spartina alterniflora* (Williams and Murdock, 1969), and the authors suggested that the decrease may be due to dilution of actively growing tissues by structural material which contained little of the elements measured.

Table 2 gives concentrations of the elements found in the leaves

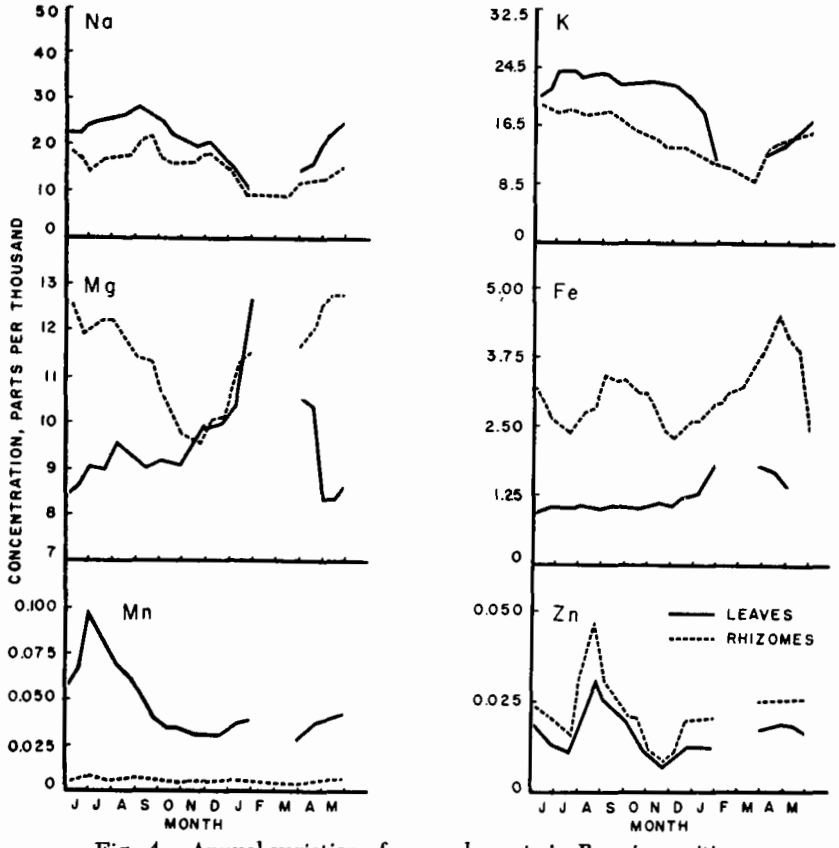


Fig. 4. Annual variation of some elements in *Ruppia maritima*.

and roots of selected plants. Concentrations in the seagrasses were always within ranges given in the table, even though annual variation was found for each element. Concentrations of zinc, manganese, and iron were also similar to those reported for *S. alterniflora* by Williams and Murdock (1969).

We cannot yet explain why the observed variations occurred because little is known about the multiple roles of each element during the year. For example, magnesium is a structural component of chlorophyll, but it was often in higher concentrations in rhizomes than in leaves. This was probably related to the fact that it is also a specific cofactor for many enzymes involved in carbohydrate metabolism and its concentration depends upon age and seasonal variation in physiology of the plant part.

TABLE 2

Concentrations, in parts per thousands, of some elements in the leaves and roots of selected plants.

Plant	K	Mg	Fe	Mn	Zn
Carrot					
leaves	13.3	2.8	0.36–0.77	0.02–0.20	0.03
roots	16.8–59.2	1.2–2.4	0.04–0.49	0.01–0.09	0.01
Soybean					
leaves	8.0	7.9	0.34	0.03–0.19	0.10
roots	14.4–15.6	10.7–31.8	–	0.02–0.15	–
Sunflower					
leaves	16.2–19.0	11.0	–	0.07–1.27	–
roots	13.6–38.0	1.3–12.7	0.03	–	0.02
Sweet potato					
leaves	16.1–23.7	4.5–5.4	–	–	–
roots	6.8–17.4	0.6–2.1	0.01–0.14	0.01–0.03	0.01
Tomato					
leaves	5.2–37.6	6.2–15.5	0.28–0.54	0.05–4.93	0.03
roots	8.0–34.1	4.6	–	–	–

From Altman, P. L. and D. S. Dittmer (eds.), Biology Data Book, 1964.

In summary, in relation to other aquatic plants and food crops, *T. testudinum* and *R. maritima* contain significant amounts of protein, carbohydrate, energy, and minerals. The nutritive value of *T. testudinum* has been established (Bauersfeld et al., 1969) and that of *R. maritima* is implied from the work reported here. Annual variation in chemical composition, however, implies that the nutritional value of seagrasses varies throughout the year.

LITERATURE CITED

ALTMAN, P. L., AND D. S. DITTMER (eds.). 1964. Biology data book. Federation of American Societies for Experimental Biology, Washington, D. C., xix + 633 pp.

BAUERSFELD, P., R. R. KIFER, N. W. DURANT, AND J. E. SYKES. 1969. Nutrient content of turtle grass (*Thalassia testudinum*). Proc. Intl. Seaweed Symp., vol. 6, pp. 637-645.

BOYD, C. E. 1970. Amino acid, protein, and caloric content of vascular aquatic macrophytes. Ecology, vol. 51, pp. 902-906.

BURKHOLDER, P. R., L. M. BURKHOLDER, AND J. A. RIVERO. 1959. Some chemical constituents of the turtle grass *Thalassia testudinum*. Bull. Torrey Bot. Club, vol. 86, pp. 88-93.

CHROMINSKI, A. 1967. Effect of (2-chloroethyl) trimethylammonium chloride on protein content, protein yield, and some qualitative indexes of winter wheat grain. Jour. Agr. Food Chem., vol. 15, pp. 109-112.

CUMMINGS, K. W. 1967. Calorific equivalents for studies in ecological energetics. Pymanting Laboratory of Ecology, University of Pittsburgh. 2nd. ed., 52 pp.

DAVID, D. J. 1958. Determination of zinc and other elements in plants by atomic absorption spectroscopy. Analyst., vol. 8, pp. 655-661.

DAVIS, L. W., W. P. WILLIAMS, JR., AND L. CROOK. 1970. Interrelationships of the protein and amino acid contents of inbred lines of corn. Jour. Agr. Food Chem., vol. 18, pp. 357-360.

FENCHEL, T. 1970. Studies on the decomposition of organic detritus derived from the turtle grass *Thalassia testudinum*. Limnol. Oceanogr., vol. 15, pp. 14-20.

GROTELUESCHEN, R. D., AND D. SMITH. 1967. Determination and identification of nonstructural carbohydrates removed from grass and legume tissue by various sulfuric acid concentrations, takadiastase, and water. Jour. Agr. Food Chem., vol. 15, pp. 1048-1051.

HALL, K. J., W. C. WEIMER, AND G. F. LEE. 1970. Amino acids in an estuarine environment. Limnol. Oceanogr., vol. 15, pp. 162-164.

HUDSON, J. H., D. M. ALLEN, AND T. J. COSTELLO. 1970. The flora and fauna of a basin in Central Florida Bay. U. S. Fish Wildl. Ser. Spec. Sci. Rep. Fish. 604, pp. 1-14.

MENZIES, R. J., AND G. T. ROWE. 1969. The distribution and significance of detrital turtle grass, *Thalassia testudinum*, on the deep-sea floor off North Carolina. Int. Rev. Ges. Hydrobiol., vol. 54, pp. 217-222.

OELSHLEGEL, F. J., JR. 1969. Potential for protein concentrates from alfalfa and waste green plant material. Jour. Agr. Food Chem., vol. 17, pp. 665-668.

OLNEY, P. J. S. 1968. The food and feeding habits of the pochard, *Aythya ferina*. Biol. Conserv., vol. 1, pp. 71-76.

PARKER, P. L. 1966. Movement of radioisotopes in a marine bay: cobalt-60, iron-59, manganese-54, zinc-65, sodium-22. Publ. Inst. Mar. Sci., Univ. Texas, vol. 11, pp. 102-107.

PHILLIPS, R. C. 1960. Observations on the ecology and distribution of the Florida seagrasses. Florida State Bd. Conser., Prof. Papers Ser., No. 2, St. Petersburg, iv + 72 pp.

RAGUSE, C. A., AND D. SMITH. 1965. Carbohydrate content in alfalfa herbage as influenced by methods of drying. Jour. Agr. Food Chem., vol. 13, pp. 306-309.

———. 1966. Some nonstructural carbohydrates in forage legume herbage. Jour. Agr. Food Chem., vol. 14, pp. 423-426.

RANDALL, J. E. 1965. Grazing effect on seagrasses by herbivorous reef fishes in the West Indies. Ecology, vol 46, pp. 255-260.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1965. A manual of sea water analysis, 2nd ed. Fish. Res. Bd. Canada. Bull. No. 125. Ottawa, viii + 203 pp.

VAN BREEDVELD, J. F. 1966. Preliminary study of seagrass as a potential source of fertilizer. Florida State Bd. Conser., Mar. Lab. Spec. Sci. Rep. 9, pp. 1-20.

VIRUPAKSHA, T. K., AND L. V. S. SASTRY. 1968. Studies on the protein content and amino acid composition of some varieties of grain sorghum. Jour. Agr. Food Chem., vol. 16, pp. 199-203.

WESTLAKE, D. F. 1965. Some basic data for investigations of the productivity of aquatic macrophytes. *In*: C. R. Goldman (ed.), Primary Productivity in Aquatic Environments. Mem. Ist. Ital. Idrobiol., 18 Suppl., University of California Press, Berkeley, pp. 229-248.

WILLIAMS, R. B., AND M. B. MURDOCK. 1969. The potential importance of *Spartina alterniflora* in conveying zinc, manganese, and iron into estuarine food chains. *In* D. J. Nelson and F. C. Evans (eds.), Symposium on Radioecology, 2nd Nat. Symp., Ann Arbor, Michigan, pp. 431-439.

YEE THONG TAN. 1970. Composition and nutritive value of some grasses, plants, and aquatic weeds tested as diets. Jour. Fish. Biol., vol. 2, pp. 253-257.

Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561. An associate laboratory of the National Environmental Research Center, Corvallis, Oregon. Contribution No. 126.

CONTRIBUTION NO. 131

In vivo Binding of p,p'-DDE to Human Serum Proteins

by W. P. SCHOOR[1]
Department of Pharmacology, Louisiana State University Medical Center, New Orleans, La. 70112

Although it is convenient to estimate chlorinated hydrocarbon pesticide levels in man by determining the amount present in serum, few investigators have questioned possible interactions of these compounds with serum proteins and the resulting consequences. DALE et al. (1965) suggested that the binding of pesticides to serum protein was the cause of incomplete pesticide recovery from human serum by hexane extraction, and GUNTHER et al. (1954) proposed molecules were held by proteins with consequent inhibition of the normal function of these proteins. Binding of dieldrin and telodrin to serum proteins has been demonstrated by MOSS and HATHWAY (1964), but the concentrations of these pesticides were considerably greater than those normally encountered in man and pH ranges during the separation deviated too far from the physiological norm to allow much speculation on the results. HATANAKE et al. (1967) attempted to recover pesticides from protein fractions after Sephadex G-50 treatment, but met with inconsistent results. Careful review of these reports emphasizes that direct evidence of serum protein binding under physiological conditions should be obtained.

METHODS

Serum was prepared from the author's blood by allowing it to clot for 2 hr at 0°C, then removing the liquid portion. Two-ml samples were kept at -15°C for not more than 10 days.

Ten g of Sephadex G-200 was soaked for 3 days in 1.0 liter of 0.9% NaCl solution at pH 7.35. This material was placed in a 2.5 cm x 46 cm glass column and allowed to settle under flow. Two ml of serum were diluted to 10 ml with 0.9% NaCl (pH 7.35), placed on the column, and eluted with the same solution. Ten-ml fractions were collected at a flow rate of 2-3 ml/min at constant

---

[1] Present address: Environmental Protection Agency, Gulf Breeze Laboratory, Sabine Island, Gulf Breeze, Florida 32561.

Bulletin of Environmental Contamination & Toxicology, Vol. 9, No. 2, © 1973 by Springer-Verlag New York Inc.

pressure. Protein concentrations were determined with a Zeiss Model PMQ-II spectrophotometer at 280 mμ.

The pooled protein fractions, usually about 150 ml, were extracted with 200-ml portions of hexane. When a 2.0-ml serum sample was diluted to 150 ml and extracted in this way, more p,p'-DDE was recovered and reproducibility was greater than when the serum was extracted directly by the method of DALE et al. (1965, 1970). Since further experimentation showed that a reduction in the volume of the diluted serum from 150 ml to 50 ml caused no significant reduction in p,p'-DDE recovery, the following procedure was adopted for the routine serum extractions:

Two ml of serum, diluted to 50 ml with 0.9% NaCl solution (pH 7.35), and 75 ml hexane were shaken vigorously in a 250-ml separatory flask for 10 sec and the flask inverted and vented. The shaking and venting process was continued for 1 min, after which the phases were allowed to separate. The process was repeated three times. Any interphase emulsion was broken by centrifugation. The extract was reduced to an appropriate volume by evaporation under a gentle stream of air at 35°C. The p,p'-DDE was detected by means of a Microtek MBT-220 gas chromatograph, using an OV-17/QF-1 column for quantitation and a SE-30/QF-1 column for confirmation at 195°C and a tritium-type electron-capture detector at 205°C. Further confirmation was achieved with thinlayer chromatography on silic acid with heptane as solvent.

RESULTS

Only p,p'-DDE was quantitated because it was present in large enough amounts to be confirmed by thin-layer chromatography. The p,p'-DDT present was in such low amounts that reproducibility between chromatograms was very poor. No polychlorinated biphenyl derivatives (PCB's) were present. Table 1 shows that compared with the method of DALE et al. (1965, 1970) the extraction method gives almost a twofold increase in p,p'-DDE recovered as well as less variability (F-test; $p<0.01$). Protein recovery after Sephadex G-200 chromatographic treatment was 95%, and the protein fractions contained approximately 80% of the original amount of p,p'-DDE. When p,p'-DDE in hexane was placed on the column and the hexane was allowed to evaporate, elution under the conditions described for serum chromatography did not yield any p,p'-DDE in the effluent in the range of the protein fractions. Preliminary data indicate that very little p,p'-DDE is found in the gamma globulin fraction. Precipitation of serum proteins with ammonium sulfate and subsequent hexane extraction of the supernatant and the precipitate yielded no p,p'-DDE in the former and only trace amounts in the latter. Serum samples kept at -15 C for more than 10 days showed a decrease in recoverable p,p'-DDE (e.g., 20% less was recovered from a sample stored for 21 days). Both

observations indicate that the degree of binding of p,p'-DDE to the proteins changes as the conformation of the protein changes.

DISCUSSION

The importance of the binding of drugs to serum proteins and the concomitant effects on their pharmacological activity cannot be overemphasized (SELLERS and KOCH-WESER 1969, MEYER and GUTTMAN 1968, CONN and LUCHI 1961, DOLLERY et al. 1961, MACREGOR 1965, BRODIE 1965, CUCINELL et al. 1965). BRODIE (1965) states, "Actually, almost all drugs are reversibly bound to proteins in plasma or tissue. The bound drug, often a high percentage of the total, acts as a reservoir, preventing wild fluctuations between ineffective and toxic levels of the biologically active unbound fraction." It is believed that the same type of mechanism can explain the behavior of p,p'-DDE and, very likely, all chlorinated hydrocarbons, including the PCB's, in the blood.

Although microsomal enzyme induction is usually cited as cause for the reduced serum levels of pesticides, the following interpretation should be considered: (1) Serum concentrations of chlorinated hydrocarbon pesticides normally encountered in human beings reflect "bound" levels that are relatively inert. (2) Any compound that can interfere with the binding of the pesticide may free it for adsorption at a site of toxic action, metabolic breakdown, or storage, depending on the distribution constant. (Large amounts of inertly bound pesticides upon liberation could in this fashion become available for binding at the site of toxic action). (3) The enzyme system responsible for the breakdown of the pesticides is always present, but cannot function because the substrate is tightly bound to the serum proteins. Pesticide metabolism is strictly governed by the difference in the distribution constants between the two sites.

This hypothesis would explain why aldrin enhanced the retention of p,p'-DDT and p,p'-DDE in the blood of dogs (DEICHMANN et al. 1969), the reduced paroxon binding capacity in rat plasma on oral pretreatment with tri-o-tolyl phosphate (LAUWERYS and MURPHY 1969), and the lowering of serum-bound iodine by o,p-DDD in humans (MARSHALL and TOMPKINS 1968). It would also explain the low levels of chlorinated hydrocarbons found in persons treated with anticonvulsant drugs by DAVIES et al. (1969) and SCHOOR (1970).

TABLE 1

Comparison of recovery of p,p'-DDE in human serum by different analytical methods.[a]

Method of DALE et al. (1965)	Present method	
	Combined protein fraction after G-200 treatment	
Serum	Serum	Serum
ppm	ppm	ppm
0.019	0.036	0.025
0.022	0.037	0.028
0.024	0.037	0.026
0.012	0.034	0.029
0.012	0.030	
0.017	0.034	
0.019	0.035	
0.015	0.033	
0.019	0.034	
0.019	0.034	
	0.034	
	0.036	

[a] Two ml of serum were extracted in each analysis

REFERENCES

BRODIE, B.B.: Proc. Royal Soc. Med., 58, 946 (1965).

CONN, H.L., and R.J. LUCHI: J. Clin. Invest., 40, 509 (1961).

CUCINELL, S.A., A.H. CONNEY, M. SANSUR, and J.J. BURNS: Clin. Pharmacol. Therap, 6, 420 (1965).

DALE, W.E., A. CURLEY, and C. CUETO: Life Sciences, 5, 47 (1965).

DALE, W.E., J.W. MILES, and T.B. GAINES: J.A.O.A.C., 53, 1287 (1970).

DAVIES, J.E., W.F. EDMUNDSON, C.H. CARTER, and A. BARQUET: The Lancet, July 5, 7 (1969).

DEICHMANN, W.B., M. KEPLINGER, I. DRESSLER, and F. SALA: Toxicol. Appl. Pharmacol., 14, 205 (1969).

DOLLERY, C.T., D. EMSLIE-SMITH, and D.F. MUGGLETON: Brit. J. Pharmacol., 17, 488 (1961).

GUNTHER, F.A., R.C. BLINN, G.E. CARMAN, and R.L. METCALF: Arch. Biochem. Biophys., 50, 504 (1954).

HATANAKA, A., B.D. HILTON, and R.D. O'BRIEN: J. Agr. Food Chem., 15, 854 (1967).

LAUWERYS, R.R., and S.D. MURPHY: Toxicol. Appl. Pharmacol., 14, 348 (1969).

MACREGOR, A.G.: Proc. Royal Soc. Med. 58, 943 (1965).

MARSHALL, J.S., and L.S. TOMPKINS: J. Clin. Endoc. and Metab., 28, 386 (1968).

MEYER, M.C., and D.E. GUTTMAN: J. Pharm. Sci., 57, 895 (1968).

MOSS, J.A., and D.E. HATHWAY: Biochem. J., 91, 384 (1964).

SCHOOR, W.P.: The Lancet, Sept, 5, 520 (1970).

SELLERS, E.M., and KOCH-WESER: The New England J. Med., 281, 1141 (1969).

CONTRIBUTION NO. 143

Reprinted from *BioScience*

Research Reports

Effects of Herbicides on Seedlings of the Red Mangrove, *Rhizophora Mangle* L.

"Mangrove" is a general term applied to a community of shrubs or trees that grow below the high-tide mark along tropical shores. The term is also used with reference to individual plant species which occur within that community (Davis 1940, Macnae 1968). The mangrove community is highly productive (Golley et al. 1962) and supports a wide variety of animals which depend upon plant detritus as a source of food (Heald 1971, Odum 1971). In Florida, many commercially important animals such as pink shrimp (*Penaeus duorarum*), blue crabs (*Callinectes sapidus*), striped mullet (*Mugil cephalus*), and spotted seatrout (*Cynoscion nebulosus*) use the mangrove for food and as nursery grounds (Idyll 1965, Idyll et al. 1968, Tabb 1966).

Susceptibility of mangrove to herbicides was first investigated by Truman (1961), who treated the grey mangrove, *Avicennia marina*, with the auxin-type herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). A concentration of only 1 percent in diesel oil distillate killed all trees when applied to the bark, and Truman concluded that the grey mangrove is very susceptible. The concept of high susceptibility of mangrove to herbicides was extended by Tschirley (1969) and Orians and Pfeiffer (1970). They stated that forests in Vietnam dominated by *Rhizophora conjugata*, *Bruguiera parviflora*, and *B. cylindrica* were destroyed after application of either a combination of 13.8 kg/ha of the n-butyl ester of 2,4-D and 14.8 kg/ha of the n-butyl ester of 2,4,5-T or 6.72 kg/ha of the triisopropanolamine salt of 2,4-D in combination with 0.61 kg/ha of the triisopropanolamine salt of 4-amino-3,5,6-trichloropicolinic acid (picloram). Westing (1971) found that treated mangrove areas in Vietnam remained uncolonized by plants 6 years after treatment and suggested that plants of the intertidal zone are highly sensitive to hormone-type herbicides.

Recolonization of mangrove in denuded areas must depend upon establishment and growth of seedlings. If herbicide residues remain in the soil, development of seedlings could be inhibited. The purpose of our research was to describe effects of a commercial formulation of 2,4-D and picloram on seedlings of the red mangrove, *Rhizophora mangle* L.

The genus *Rhizophora* is circumtropical in its distribution. *Rhizophora mangle* is common along the shores of the Gulf of Mexico, Caribbean Sea, West Africa (Chapman 1970), and Hawaii (Walsh 1967). The tree is vivaparous and, in southern Florida, produces seedlings throughout the year. The seedling consists of an elongated hypocotyl up to 30 cm long, with a short plumule approximately 0.5 cm long. The plumule is composed of the first leaves covered by the cotyledonary stipules. Leaf development does not occur until roots become established in the soil.

METHODS

Seedlings 18.2 to 26.5 cm long were picked from trees near Coral Gables, Florida, and planted in estuarine mud in plastic boxes in the laboratory. Salinity of the water which covered the sediment was 30 parts per thousand, and pH of the sediment ranged from 6.4 to 6.7. Room temperature was maintained at 27°C, and light was from gro-lux flourescent tubes beside the boxes. The lighting regime was alternate 12-hour periods of light and darkness. The herbicide formulation used was Tordon®[1] 101, which is a combination of the triisopropanolamine salt of 2,4-D (39.6%) and the triisopropanolamine salt of picloram (14.3%). This formulation is similar to that of Agent White, which was used in Vietnam. The formulation was added to the surface of the water so that the amount of each herbicide within the seedlings after uptake by the roots could be measured. Groups of seedlings were treated 3 days after planting when no leaves were extended. Later, other groups were treated after one pair or two pairs of leaves were extended. Seedlings were treated at rates of 1.12, 11.2, and 112.0 kg/ha (1, 10, and 100 lb/acre) of the commercial formulation. These rates were equivalent to active ingredient concentrations of 0.44, 4.40 and 44.0 kg/ha 2,4-D and 0.16, 1.60, and 16.0

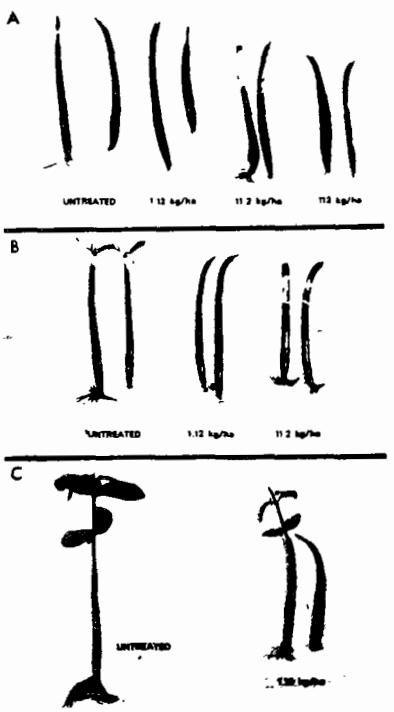


Fig. 1. Effects of Tordon 101 on seedlings of *R. mangle* treated when no leaves were present. A. 30 days after treatment; B. 40 days after treatment; C. 80 days after treatment.

Publication No. 143 from the Gulf Breeze Laboratory, Environmental Protection Agency, Gulf Breeze, Florida 32561, Associate Laboratory of the National Environmental Research Center, Corvallis, Oreg.

[1]®Registered trademark, Dow Chemical Company, Midland, Michigan. Reference to trade names in this publication does not constitute endorsement by the Environmental Protection Agency.

kg/ha picloram. Each test was performed three times and 45 seedlings were treated at each rate. Forty-five seedlings were maintained as controls for each experiment.

For quantitation of residues, herbicides were extracted from plant material with an acidified mixture of petroleum ether and ethyl ether (1:1). The acid salts were removed from the mixture with a basic aqueous wash. The aqueous solution was acidified and the free acids extracted with chloroform. After evaporation to dryness, the acids were esterified with diazomethane and the methyl ester quantitated by electron-capture gas chromatography. The limits of quantification were 0.01 parts per million (ppm) for picloram as picolinic acid and 0.02 ppm for 2,4-D. Residues are expressed as the averages for each treatment.

Sections of at least six leaves and roots from each experiment were fixed in formalin-aceto-alcohol, dehydrated in dioxane, embedded in paraffin, and cut to a thickness of 10 microns on a rotary microtome. They were affixed to slides with Haupt's adhesive and stained with safranin and fast green (Sass 1958) for histopathological studies.

RESULTS

Results are summarized in Table 1. In the first experiments, seedlings were treated while the first pair of leaves were enclosed in the cotyledonary stipules. In Fig. 1A, which shows seedlings 30 days after treatment, the leaves can be seen emerging from the hypocotyls of untreated seedlings and from one of those treated with 1.12 kg/ha. Seedlings treated with 11.2 kg/ha showed normal root development but were highly chlorotic on the upper one-half of the hypocotyl. Those treated with 112.0 kg/ha exhibited wide areas of chlorosis on their upper halves and were dead after 30 days. Some of these seedlings had longitudinal splits in the epidermis and cortex, and callosities protruded from the surface of the hypocotyl. After 40 days (Fig. 1B) growth of untreated seedlings had progressed normally, with further growth of roots and extension of the first pair of leaves. Seedlings treated with 1.12 kg/ha were alive, but root development was not as extensive as in the untreated group. The plumules of most were slightly expanded as before normal leaf extension. All seedlings treated with 11.2 kg/ha were dead. As with seedlings treated with 112.0 kg/ha, these seedlings were chlorotic, and the plumules of some had fallen from the hypocotyl. After 80 days, untreated seedlings had three pairs of leaves and appeared healthy. Those treated with 1.12 kg/ha were still alive, and some had as many as two pairs of leaves (Fig. 1C). However, stems and leaves were smaller than those of the untreated groups and root development was poor.

TABLE 1. Summary of responses of *Rhizophora mangle* to Tordon 101

Pairs of leaves when treated	Rate of application, kg/ha		
	1.12	11.2	112.0
0	Retarded development No deaths	Retarded root development Chlorosis of hypocotyl Death 40 days after treatment	Same as with 11.2 kg/ha treatment, except death 30 days after treatment
1	Positive phototrophic response No other effects noted	Positive phototrophic response Chlorosis of leaves and hypocotyl Histological abnormalities of leaves and roots Death approximately 30 days after treatment	Chlorosis of leaves and hypocotyl Histological abnormalities of leaves Defoliation Death 15 days after treatment
2	Same as when 1 pair of leaves present	Same as when 2 pairs of leaves present, except death within 40 days	Same as when 1 pair of oeaves present, except death within 10 days

Residues of herbicides in the hypocotyls are given in Table 2. Tissue concentrations were related to application rates, but residues, although present, were too low to quantitate in seedlings exposed to 1.12 kg/ha. Clearly, such low concentrations of herbicides in the hypocotyls caused impaired development of seedlings which were treated before emergence of leaves.

When seedlings with one pair of leaves were treated with Tordon 101, those which received 112.0 kg/ha became chlorotic and died within 15 days after treatment. Within a few days

TABLE 2. Concentrations of 2,4-D and picolinic acid, in parts per million (± 20%), of wet tissue in the hypocotyls of *R. mangle* seedlings treated with Tordon 101 before emergence of leaves. Residues were detected in every analysis of seedlings treated with 1.12 kg/ha but were below the level of quantification.

Treatment kg/ha	Days after treatment: 30		40	
	2,4-D	Picolinic acid	2,4-D	Picolinic acid
11.2	0.81	0.14	2.26	<0.01
112.0	4.10	0.58		

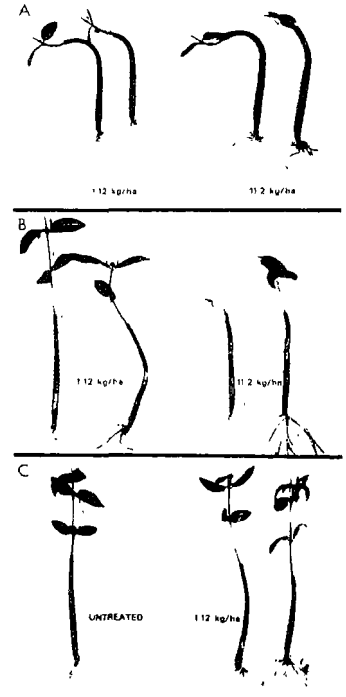


Fig. 2. Effects of Tordon 101 on seedlings of *R. mangle* treated when one pair of leaves was present. A. 10 days after treatment; B. 30 days after treatment; C. 60 days after treatment.

TABLE 3. Concentrations of 2,4-D and picolinic acid, in parts per million (± 20%), of wet tissue in organs of *R. mangle* seedlings treated with Tordon 101 when a single pair of leaves was present. Residues were detected in every analysis of seedlings treated with 1.12 kg/ha but were below the level of quantification.

Treatment kg/ha	Day	Roots 2,4-D	Roots Picolinic Acid	Hypocotyl 2,4-D	Hypocotyl Picolinic Acid	Stem 2,4-D	Stem Picolinic Acid	1st leaves 2,4-D	1st leaves Picolinic Acid
11.2	10	<0.02	<0.01	0.23	0.04	0.17	0.04	<0.02	<0.01
	30	<0.02	<0.01	0.10	0.04	0.27	0.08	0.05	0.05
112.0	10	1.04	0.23	1.55	0.41	0.91	0.19	0.46	0.10

after treatment, the hypocotyls of seedlings treated with 1.12 and 11.2 kg/ha became markedly bent toward the light source (Fig. 2A), a reaction that would be expected after treatment with hormone-type herbicides as concentration of auxin generally is greater on the dark sides of stems. Greater growth or elongation of cells on the dark side would cause bending of the stem toward the light. Thirty days after treatment, the seedlings had returned to the upright position. Table 3 shows that the amount of 2,4-D in the hypocotyls of seedlings treated with 11.2 kg/ha decreased after 30 days. However, this group was moribund at that time, with chlorosis of the leaves and hypocotyls and with callosities along most of the length of the hypocotyl. After 60 days, seedlings treated with 1.12 kg/ha appeared normal.

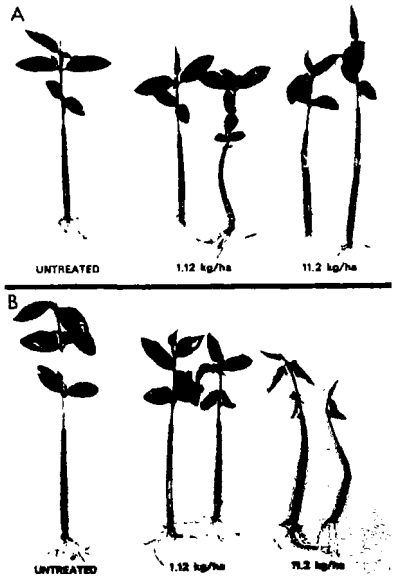


Fig. 3. Effects of Tordon 101 on seedlings of *R. mangle* treated when two pairs of leaves were present. A. 20 days after treatment; B. 40 days after treatment.

Concentrations of herbicides in various plant parts are given in Table 3. Residues were present in seedlings treated with 1.12 kg/ha but were too low to quantitate. Response to such low tissue concentrations of the auxin-type herbicides was indicated by bending of the hypocotyl toward the light. At other application concentrations, highest tissue residues were usually in the hypocotyl, which may explain why effects were greatest in that organ.

When seedlings with two pairs of leaves were treated at the two higher concentrations, chlorosis appeared on the hypocotyl at 10 to 20 days after treatment (Fig. 3A). Soon thereafter the leaves became dry and brittle, turned brown, and curled inward (Fig. 3B). The signs were those of desiccation and defoliation (Bovey et al. 1969). Seedlings treated with 112.0 kg/ha were dead within 10 days after treatment. Those exposed to 11.2 kg/ha were dead within 40 days. Bending of the hypocotyl toward the light occurred in all seedlings treated with 1.12 and 11.2 kg/ha, but this condition lasted less than 30 days. Residues were again usually greatest in the hypocotyls (Table 4), but were considerably greater in the leaves than they were in the previous tests.

Studies were made of histological abnormalities of leaves and roots associated with herbicidal treatment. The leaves of *R. mangle* have a well-developed cuticle on both surfaces. Immediately below the upper epidermis is a single or double layer of cells which contain tannin. These are underlain in turn by several layers of hypodermal cells, palisade parenchyma, spongy parenchyma, another layer of tannin cells, and the lower epidermis. Figure 4A shows the normal histology of the leaf near the midrib. Figure 4B shows early effects of treatment with 11.2 kg/ha Tordon 101 in the same area. Cell wall continuity has begun to break down in the hypodermis, palisade parenchyma, and spongy parenchyma. The normal leaf structure in the region centrally located between the midrib and the margin is shown in Fig. 4C. This is the final stage in leaf degeneration before it falls from the seedling. A total loss of structural integrity is shown.

Cortical cells of the root were also affected by treatment with 11.2 kg/ha Tordon 101. Histology of the normal root is shown in Fig. 4E and of the treated root in Fig. 4F. The figures demonstrate destruction of the cortex after treatment with herbicide.

DISCUSSION

Amounts of herbicides required to kill mangrove appear to be smaller than those required to kill other species of tropical trees. In our experiments, a combination of 4.4 kg/ha 2,4-D and 1.6 kg/ha picloram killed all seedlings. Bovey et al. (1969) treated a mixed upland tropical forest in Puerto Rico with 6.72 kg/ha 2,4-D and 1.68 kg/ha picloram and obtained 90 percent defoliation after one month, but reforestation began after that time. Truman (1961) reported that complete defoliation of the grey mangrove in Australia was caused by application of 1 percent 2,4-D to the bark. The same

TABLE 4. Concentrations of 2,4-D and picolinic acid, in parts per million (± 20%), of wet tissue in organs of *R. mangle* seedlings treated with Tordon 101 when two pairs of leaves were present. Residues were detected in every analysis of seedlings treated with 1.12 kg/ha but were below the level of quantification.

Treatment kg/ha	Day	Roots 2,4-D	Roots Picolinic Acid	Hypocotyl 2,4-D	Hypocotyl Picolinic Acid	Stem 2,4-D	Stem Picolinic Acid	1st leaves 2,4-D	1st leaves Picolinic Acid	2nd leaves 2,4-D	2nd leaves Picolinic Acid
11.1	30	<0.02	<0.01	0.10	0.03	0.02	0.01	<0.02	<0.01	0.13	0.06
	40	<0.02	<0.01	0.23	0.10	0.23	0.10	0.29	0.10	0.35	0.10
112.0	10	1.23	0.39	1.68	0.49	1.02	0.43	0.63	0.24	0.87	0.41

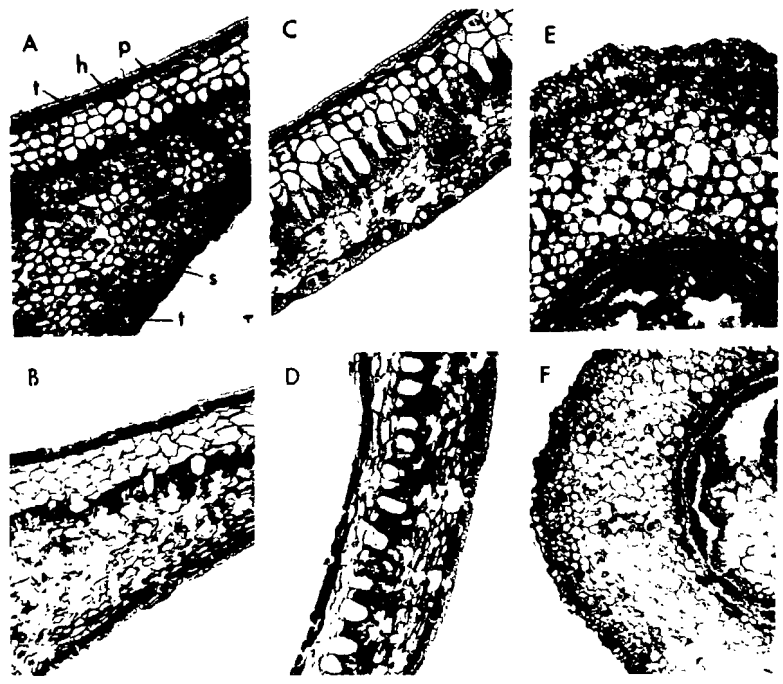


Fig. 4. Effects of Tordon 101 on microscopic anatomy of *R. mangle*. A. Section through untreated leaf near midrib (t=tannin cells, h=hypodermis, p=palisade parenchyma, s=spongy parenchyma); B. Section through leaf near midrib in seedling treated with 11.2 kg/ha; C. Section through center of normal leaf; D. Section through center of leaf of seedling treated with 11.2 kg/ha; E. Section through cortex of normal root; F. Section through cortex of root of seedling treated with 11.2 kg/ha. Magnification of all sections: 100X.

application rate caused only 9 percent defoliation of *Eucalyptus maculata* in the highlands.

The reasons for this apparent great sensitivity of mangrove to herbicides are not clear. Westing (1971) suggested that susceptibility is related to physiological attributes that permit growth in the tropical tidal environment. Scholander et al. (1966) showed that *Rhizophora* regulates ion uptake by a salt-exclusion mechanism in the roots. Our research demonstrated destruction of roots by herbicidal treatment, and it is possible that, in addition to direct effects of herbicides, death of seedlings was caused by disruption of their osmoregulatory ability. Further, physical conditions in the tidal environment could cause greater herbicidal uptake and activity than in upland regions. The mangrove environment is very fertile (Macnae 1968), and it is well known that high fertility coupled with abundant water increases the susceptibility of plants to herbicides (Hammerton 1967).

Our experiments incidate that relatively low concentrations of auxin-type herbicides inhibit mangrove development. Reclaimation of the mangrove forest may be difficult if low residues from previous sprayings persist in soil.

REFERENCES

Bovey, R. W., C. C. Dowler, and J. D. Diaz-Colon. 1969. Response of tropical vegetation to herbicides. *Weed Sci.*, 17: 285-290.

Chapman, V. J. 1970. Mangrove phytosociology. *Trop. Ecol.*, 1: 1-19.

Davis, J. H., Jr. 1940. The geologic role of mangroves in Florida. Carnegie Inst. Wash., Dept. Mar. Biol., *Pap. from the Tortugas Lab.*, 32: 303-412.

Golley, F. B., H. T. Odum, and R. F. Wilson. 1962. The structure and metabolism of a Puerto Rican red mangrove forest in May. *Ecology*, 43: 9-19.

Hammerton, J. L. 1967. Environmental factors and susceptibility to herbicides. *Weeds*, 15: 330-336.

Heald, E. J. 1971. The production of organic detritus in a south Florida estuary. *Univ. Miami, Sea Grant Tech. Bull.*, No. 6, viii, 110 pp.

Idyll, C. P. 1965. Shrimp need fresh water too. *Natl. Parks Mag.*, 10: 14-15.

Idyll, C. P., D. C. Tabb, and B. Yokel. 1968. The value of estuaries to shrimp. *Proc. Marsh Estuary Management Symp.*, La. State Univ., July 1967, pp. 83-90.

Macnae, W. 1968. A general account of the fauna and flora of mangrove swamps and forests in the Indo-West Pacific region. *Adv. Mar. Biol.*, 6: 73-270.

Odum, W. 1971. Pathways of energy flow in a south Florida estuary. *Univ. Miami, Sea Grant Tech. Bull.*, No. 7, xi, 162 pp.

Orians, G. H. and E. W. Pfeiffer. 1970. Ecological effects of the war in Vietnam. *Science*, 168: 544-554.

Sass, J. E. 1958. Botanical Microtechnique. 3rd edition, Iowa State Univ. Press, Ames, 228 pp.

Scholander, P. F., E. D. Bradstreet, H. T. Hammel, and E. A. Hemmingsen. 1966. Sap concentrations in halophytes and some other plants. *Plant Physiol.*, 41: 529-532.

Tabb, D. C. 1966. The estuary as a habitat for spotted seatrout, *Cynoscion nebulosus*. *Spec. Publ. Am. Fish. Soc.*, 3: 59-67.

Truman, R. 1961. The eradication of mangroves. *Aust. J. Sci.*, 24: 198-199.

Tschirley, F. H. 1969. Defoliation in Vietnam. *Science*, 163: 779-786.

Walsh, G. E. 1967. An ecological study of a Hawaiian mangrove swamp. Pages 420-431 *in* G. H. Lauff, ed. Estuaries, AAAS Publ. No. 83.

Westing, A. H. 1971. Ecological effects of military defoliation on the forests of South Vietnam. *BioScience*, 21: 893-898.

GERALD E. WALSH
REGINA BARRETT
GARY H. COOK
TERRENCE A. HOLLISTER

Environmental Protection Agency
Gulf Breeze Laboratory
Sabine Island
Gulf Breeze, Fla. 32561

Associate Laboratory of the
National Environmental Research
Center, Corvallis, Oreg.

CONTRIBUTION NO. 147

Avoidance of Pesticides by Grass Shrimp *(Palaemonetes pugio)*[1]

by DAVID J. HANSEN, STEVEN C. SCHIMMEL, and JAMES M. KELTNER, JR.
Environmental Protection Agency
Gulf Breeze Laboratory, Sabine Island, Gulf Breeze, Fla. 32561
Associate Laboratory of the National Environmental Research Center
Corvallis, Ore.

Some fishes can avoid certain pesticides in water. Green sunfish, Lepomis cyanellus, was repelled by chlordane but not lindane (SUMMERFELT and LEWIS 1967); sheepshead minnows, Cyprinodon variegatus, avoided DDT, endrin, Dursban®, and 2,4-D (BEE) but did not avoid malathion or Sevin® (HANSEN 1969); and mosquitofish, Gambusia affinis, avoided DDT, Dursban, 2,4-D, malathion and Sevin but not endrin (HANSEN et al. 1973).

Crustaceans are usually more sensitive to pesticides, particularly insecticides, than are fishes, but little is known about their ability to avoid pesticide pollution. The purpose of this study was to evaluate the capacity of the euryhaline grass shrimp, Palaemonetes pugio, to avoid DDT, endrin, Dursban, malathion, Sevin and 2,4-D. This shrimp was selected because of its importance in the food web (WOOD 1967) and its abundance in local waters.

Experimental Procedure

Grass shrimp, 10-40 mm rostrum-telson length, were seined from brackish-water ponds on Sabine Island. They were acclimated for at least 5 days in the laboratory at 20% salinity and 20 C before they were used in experiments.

The avoidance response was tested in a black plastic apparatus designed to allow the shrimp to move from a holding area either into a section which contained water with pesticide or into one which contained water without pesticide (HANSEN et al. 1973). A gate was lowered at the junction between the two sections and the holding area to trap shrimp for counting. When a test was in progress the apparatus was covered with black acrylic plastic to exclude light. Filtered sea water diluted with aerated tap water to 20% salinity and maintained at 20 C entered the upper end of each of the two sections at a rate of 400 ml/minute and flowed to

---

[1] Gulf Breeze Contribution No. 147

® Registered trademark: Dow Chemical Co., (Dursban) and Union Carbide Corp. (Sevin). Reference to trade names in this publication does not imply endorsement of the products by the Environmental Protection Agency.

Bulletin of Environmental Contamination & Toxicology,
Vol. 9, No. 3, © 1973 by Springer-Verlag New York Inc.

the drain in the holding area. Pesticides dissolved in acetone were metered through stopcocks at 0.5 ml/minute into the water entering one of the two sections. The same amount of acetone without pesticide was metered into the water entering the other section. The two upper "Y's" served no specific function in these tests.

Pesticides selected for avoidance testing included five insecticides and one herbicide (Table 1). Concentrations of these chemicals were selected so that one was higher and two or three were lower than the concentration that flowing water bioassays indicated would kill 50 percent of the shrimp in 24 hours (LC50). Concentrations were not checked by chemical analysis.

TABLE 1

Description of chemicals tested and 24-hour LC50's for grass shrimp, Palemonetes pugio.

Pesticide	Type	Percentage active ingredient	24-hour LC50 (ppm)[1]
DDT	Organochlorine	99	0.0007
Endrin	Organochlorine	97	0.0015
Dursban	Organophosphate	99	0.0032
Malathion	Organophosphate	95	0.032
Sevin (Carbaryl)	Carbamate	98	0.038
2,4-D (butoxyethanol ester)	Herbicide	70 (acid equivalent)	No effect at 10 ppm[2]

[1] Personal communication, Jack I. Lowe, Environmental Protection Agency, Sabine Island, Gulf Breeze, Florida 32561, May 8, 1972.

[2] Static bioassay.

The avoidance response was tested in two phases:

1. The ability of grass shrimp to choose between water that contained a pesticide and water free of pesticide was tested. Response to each concentration of pesticide was tested at least four times; twice with the pesticide entering one section of the apparatus and twice with the pesticide entering the opposite section. For each of the four replications, 50 shrimp were placed in the holding area with the gate lowered. After 30 minutes, the gate was raised to give the shrimp access to both sections. One hour later, the gate was closed and the number of shrimp in each section was recorded. This procedure was repeated when additional data were required to verify the conclusions.

2. The capacity of shrimp to discriminate between concentrations of pesticide avoided in the first phase was tested.

Experimental procedure was the same as in the first series of tests except that the shrimp were given a choice between two concentrations of the same pesticide.

The ability of grass shrimp to avoid pesticides in both phases was evaluated statistically by the chi-square test on the assumption that if there was no response to the pesticides, the shrimp that left the holding area would enter each section with equal frequency. Preliminary tests without pesticides indicated that this assumption was correct. Lack of any preference for the right or left section in avoidance tests (49 vs. 51%) further corroborated this assumption. Avoidance or preference was considered significant if the probability that observed distributions would occur by chance was 0.05 or less. Shrimp remaining in the circular area after a test was completed were not included in the statistical analysis because stationary shrimp may not have been exposed to the two choices and moving shrimp may have been in transit between areas.

Avoidance

Grass shrimp avoided 1.0 and 10.0 ppm of the butoxyethanol ester of 2,4-D by seeking water free of this herbicide but did not avoid any of the five insecticides (Table 2). The avoidance response of two fishes, sheepshead minnows and mosquitofish, to these same pesticides was tested identically and 2,4-D was the most readily avoided (HANSEN 1969, HANSEN et al. 1973). Fish in TVA reservoirs were apparently repelled by application of 2,4-D at 40-100 pounds per acre (SMITH and ISOM 1967). Concentrations of 2,4-D in reservoir water one hour after application reached 0.16 ppm; slightly less than amounts avoided by shrimp in our tests. Although statistical analysis indicated that grass shrimp preferred 0.0001 ppm of DDT, this was probably not valid because preference was observed in only one of three replications, and shrimp did not respond to greater or lesser concentrations.

Grass shrimp given a choice between two concentrations of 2,4-D selected the lower concentration (Table 3). Up to 78 percent of the shrimp that left the holding area avoided the higher of the two concentrations. Only 2,4-D was tested in this manner because it was the only pesticide that shrimp avoided by seeking water free of toxicant.

TABLE 2

Capacity of grass shrimp to seek water free of pesticides

N.S. = not significant. χ^2 = P(3.84 = 0.05; 6.63 = 0.01; 10.83 = 0.001.

Pesticide	Concentration (ppm)	Number of tests	Number of Shrimp*		Percentage in water	χ^2 value
			In pesticide	In water		
DDT	0.01	4	60	80	57.1	N.S.
	0.001	4	46	55	54.4	N.S.
	0.0001	12	218	165	43.1	7.334
	0.00001	4	78	84	48.1	N.S.
Endrin	0.01	4	60	77	56.2	N.S.
	0.001	4	79	64	44.8	N.S.
	0.0001	4	76	66	46.5	N.S.
Dursban	0.001	4	63	70	52.6	N.S.
	0.0001	4	76	88	53.6	N.S.
	0.00001	8	133	105	44.2	N.S.
Malathion	1.0	8	137	117	46.1	N.S.
	0.1	4	62	61	49.6	N.S.
	0.01	4	71	77	52.0	N.S.
Sevin	0.1	4	66	61	48.0	N.S.
	0.01	4	62	51	45.1	N.S.
	0.001	8	129	102	44.2	N.S.
	0.0001	4	57	65	53.3	N.S.
2,4-D	10.1	4	44	91	67.4	16.363
	1.0	4	51	76	59.8	4.921
	0.1	4	57	59	50.9	N.S.

*Does not include shrimp in holding area at end of test.

TABLE 3

Response of grass shrimp exposed to two different concentrations of the butoxyethanol ester of 2,4-D. χ^2 = P(3.84 = 0.05; 6.63 = 0.01; 10.83 = 0.001.

Concentrations (ppm)		Number of shrimp*		Percentage	χ^2
High	Low	In high conc.	In low conc.	in low Concentration	value
10.0	1.0	43	90	67.7	16.61
10.0	0.1	24	85	78.0	34.14
1.0	0.1	43	67	60.9	5.24

*Does not include shrimp in holding area at end of test.

Our study indicates that grass shrimp are less able to avoid and are more readily affected by pesticides than were the fishes used in earlier experiments (HANSEN 1969, HANSEN et al. 1973). Similarly, the European brown shrimp (Cragon cragon) did not avoid DDT (0.1 ppm), azinphos-methyl (1 ppm), atrazine (10 ppm) and aminotriazole (1,000 ppm) and were more sensitive to these compounds than were fishes (PORTMAN In press). These data suggest that shrimp may be extremely vulnerable to pesticide pollution because they are (1) extremely sensitive to pesticides and (2) generally are unlikely to avoid water polluted by pesticides. Consequently it is important that pesticides destined for use in and near estuaries be tested to determine their toxicity to shrimp and the capacity of shrimp to avoid them.

Literature Cited

HANSEN, D.J.: Trans. Am. Fish. Soc. 98, 426 (1969).
HANSEN, D.J., E. MATTHEWS, S.L. NALL and D.P. DUMAS: Bull. Environ. Contam. Toxicol. 8, (In press) (1973).
PORTMAN, J.E.: Proc. FAO Tech. Conf. on Marine Pollution and its Effects on Living Resources and Fishing, December 1970, Rome (In press).
SMITH, G.E. and B.G. ISOM: Pestic. Monit. J. 1, 16 (1967).
SUMMERFELT, R.C. and W.N. LEWIS: J. Water Pollut. Contr. Fed. 39, 2030 (1967).
WOOD, C.E.: Contri. Mar. Sci. 12, 54 (1967).

CONTRIBUTION NO. 154

Reprinted from
ECOLOGY OF HALOPHYTES

MANGROVES: A REVIEW[1]

Gerald E. Walsh

Environmental Protection Agency

Gulf Breeze Laboratory

Sabine Island

Gulf Breeze, Florida 32561

Associate Laboratory of the National Environmental Research Center,

Corvallis

"The beaches on that coast I had come to visit are treacherous and sandy and the tides are always shifting things about among the mangrove roots...A world like that is not really natural...Parts of it are neither land nor sea and so everything is moving from one element to another, wearing uneasily the queer transitional bodies that life adopts in such places. Fish, some of them, come out and breathe air and sit about watching you. Plants take to eating insects, mammals go back to the water and grow elongate like fish, crabs climb trees. Nothing stays put where it began because everything is constantly climbing in, or climbing out, of its unstable environment."

INTRODUCTION

The quotation above from Loren Eisley's eloquent book, "The Night Country," portrays in poetic terms the fascination of the tropical mangrove forest for those who have studied and researched that "not really natural"

[1] Publication No. 154 from the Gulf Breeze Laboratory, Environmental Protection Agency, Gulf Breeze, Florida 32561 Associate Laboratory of the National Environmental Research Center, Corvallis.

world. In the mangrove ecosystem, where tides and coastal currents bring unremitting variation to the forest, plants, and animals adapt continuously to changing chemical, physical, and biological characteristics of their environment. Many species use the environment dominated by mangrove trees for food and shelter during part or all of their life cycles. There is constant movement of living and non-living matter into and out of the mangrove swamp, and the effects of such movement may be felt miles away (Heald 1971, Odum 1971). Of course, not all tropical coasts are lined with mangrove forests; often a mangrove stand is small, or only an occasional tree dots the shoreline.

The factors which determine development of coastal forests, the ecological roles of mangroves in estuaries, and their utilization by man have been studied at length. The references at the end of this review give over 1,200 published accounts on mangroves. I am certain to have missed many publications in my search, but the number gives testimony to the importance of mangroves in estuaries. For an historical sketch of published works on mangrove, see Bowman (1917), who traced the mangrove literature back to 325 B.C. and the chronicle of Nearchus, commander of the fleet of Alexander the Great. Additional information is given in the reports of Walter and Steiner (1936) on East African mangroves, Davis (1940b) on the ecology and geologic roles of mangroves in Florida, and Macnae (1968) on the flora and fauna of mangrove swamps in the Indo-West-Pacific region. See also the excellent discussion of ecology of the Rhizophoraceae by van Steenis in Ding Hou (1958).

Davis (1940b) described "mangrove" as a general term applied to plants which live in muddy, loose, wet soils in tropical tide waters. According to Macnae (1968), mangroves are trees or shrubs that grow between the high water mark of spring tides and a level close to but above mean sea level. They are circumtropical on sheltered shores and often grow along the banks of rivers as far inland as the tide penetrates. Chapman (1939, 1940, 1944a) described silt, sand, peat, and coral reefs as mangrove habitats. On the reef, seedlings develop in holes and crevices in the porous coral rock, but the trees are usually stunted and the area occupied by the stand is not large. The reef may be a habitat only in those areas where tidal height is not great, because total inundation for extended periods of time can be fatal to seedlings (Rosevear 1947). Another mangrove habitat, the sand beach, described by Chapman (1940) supports *Rhizophora mangle* L. Later, van Steenis (1962) stated that *R. stylosa* Griffith is often found in sand in the Indo-Pacific region. Hathaway (1953) and Moul (1957) reported stands of *R. mucronata* Lamk., *Sonneratia caseolaris* (L.) Engler, and *Bruguiera conjugata (gymnorhiza?)* Lamk is sand on several atolls in the Pacific Ocean. I saw *R. mangle* growing in sand in Hawaii.

Boughey (1957) described mangroves which grew in two types of lagoons on the west coast of Africa. In open lagoons, some of the mud around the margins was exposed daily at low tide. *Rhizophora racemosa* G.F.W. Meyer

and *R. harrisonii* Leechman grew on the exposed mud. *Rhizophora* species grew only in open lagoons which were flooded daily. In closed lagoons, *Avicennia nitida* Jacq. was the dominant form in association with *Conocarpus erectus* L., *Laguncularia racemosa* Gaertn., and *Dodonea viscosa* L.

Burtt Davy (1938) classified tropical woody vegetation types according to "mature" or "apparently stable" communities. Two of his classes apply to mangrove vegetation and are given here because much of the nomenclature is in common usage today.

1. Tropical Mangrove Woodland

Name Suggested for Adoption: Mangrove woodland.

Synonyms: Mangrove, Mangrove swamp, Tidal forest.

Brief Definition: Woodland formation below high-tide mark; sometimes forest-like. Nearest in form to dry evergreen forest. A subformation of the littoral swamp forest.

Habitat: Soil flooded with water either permanently or at high tide; water usually more or less brackish; on estuarine mud.

2. Tropical Littoral Woodland

Name Suggested for Adoption: Littoral woodland.

Synonyms: Strand vegetation, Beach forest, Dune forest.

Brief Definition: Woodland formations in situations mentioned below; somewhat resembling semi-evergreen forest; open herbaceous vegetation.

General Description: The most characteristic species of this formation in India and Burma is the evergreen but very light-foliaged *Casuarina*, which often forms an almost pure fringe on sandy beaches and dunes along the sea face. Scattered smaller evergreen trees occur, with fewer deciduous trees, and these, in the absence of *Casuarine*, form the dominant canopy. On the east coast of Tropical Africa are such species as *Heritiera littoralis* Dryand, *Barringtonia racemosa* L., *Terminalia catappa* L., *Phoenix reclivata*, and *Diospyros vaughaniae*; species of *Pandanus* and *Cocos nucifera* L. are characteristic of this formation, which naturally includes several species whose seeds or fruits are current-borne. *Ipomea pescaprae* commonly occurs as a surface creeper on exposed sand dunes. Xerophytic herbs such as *Sansevieria, Opuntia, Kalanchoe,* and *Euphorbia* are common.

Habitat: Sandy and gravelly seashores; not subject to immersion, but under constant maritime influence. All around the coast wherever a fair width of sandy beach occurs, including sandy bars on the sea face of river deltas.

In this discussion, I shall follow Macnae (1968) and use the word "mangrove" with reference to individual kinds of trees, and the word "mangal" with reference to the swamp forest community.

It has been estimated that between 60% and 75% of the tropical coastline is lined with mangrove trees (McGill 1958) though some stands are more extensive than others. There seem to be five basic requirements for extensive mangal development. They are:

1. **Tropical temperatures.** Well developed mangals are found only along coastal areas where the average temperatures of the coldest month is higher

than 20°C and the seasonal temperature range does not exceed 5°C (West 1956, van Steenis 1962).

2. **Fine-grained alluvium.** Mangrove stands are best developed along deltaic coasts or in estuaries where soft mud comprised of fine silt and clay and rich in organic matter, is available for growth of seedlings. Quartzitic and granitic alluvia are generally poor substrata, whereas volcanic soils are highly productive of mangroves (Schuster 1952, West 1956, Haden-Guest et al. 1956, Macnae and Kalk 1962, Macnae 1968).

3. **Shores free of strong wave and tidal action.** Mangroves develop best along protected shores of estuaries because strong wave and tidal actions uproot seedlings and carry away soft mud (Young 1930, Cockayne 1958).

4. **Salt water.** Salt water per se is not a physical requirement of mangroves (Bowman 1917, Warming and Vahl 1925, Rosevear 1947, Egler 1948, Daiber 1960). Mangroves are faculative halophytes that occupy tidal areas where fresh-water plants, which are intolerant to salt, cannot live (West 1956).

5. **Large tidal range.** A wide, horizontal tidal range has been cited as requisite for extensive growth of mangrove (Foxworthy 1910, West 1956) and Chapman and Trevarthen (1953) stated that a universal scheme for comparison of different shores can be based only on the tides as a universal controlling factor. Although the tide per se is probably of little importance in determining the extent of mangal development, on a shore of gentle gradient and large tidal range, a wide belt of alluvium will be formed, and with it, a wide belt of mangrove. Deep tidal penetration would also cause saline water to be distributed far inland. Davis (1940) described the action of wind in driving salt water inland in Florida.

These five factors can determine the occurrence of mangroves, the species present, and the area occupied by a mangal. Once established, mangals throughout the tropics have many ecological similarities. In the following pages I attempt to summarize from accounts available to me, what is known about mangroves and mangals.

GEOGRAPHICAL DISTRIBUTION

Geographical distribution of mangroves is similar in many ways to that of sea grasses (Den Hartog 1957) and marine angiosperms in general (Good 1953). The main difference is that some mangrove species occur on both sides of the Atlantic Ocean and on the Atlantic and Pacific coasts of the Americas.

Fig. 1 shows the general geographic distribution of mangroves. Among individual genera and species, distribution is undoubtedly influenced by whether or not the plant is viviparous, and the ability of the seedling to survive in sea water for an extended period of time. Dispersal of resting seedlings by drift in the open ocean and by alongshore surface currents permits wide geographic range, and temperature and geomorphological characteristics determine distribution along individual coasts.

Geographical distribution is restricted, in general, to the tropics, but Oyama (1950) reported a small stand of mangrove on the southern tip of Kyushu Island at 35°N latitude. Later, van Steenis (1962a) identified the species there as *Kandelia kandel* (L.) Druce. Vu Van Cuong (1964) reported the Ryukyu Islands (about 27°N latitude) to be the northern limit of *R. mucronata, B. gymnorhiza, Avicennia marina* (Forsk.) Vierth., *Xylocarpus moluccensis* (Lamk.) Roem., *X. granatum* Koenig, *Lumnitzera littorea* (Jack) Voigt, and *Lumnitzera racemosa* Willd. In the southern hemisphere van Steenis (1962a) reported the southernmost stand to be on North Island of New Zealand at "less than 40° south." Chapman and Ronaldson (1958) reported that dwarfed *A. marina* grew in abundance in Auckland Harbor at 37°S latitude.

Mangroves are present on the Pacific coast of South America only to about 4°S latitude due to lack of sedimentation below that point. It was once thought that atmospheric drought caused absence of mangroves from that and other areas. Van Steenis (1962a) pointed out, however, that drought is not a factor in distribution as mangroves grow in the Arabian Gulf, the delta of the Indus River, southern Timor, and western Australia, where large silt deposits are found on arid coasts. The major differences between mangals on arid coasts and those on humid coasts is the paucity of the epiflora in the former.

Every mangal is composed of two classes of plants: (a) genera and higher taxa which are found only in the mangrove habitat and (b) species that belong to genera of inland plants but which are adapted for life in the swamp forest. World distribution of genera that occur in mangrove swamps is given in Table 1. For a detailed listing of many forms in class "b" above, see Vu van Cuong (1964). The fern *Acrostichium aureum* appears to be a circumtropical associate of mangroves since it has been reported in mangles of Ceylon (Abeywickrama 1964), India (Biswas 1927), Africa (Bews 1916, Boughey 1957), and the West Indies (Borgesen 1909).

Geographically, mangrove vegetation may be divided into two groups: that of the Indo-Pacific region and that of western Africa and the Americas. The Indo-Pacific region is comprised of East Africa, the Red Sea, India, Southeast Asia, southern Japan, the Philippines, Australia, New Zealand, and the southeastern Pacific archipelago as far east as Samoa. The West Africa-Americas region includes the Atlantic coasts of Africa and the Americas, the Pacific coast of tropical America, and the Galapagos Islands. Mangroves are not native to Hawaii, but *R. mangle, B. sexangula* (Lour.) Poir., *S. caseolaris*, and *Conocarpus erectus* have been introduced.

Distributions of several species found only in mangrove swamps are shown in Fig. 2-8. These figures, with Table 1, show that (a) the greatest number of genera and species occur along the shores on the Indian and western Pacific oceans, (b) there are no species common to East and West Africa, and (c) the species of the Americas and West Africa are related taxonomically. Species found on both the eastern shores of the Americas and the western shore of

Table 1. Distribution of plant genera that occur only in mangrove swamps (Chapman 1970).

Families and Genera	Total species	Indian Ocean W. Pacific	Pacific America	Atlantic America	West Africa
Rhizophoraceae					
Rhizophora	7	5	2	3	3
Bruguiera	6	6	0	0	0
Ceriops	2	2	0	0	0
Kandelia	1	1	0	0	0
Avicenniaceae					
Avicennia	11	6	3	2	1
Myrsinaceae					
Aegiceras	2	2	0	0	0
Meliaceae					
Xylocarpus	?10	?8	?	2	1
Combretaceae					
Laguncularia	1	0	1	1	1
Conocarpus	1	0	1	1	1
Lumnitzera	2	2	0	0	0
Bombacaceae					
Camptostemon	2	2	0	0	0
Plumbaginaceae					
Aegiatilis	2	2	0	0	0

Con't on next page

Table 1 con't

Palmae					
Nypa	1	1	0	0	0
Myrtaceae					
Osbornia	1	1	0	0	0
Sonneratiaceae					
Sonneratia	5	5	0	0	0
Rubiaceae					
Scyphiphora	1	1	0	0	0
	55	44	7	9	7

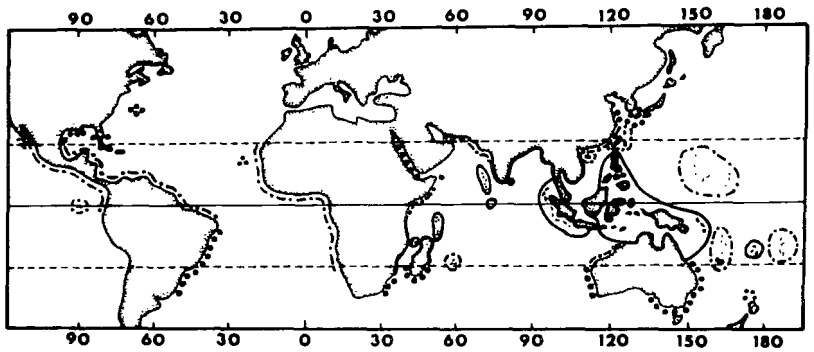


Figure 1. *World distribution of mangroves* (after Chapman 1970). • • • • less than five species present; – • – • – • five to twenty species present; ——— more than 20 species present.

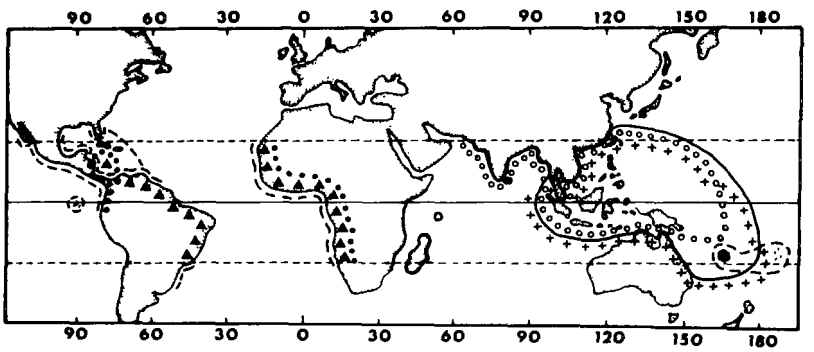


Figure 2. World distribution of Rhizophora species (after Ding Hou 1960, Vu Van Cuong 1964, and Chapman 1970). – – – R. mangle L.; ▲▲▲ R. racemosa F.F.W. Meyer; • • • R. harrisonii Leechm.; ——— R. mucronata Lamk.; ○○○ R. apiculata Blume; + + + R. stylosa Griffith; ⬢ ⬢ R. lamarckii Montr.

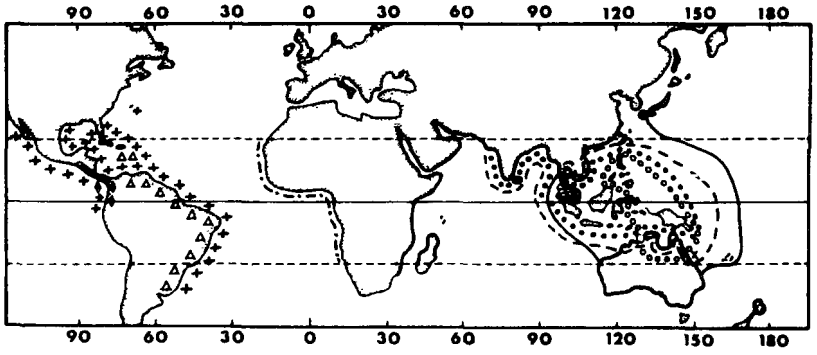


Figure 3. World distribution of Avicennia species (after Vu Van Couong 1964 and Chapman 1970). + + + A. nitida (germinans?) Jacq.; ΔΔΔ A. schauerana Stapft; ⊠⊠⊠ A. bicolor Standl.; ◆◆◆ A. tonduzii Moldenke; – • – •– A. africana P. Beauv.; ——— A. marina (Forsk.) Vierh.; ••••• A. officinalis L.; – – – A. alba Blume; X X A. balanophora Stapft and Modlenke; ○○○ A. eucalyptifolia Zipp; ▤▤▤ A. lanata Ridly.

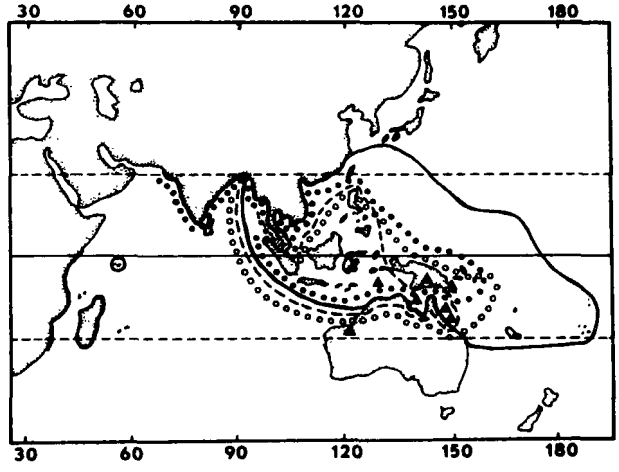


Figure 4. Distribution of Bruguiera species (after Vu Van Cuong 1964). ——— B. gymnorhoza Lamk.; •••• B. sexangula (Lour.) Poir.; – – – B. Cylindrica (L.) Blume; ○○○ B. parviflora (Roxb) W. and A; + + + B. hainesi C. G. Robers; ▲▲▲ B. exaristata Ding Hou.

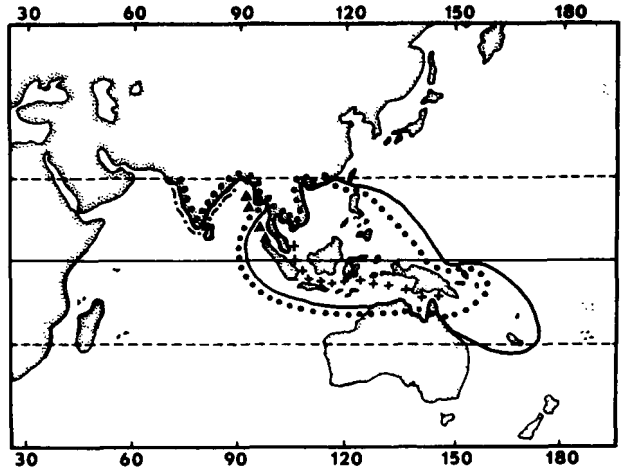


Figure 5. Distribution of Sonneratia species (after Vu Van Cuong 1964 and Chapman 1970). ——— S. alba J. Smith; ●●●● S. caseolaris (L.) Engler + + + S. ovata Backer; ▲▲▲ S. griffithii Kurz.; – ● – ● – S. apetala Buch.-Ham.

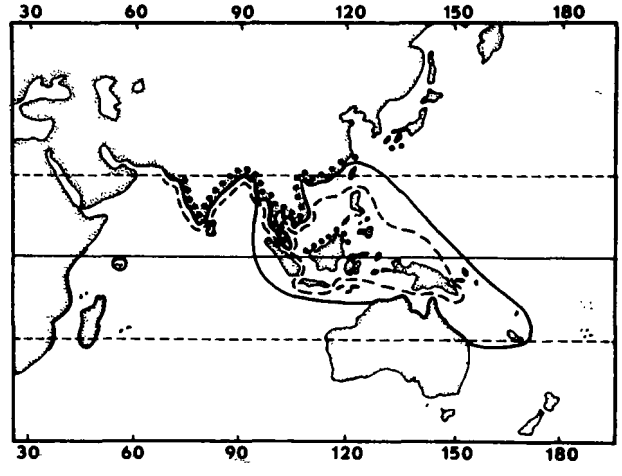


Figure 6. Distribution of ——— Ceriops tagal (Perr.) C. B. Rob.; – – – C. decandra (Griffith) Ding Hou; ●●●● Kandelia Kandel (L.) Druce (after Vu Van Cuong 1964).

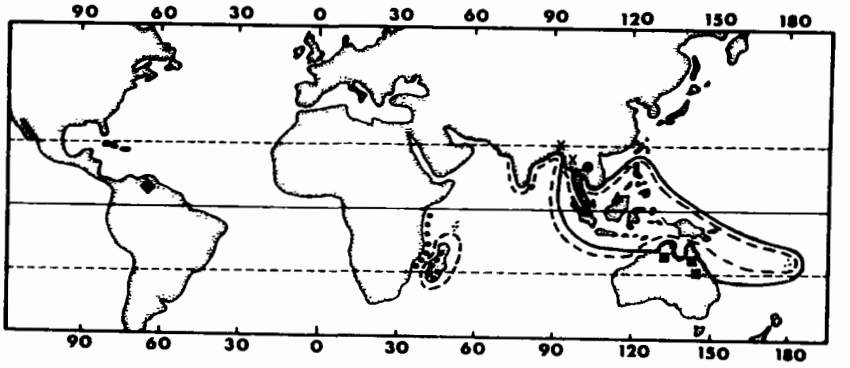


Figure 7. World distribution of Xylocarpus species (after Vu Van Cuong 1964). ——— X. granatum Koenig; – – – X. moluccensis (Lamk.) Roem.; X X – X. gangeticus Park; ▲▲▲ X. minor Ridley; ⊗⊗ X. parvifolius Ridley; ■■■ X. australasicum Ridley; ◆◆ X. guianensis; ●●●● X. benadirensis Moll.

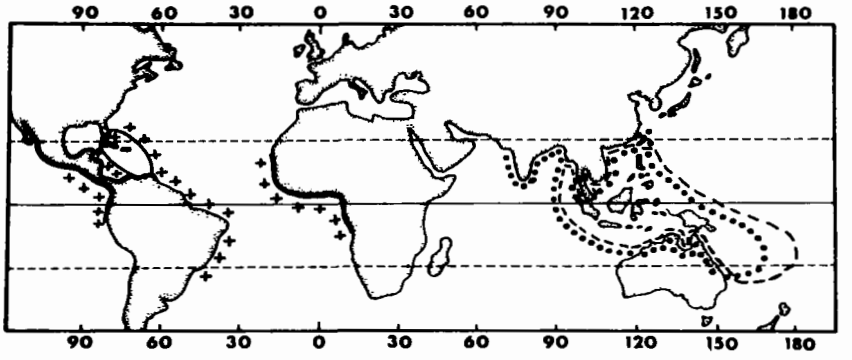


Figure 8. Distribution of ——— Conocarpus erectus L.; + + + Laguncularis racemosa Gaertn.; ●●●● Lumnitzera racemosa Willd.; and – – – Lumnitzera littorea (Jack.) Voigt.

Africa are *R. mangle, R. racemosa, R. harrisonii, Laguncularia racemosa*, and *C. erectus. Pelliciera rhizophorae* Planchon and Triana, a member of the tea family (Theaceae), is found only on the Pacific coast of tropical America. It occurs in small communities on exposed areas such as the seaward tips of point bars in estuaries or in spots having hard, clay soils (West 1956). Fuchs (1970) reported pure stands of *P. rhizophorae* on firm, sandy ground of low salinity. *Pelliciera* was associated with *Rhizophora* on low, muddy ground but in this habitat, trees of both genera were small.

There is confusion concerning taxonomy and distribution of *Avicennia* species on the shores of the eastern and western Atlantic Ocean. It was commonly held that a single species, *A. nitida*, occurred on both sides of the Atlantic Ocean. Moldenke (1960), however, recognized *A. nitida* in the Americas and *A. africana* Moldenke in West Africa. Vu van Cuong (1964) discarded the species *nitida* and recognized *A. germinans* in the Americas and *A. africana* in West Africa. Both Chapman (1970) and Vu van Cuong (1964) recognized four species in the Americas and only one, *A. africana*, in West Africa, and toxonomists at this time seem to agree that the American and West African species are closely related. Chapman (1970) speculated that the reason for confusion is that speciation is now occurring within the genus on both sides of the Atlantic.

There is also confusion in the common names of some mangroves. Those of the genus *Rhizophora* are called "red" mangrove in both the Americas and Africa. *Avicennia*, called the "black" or "honey" mangrove in the Americas, is known as the "white" mangrove in West Africa. *Laguncularia* is called the "white" mangrove in America.

From taxonomic and distributional considerations, Ding Hou (1960) and van Steenis (1962) concluded that *Rhizophora, Avicennia, Xylocarpus, Lumnitzera*, and *Laguncularia* arose in the Indo-Malaysian region and spread westward to East Africa and (except *Laguncularia*) eastward to the Pacific coast of the Americas. The genera reached the Caribbean Sea sometime between the Upper Cretaceous Period and the Lower Miocene Epoch, when the Isthmus of Panama was an open seaway. After establishment on eastern American shores, the trees reached West Africa when seedlings were carried across the ocean by surface currents.

To explain why the mangrove floras of East and West Africa are separate, van Steenis (1962a) postulated that the climate of South Africa during the Upper Cretaceous Period was not tropical and that mangroves could not have been distributed from east to west around the Cape of Good Hope.

As an interesting sidelight to the problem of distribution, Ding Hou (1960) pointed out that broad areas of the Pacific Ocean, to which favorable currents flow, do not contain mangrove. He attributed this to lack of suitable coasts for successful implanting of seedlings. He also described *R. mangle*, a native of the Americas, as present in New Caldonia, Fiji, and Tonga (see Fig. 2). Chapman (1970) speculated that early man carried seedlings from Pacific

America to those islands for growing trees to serve as a source of tannin.

ECOLOGY

Mangrove swamp forests are complex ecosystems that occur along intertidal accretive shores in the tropics. Dominated by estuarine trees, they draw many of their physical, chemical, and biological characteristics from the sea, inflowing fresh water, and upland forests. Mangrove swamps serve as ecotones between land and sea, and elements from each are stratified both horizontally and vertically between the forest canopy and subsurface soil.

The canopy is inhabited by floristic and faunistic elements from the tropical rain forest, including epiphytes, insects, reptiles, birds and mammals. Phytotelmata, filled with rain water, support a variety of algae, protozoa, and immature insects. Below the canopy, portions of tree stems are immersed, in relation to the tidal cycle, for various periods throughout the day. An extreme example is Inhaca Island in southeastern Mozambique where stems are often immersed for 8 to 12 hours per day at depths up to 2 m (Mogg 1963). The surface soil of swamps is alternately inundated and drained. It supports animals such as crabs, amphibians, reptiles, air-breathing fishes, and mammals, whose distributions are governed by degree of tidal penetration and by the nature of the substratum. At the landward edge of the swamp, typically fresh-water forms such as frogs, monitors, and crocodiles may be found (Macnae 1968), and I have observed the toad *Bufo marinus* in salinities up to 10 ppt (parts per thousand) in a mangal in Hawaii.

At the seaward edge, the mud surface is often a truly marine mid-littoral soft-bottom environment (Rutzler 1969) and supports crabs, shrimp, shellfish, etc. Numerous permanent and semi-permanent pools contain insects, shellfish, amphibians, and fish. Throughout the mangal is a network of rivulets, creeks, channels, and often rivers which change in depth with tidal ebb and flow. These contain numerous sessile forms such as algae, fungi, tunicates, sponges, and shellfish which live on mangrove prop- and aerial-roots. Mobile forms such as worms, crabs, shrimp, and fish migrate within the waterways in relation to the tidal cycle and nature of the substratum.

Jennings and Bird (1967) gave six environmental factors which affect geomorphological characteristics in estuaries and, therefore, the flora and fauna. The characteristics were: (1) aridity, (2) wave energy, (3) tidal conditions, (4) sedimentation, (5) mineralogy, and (6) neotectonic effects. All have been cited as factors in mangrove establishment. Troll and Dragendorff (1931) stated that water, salt, and oxygen contents of the soil are also important. On a short-term basis, tropical storms are very disruptive to mangals (West 1956, Alexander 1967) and are the greatest single sources of repeated set backs to the vegetation (Exell 1954). On the other hand, storms may carry propagules further inland than would normal tides (Egler 1952), and Mullan (1933) stated that seeds of mangroves are dispersed widely during the monsoon in Malaya.

Tides and type of substratum are probably the most important factors that govern the nature of intertidal communities (Chapman and Trevarthen 1953). In the case of mangroves, salinity of the surface and soil waters are also very important (Davis 1940), as are temperature, rainfall, rate of evaporation, topography, and geomorphology.

Surface Water

One of the distinctive features of mangrove vegetation is the ability to live in salt water as faculative halophytes. In such situations as reefs, lagoons, and the Florida Everglades, the surface water environment of mangroves is fairly stable in terms of physical and chemical composition. In mangals, salt and nutrient concentrations of surface water, whether in waterways or covering the swamp floor at high tide, is regulated by (1) inflow of fresh water from upland areas, (2) inflow and outflow of seawater with each tidal cycle, (3) precipitation, and (4) humidity.

Chemical and physical data on the surface waters of mangrove swamps have been reported from the Great Barrier Reef (Orr and Moorehouse 1933); Inhaca Island, Mozambique (Macnae and Kalk 1962); Cananeira, Brazil (Teixeria and Kutner 1963, Teixeria et al. 1965, Okuda et al. 1965); Tabasco, Mexico (Thom 1967); Hawaii (Walsh 1967); and Trinidad (Bacon 1968, 1971). Davis (1966) reported salinities up to 43 ppt and temperatures to 39.5°C in a mangrove salt-water pool in Jamaica. Examples of extreme conditions in a single swamp were given by Walsh (1967) who analyzed the water at six stations located between the landward and seaward edges of a swamp in Hawaii. At the landward edge, tidal effect was minimal or non-existant between August 1961 and November 1962, and the water was always fresh. Oxygen content of the water was that of a dystrophic body, averaging 0.67 ml/L throughout the sampling period. None of the factors measured were subject to large diel, monthly, or annual changes. Proceeding from the landward to the seaward stations in the swamp, diel changes became greater. At the seaward edge, water chemistry at high tide was similar to that of open bay water with great variations in relation to the tidal cycle. At low tide the water was fresh, whereas at high tide salinity was always greater than 25 ppt. In spite of such great differences in physical and chemical properties of the surface water, *R. mangle* grew in a dense stand between the landward and seaward edges of the swamp.

Bacon (1968) gave similar data for a mangrove swamp in Trinidad. In addition, he reported diel variations in concentrations of dissolved nitrate, phosphate, silicate, and suspended solids. Increased concentrations of nitrate and silicate occurred at low tide. Bacon suggested that either the inflowing fresh water was richer in nutrients than tidal water or that nutrients were released from the mud at low water. Walsh (1967) found a similar phenomenon for both nitrate and phosphate, and ascribed this to greater solubility of the substances in fresh water than salt water. He also demonstrated the affinities of the various types of swamp substrata for

nitrate and phosphate. In addition, nitrate and phosphate contents of swamp waters at low tide were greater than those of inflowing water. All of this indicates a dynamic system in which nitrate and phosphate are released from or taken up by sediments covered by surface water.

Watson (1928), DeHaan (1931), Walter and Steiner (1936), Macnae and Kalk (1962), and Macnae (1966) proposed schemes for classification of zonation of vegetation within mangals based upon tidal inundation and salinity. The details of these proposals were reviewed by Macnae (1968). In each scheme, vegetation of southeastern Asia was related to salinity of the water and Macnae (1968), using the data of Watson (1928), showed the general preference of *Avicennia intermedia* for coastal seawater and of *A. alba* for less saline water around the mouths of rivers. *Rhizophora mucronata* lived in water of greater salinity than *R. apiculata. Bruguiera sexangula* occurred in water of greater salinity than *B. cylindrica, B. parviflora*, and *B. gymnorhiza*. The three species of *Xylocarpus* lived in the less saline areas of the swamp.

Davis (1940) related salinity of surface water to distribution of trees in a swamp in southern Florida. American swamps are simpler floristically than those of Malaya and Davis demonstrated the relationship between tidal penetration and salinity to horizontal zonation. In Florida, *R. mangle* and *Laguncularia racemosa* are mixohaline, but the former is the pioneer species on seacoasts. *Rhizophora mangle* was found by Davis to grow in salinities that ranged from fresh water in the Everglades to 34.9 ppt along the seashore. *Laguncularia* grew in "nearly fresh water" to water of 45.8 ppt, and was usually found in association with the other mangrove species. Davis stated "no particular habitat is definitely most suitable for *Laguncularia.*" This concept was extended by Thom (1967), who observed that *Laguncularia racemosa* in Tabasco formed communities with other mangroves and had less stringent habitat requirements than they.

Davis (1940) reported *A. nitida* growing in the field in salinities between 36.8 ppt and 38.6 ppt although it can grow in fresh water in the laboratory. This species seems to be adapted for survival in swamp areas with great salinity fluctuations. The community is not flooded deeply by tidal water and salt is concentrated by evaporation during dry periods. During periods of rain, the surface water is diluted greatly so that the *Avicennia* zone has a greater range of salinity than any other. Conversely, *Conocarpus erectus* grew only where salinity was low and the ground covered only occasionally by tidal water. Many *Conocarpus* localities had no surface water; where there was surface water, salinity averaged less than 2 ppt. An important factor for survival of *C. erectus* seemed to be high salinity of the soil water. This will be discussed in the next section.

Sedimentation and Soil

According to the nature of the substratum, mangroves may be classified as reef, sand, mud, and peat types (Chapman 1940, Rutzler 1969). Also, some are found occasionally among boulders, having roots within cracks or other niches, and use tidal water as the source of nutrients. The typical sediments

of swamps are composed of peaty, soft, sandy or clayey mud. They are similar to the sediments of salt marshes, which occupy the same sedimentological position at higher latitudes. Mineralogy of mangrove sediments is concerned mainly with clastic detritus from rivers and calcareous debris formed either biologically by shelled organisms or by inorganic precipitation. Algae and bacteria can also function in precipitation and pyrite is often abundant in swamps, usually embedded within or attached to plant remains. Along some shores, where tidal and alongshore currents control the character of the sediments, siliceous and quartzitic sand may predominate.

Mangroves advance seaward only where sedimentary processes prepare shallow water areas for growth of seedlings Mangals often are associated with lateral accretion of sediment along tropical shores, and location, size, and shape of swamps are influenced strongly by the pattern of coastal sedimentation. Hagen (1890) and van Steenis (1941) stated that natural coastal accretion by mud-silting is the major factor responsible for development of large mangals. Although the trees do not aid appreciably in lateral extension of shores, they do aid in accumulation of sediment with subsequent build-up of soils (Curtiss 1888, Vaughan 1909, Watson 1928, Holdridge 1940, Egler 1952, West 1956, Boughey 1957, Vann 1959, Stoddart 1962, Scholl 1963, Thom 1967, Macnae 1968). During high tide, brackish, sediment-laden water overflows the numerous creeks and channels of the swamp. Alluvium is deposited on the swamp floor and, with autochthonous organic and inorganic detritus, aids in land elevation. Freise (1938) stated that the black color of mangrove mud in Brazil was due to the presence of iron sulphide. The black mud was often covered by a 1-5 cm deep layer of grey-brown mud which was either deposited during tidal inundation or affected chemically by oxygen in tidal waters.

Accretion of sediment along alluvial coasts is regulated mainly by physiographic-geomorphic processes such as (a) the rate at which sediment is brought into an area by rivers and tides, (b) the angle of slope of the shore, (c) sedimentary distributional patterns, (d) subsidence or emergence of the coast, (e) other factors associated with changes in sea level, and (f) tidal-river channel development. As in other estuaries, the coarser sediments of mangrove swamps are generally in the channels, the finer sediments along the shores of the channels (Walsh 1967). Also, when a river reaches the estuary, the heavier particulate elements have been sorted out above the mangal, so that the predominent sediments within the swamp are of fine-grained alluvium. Near the mouth of the estuary, coarser sediments may again be found. These originate from tidal and alongshore currents which have enough energy for deposition of small sand particles and calcareous detritus. The contribution of inorganic detritus from seawater is usually small, however, because strong currents do not allow seedling development. River-borne sediment is the greatest source of allochthonous material in most waamps and appears to be especially important in the Indo-Malayan region (Watson 1928, Schuster 1952, Macnae 1968).

Sedimentation of autochthonous matter is an important factor for mangroves which are not influenced greatly by fresh-water inflow. Davis (1940) stated that the authochthonous mangals of Florida develop over three primary soil types; namely, (1) siliceous sands, (2) calcareous sands, and (3) calcareous mud marls. Mature *R. mangle* trees are thus sometimes found on nearly bare rock with only small pockets for rooting, but more often grow on deep peat soils. Although the general physical and morphological features of soils vary greatly between mangals, the halotropic peats so often found with mangroves are composed mainly of calcium compounds from shells, biologically precipitated calcite and aragonite, and organic matter of floral and faunal origin.

Extensive autochthonous mangrove swamps have developed along the western side of Andros Island in the Bahamas where the rate of carbonate mud precipitation is great. Burkholder and Burkholder (1958) described the autochthonous sediments of Bahia Fosforescente in Puerto Rico. The sediments contained large amounts of mangrove roots, stems, and leaves, and the authors stressed the important influence of mangrove detritus on the chemistry and biology of the bay. At the present time, autochthonous peat swamps are developing along the southwestern coast of Florida because of the paucity of sediments from rivers and streams.

In southeast Asia, where large numbers of rivers drain uplands of volcanic origin, large allochthonous swamps form in deltas, estuaries, lagoons, and along sheltered open coasts. These allochthonous swamps are the most highly developed mangals in the world (Watson 1928, Macnae 1968).

Mixed authochonous-allochthonous swamps occur along the Pacific coast of Colombia where there is low to intermediate supply of river-borne sediment (West 1956).

Several systems have been proposed for the classification of mangrove swamp soils. Aubert (1954) and Dubois (1954) classified mangrove soils in relation to hydromorphic characteristics and salinity. Bonfils and Faure (1961) related halomorphic soil types to the degree of salt-and fresh-water flooding and to the relative concentrations of chlorides and sulphates. D'Hoore (1963) typed mangrove swamp soils as "juvenile soils on marine alluvium" and called them "weakly developed soils." This general classification was accepted by Giglioli and Thornton (1965), who suggested further subdivision for agricultural purposes according to soil texture, water regime, degree of gleying and/or mottling, amount of oxidizable sulfur, and relative amounts of chlorides and sulphates.

Grant (1938), Davis (1940b), Chapman (1940), Thom (1967), Walsh (1967), and Giglioli and King (1965) discussed the evolution of mangrove swamps in relation to silting and plant succession. Davis (1940b) listed three main factors which promote soil accretion: (1)molar, (2) physicochemical, and (3) biotic.

Molar factors are mainly tides, littoral currents, and winds. The first stages of accretion consist of marine and estuarial sedimentation, resulting in

formation of shoals, bars, and flats in the shallow water. At the same time, deposition of sediments and physico-chemical precipitation of dissolved substances occurs when fresh and salt water mix (Jackson 1958). This adds carbonates, phosphates, nitrates, and other substances to the developing soil.

Hesse (1961b) reported that *R. racemosa* swamps in Sierra Leone were comprised of fibrous mud, whereas *A. germinans* swamp soils were non-fibrous. Also, *Rhizophora* swamps had higher pH values, C/N ratios, and contents of oxidizable sulfur, nitrogen, phosphorus, and carbon. Giglioli and Thornton (1965) described the early phases of swamp evolution in the Gambia, West Africa, where *R. racemosa* pioneers in virgin alluvia composed of soft, silty soil. Proliferation of fibrous roots at the soil surface produces a "felt-like" layer which entraps sediment and increases the rate of deposition of both alluvium and leaf litter. Rosevear (1947) suggested that the fibrous mat formed by *R. racemosa* prevents further establishment of that species and conditions the soil for colonization by *Avicennia*, which requires a more consolidated and elevated substratum (Jordan 1964). As the soil surface becomes elevated, the rhizophoretum dies and *A. germinans* replaces *R. racemosa* (Giglioli and Thornton 1965). The many pneumatophores of *Avicennia* further accelerate deposition and the forest floor becomes even more elevated. According to Giglioli and Thornton, if the amount of drainage from higher ground is large and the swamp near the main river or a tributary, a balance occurs between erosion and drainage of the swamp and land elevation.

Zieman (in press) found that in Biscayne Bay, Florida, circular beds of *Thalassia testudinum* Koenig and Sims laid over depressions in the bedrock. The depressions were over 5 m deep and filled with mangrove peat dated to be 3,680 years old. Wharton (1883) suggested that living mangroves and their peat produce organic acids that dissolve bedrock. Zieman suggested that bedrock was dissolved under mangrove hammocks and hypothesized that as the mangrove shoreline receded and sea level rose, *Thalassia* colonized the old mangrove areas. Dodd and Siemers (1971) described a very similar situation on Bahia Honda and Big Pine Keys in the lower Florida Keys. They stated that the topography developed during the lowered sea level of the Pleistocene strongly controls Holocene sediment thickness and present biotic distribution. They said, however, that the depressions were sinkholes and thick sediment in underwater sinkholes promoted growth of *Thalassia*, whereas depressions in shallow water or in the tidal zone supported growth of *R. mangle* and *A. nitida*.

Schuster (1952) reported deposition of sediment on the mangrove forest floor at every spring tide in Java, the processes of land elevation and soil formation being accelerated by growth of beach thistles (*Acanthus* sp.) which produced large quantities of organic matter.

Soil derived by sedimentation from river water is often poor in calcium and potassium and, in mangrove swamps, tidal water is the main source of

salt. In the early stages of mangal development, the clay particles absorb calcium and potassium salts from seawater and a fine-grained soil, rich in minerals, results. As evolution of the swamp continues, shelled animals invade and grow on the trees and substratum. The organic content of the soil increases and in the moist environment, decay processes are rapid and calcareous particles are dissolved (Abel 1926). Wharton (1887) reported rapid corrosion of $CaCo_3$ in mangrove swamps at Aldabra. Fairbridge and Teichert (1947) concluded that pools on reef flats at Low Isles (Marshall and Orr 1931) were caused by solution of $CaCO_3$ by mangrove swamp acids. Reville and Fairbridge (1957) suggested that the principal agent for destruction of $CaCO_3$ in mangrove swamps is carbonic acid produced by decomposition of organic matter. They also suggested that tannic acid from mangrove bark and "humic acids" aid in decomposition.

Very little is known about the factors that form and condition mangrove mud, which may lie in an unconsolidated state to a depth of 1.5 m. Schuster (1952) discussed breakdown and modification of the substratum by bacteria, fungi, actinomycetes, and myxomycetes. He mentioned the occurrence of the bacteria *Clostridium* sp. and *Azobacter* sp. and the algae *Nostoc* sp. and *Anabena* sp. in mangrove swamps and speculated that those organisms are important in nitrogen fixation.

Most of the organic debris on and within mangrove soils is authochonous. Because of the saline water, relatively high pH of surface soil water (often as high as 7.8) and anaerobic conditions at low tide, plant detritus is only partially broken down by bacteria, fungi, and algae. This causes formation of peat, which is composed mainly of plant remains.

The role of birds in composition and fertility of mangrove soil has not been investigated adequately. Birds in mangrove have been described by Cawkell (1964), Haverschmidt (1965), Ffrench (1966), Parkes and Dickerman (1967), Nisbet (1968), Field (1968), Dickerman and Gavino T. (1969), Ffrench and Haverschmidt (1970), Dickerman and Juarez L. (1971), and Ricklefs (1971). Large numbers of birds (Ffrench reported 94 species in mangrove in Trinidad), including egrets, ibis, herons, spoonbills, anhingas, pelicans, storks, ospreys, and eagles, roost in mangrove trees but feed elsewhere. In this way, nutrients are brought into the swamps and the functions of such nutrients should be investigated.

Analyses of mangrove peat have been reported by several workers. Davis (1940) gave detailed accounts of soil profiles in the swamps of southern Florida and classified them according to their general composition, i.e. homogeneous, heterogeneous, or layered. He also classified them on the basis of the type of vegetation which covered the soil and the probable types of vegetation that formerly were present and contributed most to the accumulated materials. Davis reported various types of soil profiles, some of which indicated progressive soil accretion, while others did not. Giglioli and Thornton (1965) gave soil profiles from the Gambia River basin in West Africa. The profiles indicated typical alluvial soils in the process of silting.

The composition of mangrove swamp substratum is dependent upon its source, age, position in the swamp, organisms present, and scouring by water flow in the channels and over higher ground at high tide. Walsh (1967) reported that up to 74.6% of the substratum in the center of the channels of a swamp in Hawaii was composed of shells, pebbles, and gravel with diameter greater than 3.35 mm. All of the alluvial particles, however, were less than 0.23 mm in diameter, and most sediments in mangrove swamps are of small grain size.

Scholl (1963) compared grain-size distribution of clastic sediments in two mangrove swamps in southwestern Florida, where *R. mangle* was the dominant species. In the swamp of the Ten Thousand Islands area, the sediment was composed of fine to very fine calcareous-quartzitic sand and coarse silt. The quartz content was approximately 70%, carbonate mineral 10-20%, and organic matter usually less than 10%. Isopleths of grain size showed a zone of coarser-grained sediment (approximately 0.100-0.200 mm diameter) along the shore flanking a belt of finer-grained sediment (0.062-0.125 mm) inland. Another belt of coarser sediment (0.125-0.250 mm) lay landward of the finer-grained belt. In contrast, the sediments of the Whitewater Bay area swamp were composed mainly of mollusc shells and shell fragments. Less than 15% of the sediment was quartz and "little organic detritus" was present. The grain sizes of surface sediments fell between 0.054 and 0.540 mm in diameter. Distribution of grain size was variable throughout the swamp. Scholl attributed the differences in sediment characteristics between the Ten Thousand Islands and the Whitewater Bay areas to differences in patterns and strengths of the tidal currents. Strong tidal currents which washed the former were lacking in the latter. Tables 2 and 3 give grain-size distributions in several swamps.

There is a paucity of data on physical and chemical characteristics of mangrove soil. Values for physical and chemical factors from forests dominated by different trees with different substrata overlap (Doyne 1933; Davis 1940; Bharucha and Navalkar 1942; Chapman 1944a, b, c; Navalkar and Bharucha 1948, 1949; Schuster 1952; Wyel 1953; Hesse 1961a, b; Scholl 1963; Giglioli and Thornton 1965a; Giglioli and King 1966; Clarke and Hannon 1967; Kassas and Zahran 1967; Walsh 1967; Lee and Baker 1972a). The ranges of some factors reported are: pH 5.0-9.0, Chloride 1.9-87.0 ppt, carbon 0.05-11.9%, loss on ignition 3.0-72.8%, and C/N ratio 0.4-36.0. Diel and seasonal variations occur in relation to the tide, rainfall, and rate of evaporation (Navalkar 1941; Navalkar and Bharucha 1948, 1949, 1950; Barucha and Navalkar 1942; Clarke and Hannon 1967, 1969; Giglioli and Thornton 1965a, b) and at this time, it is impossible to relate plant distributional patterns to specific physical or chemical properties of the soil. In a study of the plant communities of the Sydney District, Australia, Clarke and Hannon (1967, 1969) found that variations in the physical and chemical properties of mangrove soils were similar to those of other plant stands.

Table 2. Percentage grain-size distribution (mm diameter) in mangrove swamp surface sediments.

El Salavador (Wyel 1953)	
>1.0 mm	0.0-5.2%
1.0-0.5	0.0-4.2
0.5-0.4	0.0-8.0
0.4-0.3	0.0-6.0
0.3-0.2	0.0-18.0
0.2-0.1	0.0-18.0
0.1-0.06	0.0-16.6
0.06-0.03	22.0-43.8
0.03-0.017	7.0-32.0
0.017-0.007	3.0-27.0
0.007-0.003	0.5-8.0
<0.003	0.5-5.0

Java (Schuster 1952)	
2 mm	0%
2.0-0.1	2
0.1-0.05	5
0.05-0.01	30
0.01	63

Jamaica (Chapman 1944)	
Coarse sand	39.9%
Fine sand	26.5
Clay	5.1
Silt	15.7

Brazil (Friese 1937)	
>0.2 mm	4.7-7.0%
0.2-0.06	7.2-11.8
0.06-0.03	6.7-10.1
0.03-0.006	6.6-10.4
0.006-0.003	22.1-24.5
<0.003	43.6-46.6
India (Navalkar 1941)	
2.0	3.9-4.0%
0.2	38.2-38.6
0.02	29.5-33.1
0.002	3.9-4.3

Florida (Scholl 1963)

Median grain-size from 10 stations varied between 0.006 and 0.700 mm.

Table 3. Grain-size distribution in surface sediments of swamps dominated by *Avicennia alba* (Navalkar 1941) and *A. marina* (Clarke and Hannon 1967). Coarse sand = 2.0 mm diameter, fine sand = 0.2 mm, silt = 0.02 mm, clay = 0.002 mm.

Species	Percentage of Dry Soil			
	Coarse sand	Fine sand	Silt	Clay
A. alba	4.0	38.4	31.3	4.1
A. marina	75.2	3.8	1.7	4.8

However, *Avicennia marina* and *Arthrocnemum australasicum* occurred only where high salinity of the soil water endured for long periods of time or where there were wide variations in salinity. Giglioli and King (1966) pointed out that *A. germinans* grew in old soils of high salinity and that this high salt content was a function of time. *Avicennia* was apparently able to exclude *Rhizophora racemosa* because it was better adapted to high concentrations of salt. *Avicennia*, unlike *Rhizophora*, absorbs large quantities of salt through its roots and excretes them through the leaves (Scholander et al 1962). As shown above, the fibrous nature of the substratum also appears to be important in colonization, and it is most likely that combinations of factors, including soil salinity, regulate species distribution.

Clarke and Hannon (1969) concluded that tidal action, as modified by microtopography, was the major factor which affected soil salinity over long periods of time, and that succession was mainly allogenic rather than autogenic. This concept was shown to be true by Thom (1967), who demonstrated that although biotic and geomorphic processes are effective in short-term changes on actively accreting shores, physiographic processes of sedimentation and subsidence are more important over a long period of time. Physiographic changes influence salinity of the soil, degree of water saturation, soil type, and drainage, and therefore greatly influence the species present.

Zonation and Succession

In general, in areas of large mangals, five geographic belts can be distinguished (West 1956): (1) a belt of shore water and mudflats along the coast, (2) a series of discontinuous sand beaches, variable in size, which are interrupted by tidal inlets and mudflats, (3) a zone of mangrove forest, usually one-half to three miles wide, (4) a fresh-water swamp, and (5) equatorial rain forest. Although the beach zone is frequently absent, this zonation was described in the Malay Peninsula (Watson 1928), western Africa (Grew 1941), the Congo (Pynaert 1933), and in Guiana (Martyn 1934).

Within the mangrove belt, there is usually a seral succession of vegetation in relation to hydrological and climatic conditions. Day et al. (1953) held the salinity gradient to be of great importance to distribution in South Africa, and described correlations between rainfall, evaporation, upflow of salt water from the sea, and seral change.

There have been several attempts to classify mangrove vegetation according to physical characteristics of the environment. Watson (1928) described two general classifications: (1) mangroves that grow on accretive shores and (2) those that grow on sand. Watson also related species to the tidal cycle and described five classes: (1) inundated by all high tides, (2) inundated by medium high tides, (3) inundated by normal high tides, (4) inundated by spring tides, and (5) occasionally inundated by exceptional or equinoctial tides.

Stevenson and Tandy (1931), working at Low Isles in Australia, described the mangrove habitat as (1) dense woodland, (2) muddy glades, and (3) shingle tongues. At present, mangrove types are sometimes considered to be related to the type of soil present. Troll and Dragendorff (1931) and Walter and Steiner (1937) described mud and reef mangroves; Chapman (1944a) added the categories of sand and peat mangroves. These four types are generally recognized today.

Tansley, Watt, and Richards (1939) suggested that mangrove vegetation be considered as a formation type on a world-wide basis. They recognized two subformations: (1) the New World subformation, including western Africa, and (2) the Old World subformation. Chapman (1944a) recommended that a third subformation, the Australasian, be recognized because the species of *Avicennia* are very distinct in their distribution and segregate into these three geographical regions.

Davis (1940) said that the mangrove formation is composed of seral communities. Although reef and sand communities appear to be climax stages, the statement of Davis is generally true. The order of zonation varies considerably even in geographically related areas. For example, in the Old World subformation, *Rhizophora* is the pioneer species along river banks and in the more protected regions along oceanic shores, whereas *Avicennia* or *Sonneratia* pioneer on shores of greater wave and tidal action. In Jamaica, *R. mangle* pioneers along protected shores, while *Laguncularia racemosa* pioneers on sand spits where wave action is greater (Chapman 1944a).

Chapman (1940) pointed out that the presence of sea grass in submerged areas accelerates the seaward extention of mangrove because it raises the height of the sea bed, allowing *R. mangle* seedlings to grow.

The seral nature of mangrove vegetation in the Indo-West-Pacific region was described in detail by Macnae (1968), who recognized succession in every mangal he visited. Macnae stated that variation in development was often found, succession being complete only where the amount of available fresh water exceeded that lost through evaporation and transpiration. When losses through evaporation and transpiration exceed income from rain and rivers,

the soil becomes hypersaline and zonation is interrupted.

Macnae described the effects of fresh-water imbalance in a zone of *Ceriops tagal* (Perri) C. B. Rob. This zone was located between a seaward fringe of *Bruguiera gymnorhiza* and a landward fringe of *A. marina*. Where fresh-water loss exceeded gain, *C. tagal* became stunted. With increasing excess of evaporation, the *Ceriops* bushes died, forming a bare area which expanded both landward and seaward until only a few bushes grew near the *Avicennia* and *Bruguiera* fringes.

In Florida, Davis (1940b) was the first investigator to give a detailed account of succession in a mangal. He recognized seven principal communities: (1) The pioneer *Rhizophora mangle* zone. This seaward stage was composed of mangrove seedlings of various age growing in marl soil below the level of low tide in shallow undisturbed water. *Thalassia testudinum* Koenig and Sims and *Cymadocea manatorum* Aschers grew in shoal areas near this zone, and *Spartina alterniflora* Loisel was present in some parts of Florida. (2) Mature *Rhizophora* consocies. This stage was composed of mature *R. mangle* with well-developed prop roots growing in mangrove peat. (3) *Avicennia*-salt marsh consocies. This stage was composed of the tree *A. nitida* and the salt marsh plants *Batis maritima* L., *Salicornia perennis* Mill, *Spartina alterniflora*, *S. spartinae* (Trin.) Merv, *Monanthochloe littoralis* Englem., and *Sporobolus virginicus* (L.) Kunth. This mangrove salt marsh consocies grew on peaty soil and accumulated large amounts of organic and inorganic detritus. During dry periods, soil salinity was very high, whereas salinity was very low during rainy periods. In some places, *Avicennia* was more than 30 cm in diameter, but in other places was a small gnarled bush. (4) Mature mangrove associes. This stage consisted of large trees of *R. mangle* and *A. nitida* growing together on peat soils in water of low salinity at approximately the mean high tide mark. (5) *Laguncularia racemosa* consocies. This stage did not occupy a specific habitat, but was found with both the mature mangrove associes or between the *Avicennia*-salt marsh associes and a *Conocarpus* associes when the natural mangrove associes was not present. (6) *Conocarpus erectus* transition associes. This was the final stage of mangrove succession in Florida, as it was bordered on its landward edge by sand dunes, upland tropical forest, or fresh-water marsh. Davis considered the *Conocarpus* associes to be an ecotone, not a definite seral community. This was disputed by Chapman (1944a) who reviewed the work of Borgeson (1909) and concluded that *Conocarpus* formed a true seral stage. Chapman later (1970) considered the *Conocarpus* community to be an ecotone between saline and fresh-water communities. West (1956) described the final stage in seral succession in Colombia as dominated by *C. erectus* in the drier and less saline areas. (7) Dwarf-form mangroves. Davis recognized a scrub-mangrove facies of dwarfed *Rhizophora*, *Avicennia*, and *Laguncularia*, which grew above the high tide mark in fresh water. This dwarfed form was common in the Everglades region.

Thorne (1954) listed many other plants in the mangals of Florida. Holdridge (1940) gave an extensive description of the vegetative characters and general characteristics of *R. mangle*, *Langucularia racemosa*, *C. erectus*, and *A. nitida*, and reported *Petrocarpus officinale* Jacq., *Anona glabra* L., *Bucida buceras* L., and *Drepanocarpus lunatus* G.F.W. Meyer living in the mangals of Puerto Rico.

Chapman (1944a) compared succession in the swamps of Jamaica with that in Florida (Figs. 9 and 10). In both cases, *Rhizophora* was the pioneer form, with *Avicennia*, *Laguncularia*, and *Conocarpus* inland. Asprey and Robbins (1953) stated that there were few associates with mangrove in Jamaica, a pattern similar to mangals in other parts of the world. *Batis maritima*, *Salicornia ambigua* Michx., *Acrostichium aureum* L., *Alternanthera ficoides*, and *S. virginicus* occurred in the swamps of Jamaica. In other parts of the world, other genera and species occupy similar positions. For example, Taylor (1959) described the mangals of Papua, New Guinea, as follows:

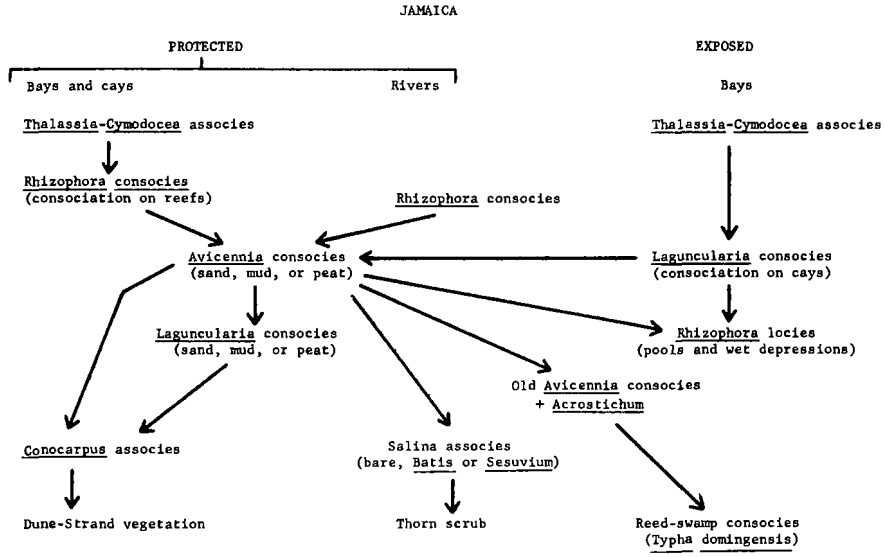


Figure 9. Succession in the mangrove swamps of Jamaica (Chapman 1944a).

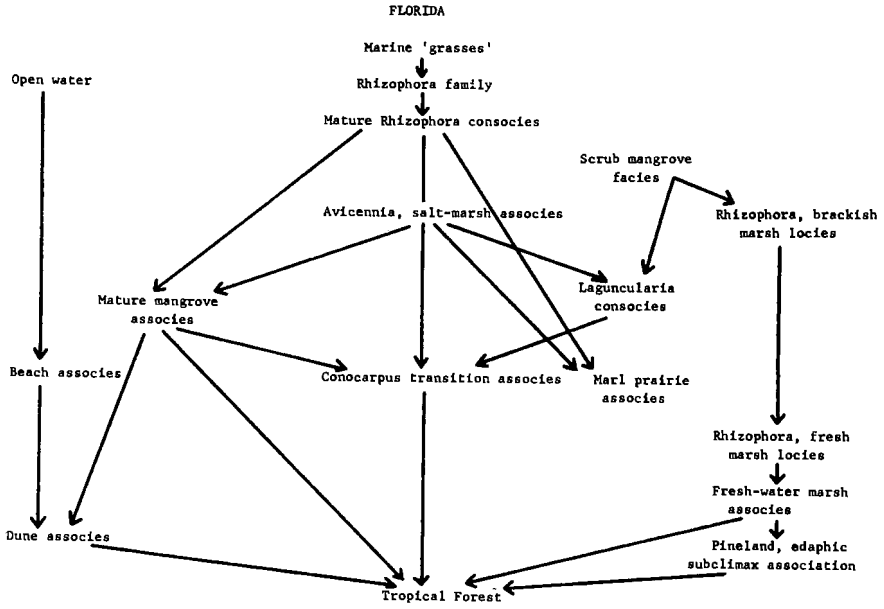


Figure 10. Succession in the mangrove swamps of Florida (Chapman 1944a).

1. Salt water swamps
   a. Tidal mangrove sequence
   b. Mangrove marsh sequence
2. Brackish water swamps
   a. Brackish swamp sequence
   b. Estuarine sequence.

In the tidal mangrove sequence, each succeeding community occurred at sites with successively longer periods of tidal inundation. The shore pioneer species was *C. tagal*, followed by a broad zone of *R. mucronata* and *B. gymnorhiza*. The final stage was dominated by *Heritiera littoralis*. The boundary between the *H. littoralis* zone and the rain forest was dominated by *Intsia bijuga* (Colebr.) O. Kunz. and was very sharp. All of the mangrove species occurred as scattered individuals in all of the communities, although the dominant species comprised over 50% of the number of species, and there were sharp transitions between the zones.

In the mangrove marsh sequence, there was a fringing area of *A. alba* trees up to 12 m high, which changed gradually to a thicket of the same species up to 6 m high. The thicket was bordered by swampy soil devoid of vegetation and Taylor suggested that the sequence from tall trees to bare swampy ground was regulated by soil salinity.

Brackish water swamps occurred where mangrove forest was bordered inland by fresh-water swamps. There were only two sharply-defined zones: (1) a zone composed predominantly of *A. alba* and *B. gymnorhiza*, with a dense ground cover of the fern *Acrostichium speciosum* Thunb.; and (2) a zone dominated by *Parinari corymbosum* (Bl.) Mig. *Hibiscus tiliaceus* L. was present in small numbers. *Acrostichium speciosum* was generally abundant in this zone, but *Acanthus ilicifolius* L. sometimes made up 50% of the ground cover.

The estuarine sequence was similar to the tidal mangrove sequence, except the palm *Nypa fruticans* Wurmb. dominated the zone which bordered the fresh-water swamp.

In West Africa (Nigeria), Jackson (1964) recognized six groups of mangrove on the dual basis of range of habitat and dispersal of seeds.

Group 1. Species restricted to the tidal areas and with specialized seed habits. This group included *R. racemosa*, the pioneer species at the water's edge and on the storm beach, and *A. nitida* dominant along the inner edges of closed lagoons. Boughey (1957) found *Rhizophora* only in open lagoons and *Avicennia* only in closed lagoons in West Africa. Bews (1912) reported *B. gymnorhiza* from lagoons in Natal. In the case of *Rhizophora* species, Keay (1953) considered *R. racemosa* to be the pioneer species, with *R. mangle* following on drier ground and *R. harrisonii* on wet ground. These were followed inland by *A. nitida*, *Laguncularia racemosa*, and *C. erectus*. Gledhill (1963) pointed out that the propagules of *R. racemosa* are between 30 and 65 cm long and, by virtue of their length, are suited for establishment in flooded mud. The seedlings of *R. mangle* are approximately 20 cm long and those of *R. harrisonii* 30 cm long. Gledhill felt these were adapted for establishment on less heavily silted soil under more vigorous water current conditions.

Group II. Species found normally in tidal areas and with specialized fruits but normal seeds, or with buoyant seeds. Genera in this group were *Drepanocarpus*, *Dalbergia*, *Ormocarpum*, and *Hibiscus*. The seeds of these groups germinate in the water.

Group III. Species widely distributed along water courses and in swampy areas, usually with unspecialized fruits. The genera here were *Pterocarpus*, *Cynometra*, *Lonchocarpus*, *Phyllanthus*, and *Phoenix*. Seeds of the first four are buoyant and are found floating in water. Seeds of *Phoenix* have never been found in the drift.

Group IV. Species not restricted to water courses in the forest areas and with no marked specializations. These species are associated with high or well-distributed rainfall. The fruits and seeds are not associated with dispersal by water. This group included *Anthocleista*, *Elaies*, *Combretum*, *Alchornea*, and *Paullinia*.

Group V. Cultivated plants. These are plants which occur in strand vegetation and have floating fruits which are dispersed by water. Genera include *Cocos, Terminalia*, and *Anacardium*.

Group VI. Species whose seeds and seedlings are found in the swamp but with few or no mature individuals present. These include *Lonchocarpus, Halomosia, Spondias, Cleistopholis, Dioclea,* and *Entada.*

It is clear that mangrove swamps are not the simple communities some writers thought them to be. Macnae (1966) described in detail a complicated succession in the swamps of Queensland, Australia. There, the pioneer tree was *A. marina* where there was a large amount of fresh-water inflow, or *Sonneratia alba* where the influence of saline water was strong. The *Avicennia* zone was composed of a row of mature trees, two or three trees deep, with thickets of seedlings and saplings extending out onto a beach. Often, where the influence of fresh water was strong, *Aegiceras corniculatum* Blanco occurred in large numbers in the seedling and sapling thickets. On the other hand, where *S. alba* fringed the shore, the pioneer belt was well-developed. The alga *Catenella nipa* Zanard colonized the pneumatophores of both *Avicennia* and *Sonneratia*. Macnae found no other algae there. This was exceptional as large numbers of algae are present on the pneumatophores of both genera in southern Australia and eastern Africa. The substrata of shoreline fringes were considerably firmer than either the foreshore in front or the *Rhizophora* forest behind because both *Avicennis* and *Sonneratia* have a mass of intertwining absorptive roots which lie 20 to 40 cm below the surface.

Behind the ocean fringe, there occurred an ocean shore sub-fringe composed mainly of *R. stylosa* and occasionally of *R. mucronata. Rhizophora* formed the fringing zone along creeks. Ding Hou (1958) and van Steenis (1962) held that *R. stylosa* was found only on sandy shores and coral terraces. Macnae stated that both *R. styloas* and *R. mucronata* grew in mud, sand, and on coral debris, and speculated that the two forms are actually variants of a single species. Whatever the taxonomic position may be, it is clear that in contrast to the New World genera the southeastern-asiatic and eastern Africa forms of *Avicennia* are better adapted for pioneering than Rhizophorous forms (see Watson 1928; Macnae 1963, 1968).

In contrast to the pioneer fringe, the substratum in the well-developed *Rhizophora* forest was always very soft and muddy due to entrapment of sediments between the prop roots.

Landward of the *Rhizophora* forest lay broad areas of either (1) thickets dominated by *C. tagal*, where the amount of rainfall was intermediate, or (2) forests dominated by *Bruguiera*, where the amount of rainfall was large. In the thickets, *C. tagal* was ordinarily the only species present, but *Bruguiera exaristata* Ding Hou was sometimes subdominant. Occasionally, *A. marina, B. gymnorhiza, R. apiculata, R. stylosa, Xylocarpus granatum* Konnig, and *X.*

australasicum Ridley were present. In areas of much rainfall, the *Ceriops* thickets were narrow and bordered by very dense forests dominated by either *B. parviflora* or *B. gymnorhiza*. In *B. gymnorhiza* forests, scattered specimens of *X. australasicum* occurred and the fern *A. speciosum* grew between the trees. The *Bruguiera* forests were the tallest of the Australian mangrove. Height of the trees appeared to depend upon the amount of fresh water available, with tallest trees in areas of highest rainfall.

The landward fringe of the Queensland mangals was the most diverse of all seral stages. *Avicennia marina* was the most abundant tree, but *B. exaristata* Ding Hou was common, and *C. tagal, C. decandra, Lumnitizera agallocha, L. littorea, R. apiculata, and Exocoecaria agallocha* L. were present. *Xylocarpus granatum* and *X. australasicum* were present occassionally. Where the landward fringe bordered a rain forest, many of the forest epiphytes grew on the mangrove trees. These included the orchids *Dischidia nummularia* R. Br. and *Dendrobium* sp., the ant plant *Myrmecodia (beccari* Hook.?), and the ferns *Drynaria rigidula* Bedd., *Platycerium* sp., *Polypodium acrostichoides* Forst., and *P. quercifolium* L.

The above description by Macnae of seral succession in a mangal is, in general, typical of the large swamps of the Indo-Pacific region. This author (1966, 1968) described characteristics of large mangals in detail, and concluded that zonation of mangrove trees was due to the interaction of (1) frequency of tidal flooding, (2) salinity of the soil water, and (3) water logging of the soil. All three are modified by the presence of creeks, gullies, channels, and rivers. The second and third depend upon rainfall and/or the supply of fresh water, evaporation, transpiration, and the nature and quality of the soil. Chapman and Trevarthen (1953) stated that on muddy or sandy shores, distribution of organisms is related to the nature of the substratum which controls drainage, aeration, and penetrability.

The possible role of tidal flooding in relation to succession of Jamaican mangroves was shown by Chapman (1944b), who related vegetation types to the number of tidal submergences per year. His data showed decreasing tidal influence between the seaward *R. mangle* stand and the landward *C. erectus* stand (Table 4).

Tidal flooding alone does not determine species composition, zonation, or succession in mangals. Clarke and Hannon (1967, 1969, 1970, 1971) studied the physical habitat of mangroves in Australia in great detail. They concluded that soil did not play a major role in control of plant distributional patterns and that plant reaction on the soil did not regulate seral change. Microclimate was important in providing conditions necessary for seedling development, determining soil characteristics, and influencing competition between species. Clark and Hannon showed that the holocoenotic complex (Fig. 11) was intricate and that variation in any of the components affected species distribution. The main factors were degree of tidal flooding, elevation of the land, and salinity of the soil water. Plant zonation was associated closely with elevation above mean sea level, seasonal patterns of soil salinity, and small

Table 4. Number of tidal inundations in a mangrove swamp in Jamaica (Chapman, 1944b).

Vegetation	Inundations per year
Rhizophora swamp	700+
Rhizophora/Avicennia boundary	524
Avicennia swamp	432
Avicennia/Laguncularia boundary	213
Center of salina	150
Laguncularia/Conocarpus boundary	4

differences in microtopography. Also, light and water-logging of the soil were important to distribution, and a comparison of environmental requirements of coastal halophytes was made (Fig. 12). The authors stated that the sharpness of zonation depended upon the intensity of species interaction at ecotones. Slight environmental changes related to topography produced intense competition which made significant factors that were normally of secondary importance. Generally, the severity of the environment, including covering by mud and tidal scouring, determined the success of a species in advancing seaward, whereas landward extension was governed by ability to compete with other species in relation to salinity, availability of fresh water, temperature, light, and humidity.

Macnae (1966) criticized the Watson (1928) scheme of classification based on frequency of tidal flooding (described above) because it applied only to ever-wet forests in Malaya. Instead, Macnae proposed that zonation be based on the dominant tree. Dansereau (1947), in a phytosociological study of mangrove in Brazil, described three natural associations: *Rhizophoretum manglei, Avicennietum tomentosae,* and *Laguncularietum racemosa.* Cuatresasas (1958) described the mangrove associations of South America as *Rhizophoretum brevistylae, Rhizophoretum mangleae, Brugieretum gymnorrhizae, Sonneratietum albae,* and *Avicennietum nitidae.* Schnell (1952) described five "edaphic" associations in West Africa: *Rhizophoretum racemosae, Avicennietum nitidae, Drepanocarpeta-Rhizophoretum, Ecastophylletum (Dalbergiaetum) brownei,* and *Cyperteum articulati.* Chapman (1970), in a very important paper on mangrove phytosociology, compared succession in mangals throughout the tropics and gave eight schemata that depicted zonation (Figs. 13-21). Chapman concluded that there is great similarity in the vegetational communities and suggested an extensive classification of natural associations according to the Braun-Blanquet system. The classification consisted of 8 alliances, 15 orders, and 40 associations, but must be considered tenuous at this time because of lack of taxonomic and systematic data from many localities.

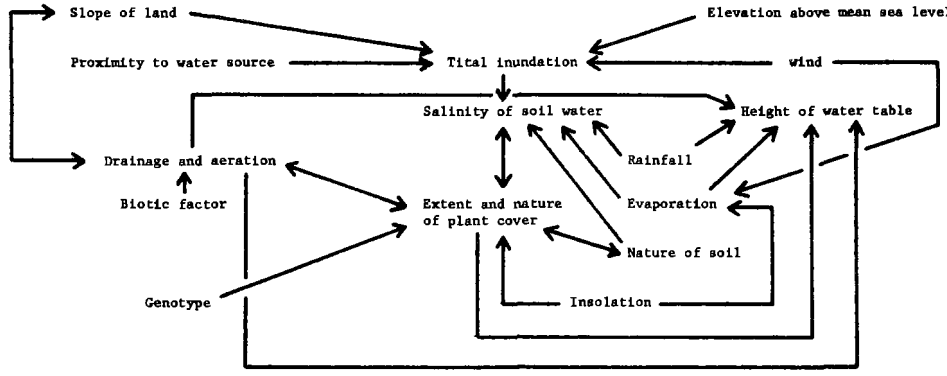


Figure 11. **The holocoenotic complex in mangrove swamps and salt marshes of the Sydney District, Australia (Clarke and Hannon 1969).**

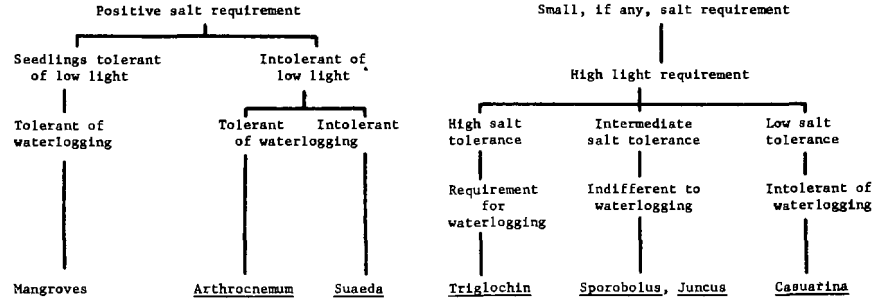


Figure 12. **Environmental requirements of coastal halophytes of the Sydney District, Australia (Clarke and Hannon 1971).**

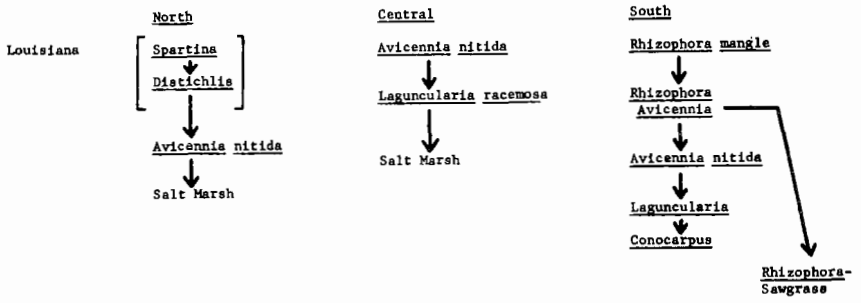


Figure 13. Succession in the mangrove swamps of the Gulf of Mexico and the Caribbean Sea (Chapman 1970, Schema 1).

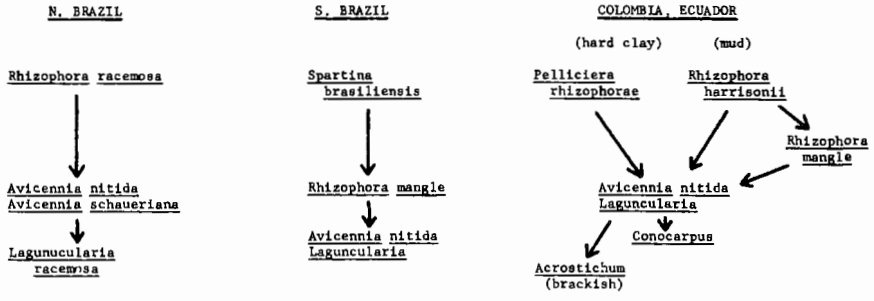


Figure 14. Succession in the mangrove swamps of South America (Chapman 1970, Schema 2).

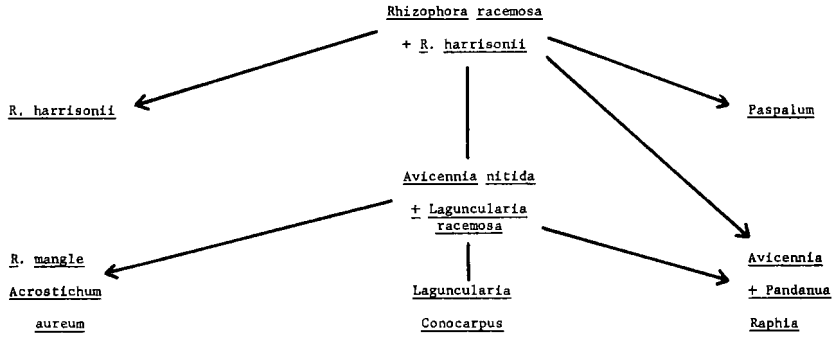


Figure 15. Succession in the mangrove swamps of West Africa (Chapman 1970, Schema 3).

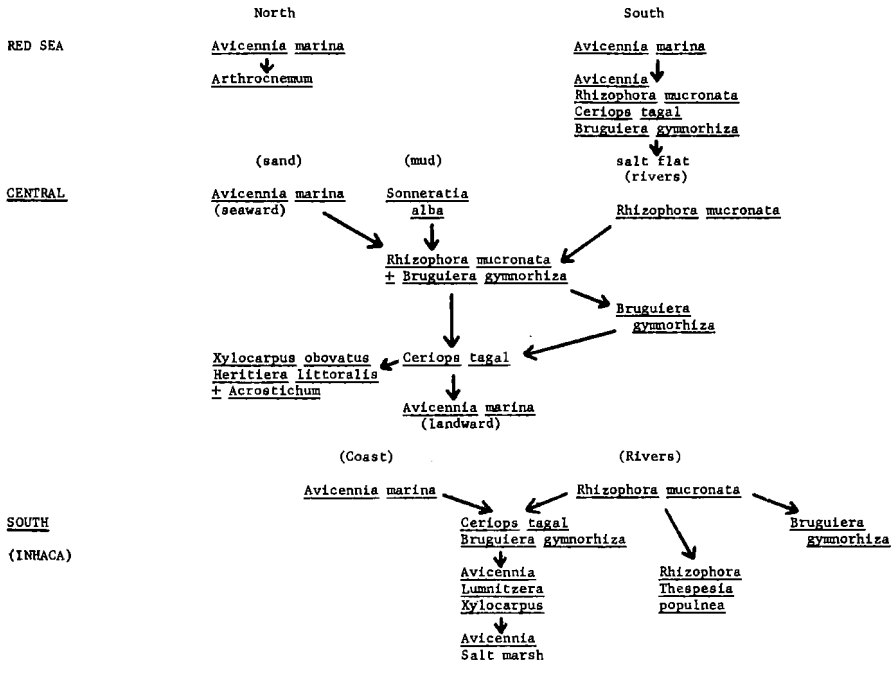


Figure 16. Succession in the mangrove swamps of East Africa (Chapman 1970, Schema 4).

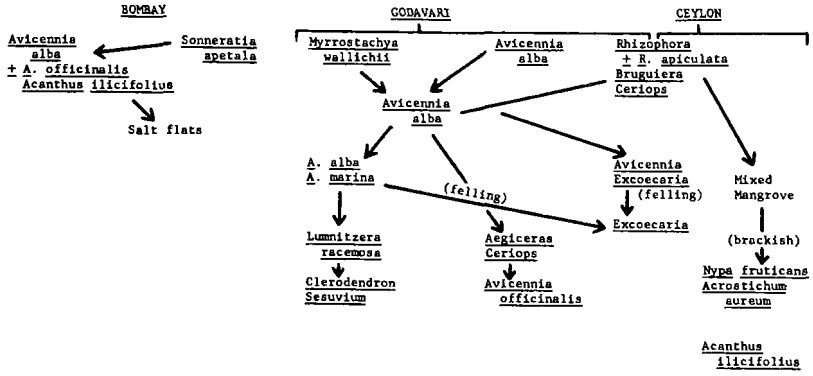


Figure 17. Succession in the mangrove swamps of India (Chapman 1970, Schema 5).

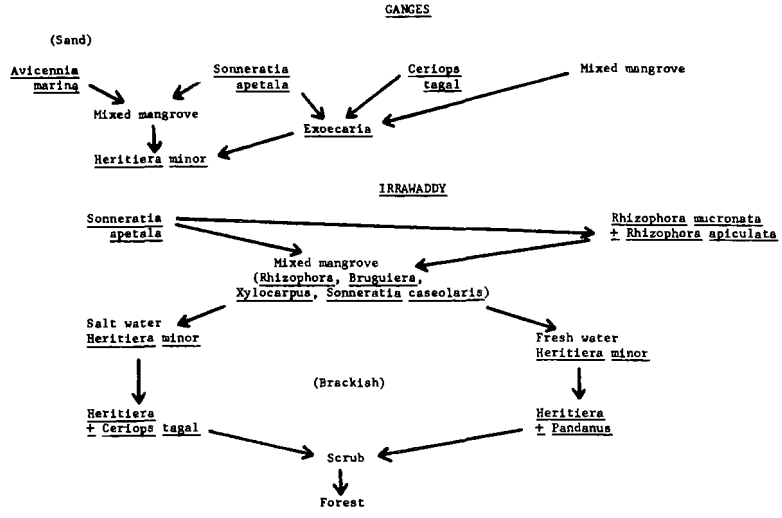


Figure 18. Succession in the mangrove swamps of India (Chapman 1970, Schema 6).

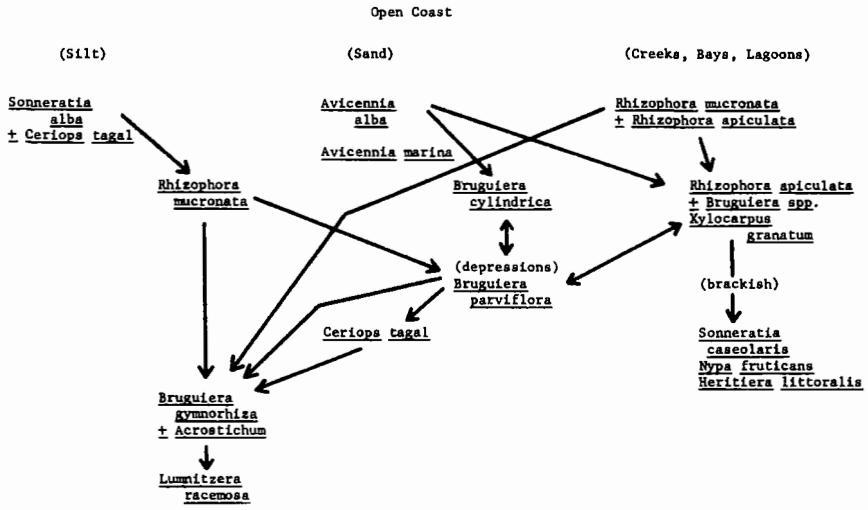


Figure 19. Succession in the mangrove swamps of Malaysia, Indonesia, and Borneo (Chapman 1970, Schema 7).

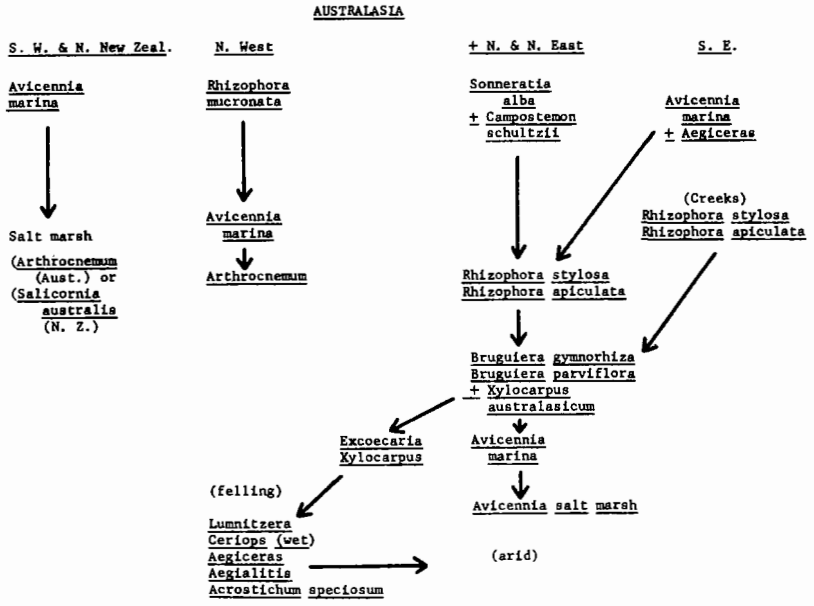


Figure 20. Succession in the mangrove swamps of Australia (Chapman 1970, Schema 8)

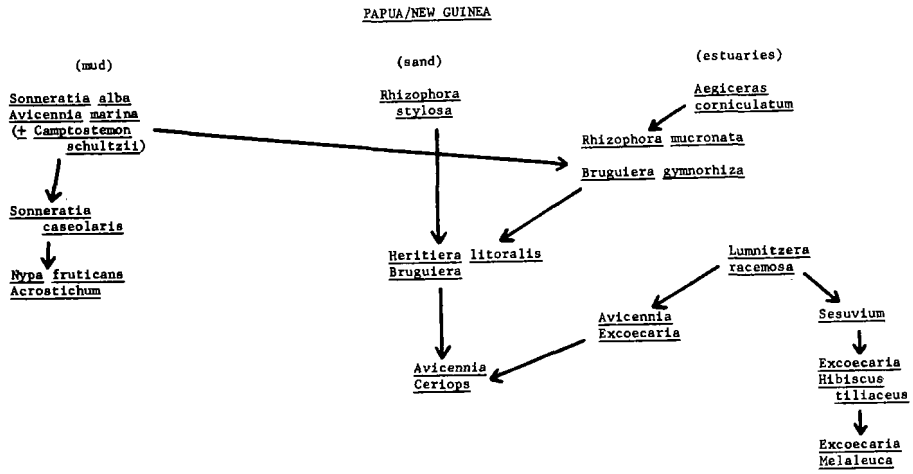


Figure 21. Succession in the mangrove swamps of Papua, New Guinea, the Philippines, and Oceania (Chapman 1970, Schema 9, part 1).

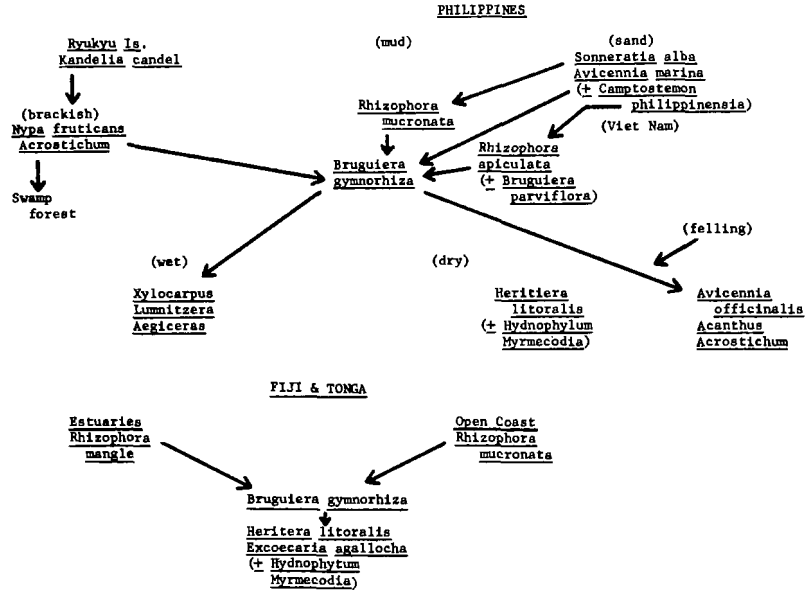


Figure 21. (Continued). Succession in the mangrove swamps of Papua, New Guinea, the Philippines, and Oceania (Chapman 1970, Schema 9, part 2).

ADAPTATIONS

Warming (1883) stated that mangroves have adapted to their environment through (1) mechanical fixation in loose soil, (2) respiratory roots and aerating devices, (3) viviparity, (4) specialized means of dispersal, and (5) development of xerophytic structures in relation to soil salinity. Walter (193la, b; 1936a, b) and Walter and Steiner (1934) concluded from studies in East Africa that zonation was related to the capacity of mangroves to compete and survive in saline soils. Thus, they distinguished zones of *Rhizophora*, *Avicennia*, and *Sonneratia* and stated that *Rhizophora* and *Avicennia* bore great fluctuations in soil salinity, whereas *Sonneratia* required a constant chloride content. It is clear, however, that zonation depends also upon morphological and physiological adaptations. Wenzel (1925) gave detailed descriptions of the anatomy of *R. mucronata*, *R. mangle*, *B. gymnorhiza*, *C. candolleana*, *A. officinalis*, and *X. granatum*. Chapman (1944c) described functional morphology of *A. nitida* in detail and presented data on physiology of the pneumatophores. The gross morphology of most species is described in many manuals of tropical trees.

Anatomical

Marco (1935) described the anatomy of the woods of rhizophoraceous species from both mangals and upland forests of the Indo-West-Pacific region. He divided the family into three groups and stated that the mangrove genera formed a well-defined, natural aggregation that was readily separable from all other members of the family. He suggested that *Rhizophora*, *Bruguiera*, *Ceriops*, and *Kandelia* be placed in an independent family. Marco placed the four genera in the anatomical division Rhizophoreae, characterized by (1) heavily barred, exclusively scalariform perforation plates, (2) characteristic scalariform intervascular pitting, (3) little vasicentric parenchyma, (4) numerous fine-celled multiseriate rays and very few uniseriate rays, (5) libriform fibers with inconspicuous pits, and unilaterally and bilaterally compound pitting between rays and vessels. These features segregated the four genera from all other groups of the Rhizophoraceae, but their significance as adaptive features has not been determined.

Reinders-Rouwentak (1953) stated that, in the Sonneratiaceae, the mature wood of species from more saline environments contained a larger number of smaller vessels than species from less saline areas. For example, *S. griffithii* from the seashore of Bengal had 34-50 vessels/mm^2 and the diameter range was 85-100 u. *Sonneratia apetala* from the river had 18-32 vessels/mm^2 with diameter range of 135-150 u. Heiden (1893) gave a detailed account of anatomy of the Combretaceae, including the genera *Conocarpus*, *Lumnitizera*, and *Laguncularia*.

Macnae (1968) reviewed adaptations of mangroves with regard to growth in ill-consolidated mud, specializations of stems and leaves, relationships between root and shoot systems, and vivipary. Robyns (1971) considered mangroves to be the only truly viviparous plants. He defined vivipary as the process in which the seed remains attached in the fruit to the mother tree, germinates into a protruding embryo with a long hypocotyl, and finally falls from the tree. Genkel' (1962) speculated that mangroves evolved in the ancient tropical forest from xerotic plants in which the seeds had no dormant period and loss of fruit from the tree was delayed by chloride in the soil water.

The roots of mangroves are not deep and tap roots are not present. For descriptions of mangrove root systems see Goebel (1886), Schimper (1891), Troll (1930), Troll and Dragendorff (1931), Uphoff (1941), and Macnae (1968). Zieman (in press) found that height of *R. mangle* was related to root length. In *Rhizophora*, the primary roots of the hypocotyl function for only a short period of time and root functions are assumed by secondary roots which extend from the main trunk. The cause of cessation of growth of primary roots is not known, but Warming (1877), Johow (1884), and Schimper (1891) suggested that they are injured mechanically by crabs and snails. There are two kinds of roots in *Rhizophora:* (1) aerial roots that arise from the main trunk and form arched stilts which penetrate the ground (prop roots) and (2) subterranean roots that arise from the prop roots. Aerial roots also arise adventitiously from the lower branches of trees. The prop and aerial roots function in aeration and ventilation of the tree in general and of the subterranean roots in particular. Most mangroves have schizogenous lacunae in the cortex of the roots. The main function of the subterranean roots is absorption of water and nutrients.

The anatomy of aerial roots has been described by Warming (1883), Schenck (1889), Karsten (1891), Leibau (1914), Bowman (1917), Mullan (1931, 1932, 1933), and Gill and Tomlinson (1971). According to Gill and Tomlinson, aerial roots first appear on the hypocotyl or lower internodes of seedlings after 1 to 3 first-order branches have been produced. Later, they arise on higher internodes and lower branches. Aerial roots also develop on the high branches of mature trees. In general, the aerial roots originate on the shoot in acropetal sequence. Gill and Tomlinson (1971) gave a detailed account of root growth and anatomy.

When aerial roots reach and penetrate the ground, they undergo marked changes which relate to subterranean function. According to Bowman (1917), the absorptive subterranean roots are thick, spongy, and gas-filled due to great development of the primary cortex. The primary cortex of absorptive roots is composed of large cells and very large intercellular spaces in which idioblasts, trichoblasts, and root hairs are lacking (Bowman 1921). The periderm of the absorptive root consists only of cork cells, whereas that of the aerial root consists of both cork and "parenchymatic" tissue (Bowman 1921). Bowman also reported stone cells and idioblasts in all parts of *R.*

mangle except the flower. These were frequently associated with tannin cells. Sclerenchymatous tissue occupies a large portion of the stem and hypocotyl of mangrove and makes anatomical study very difficult.

Two other rhizophoraceous genera, *Bruguiera* and *Ceriops*, do not have aerial roots. Instead, they have subterranean cable roots which differentiate into knee roots that penetrate the soil surface, and absorptive roots (Marco 1935).

Troll and Dragendorff (1931) gave an extensive account of the cable root system of *Sonneratia*, and similar roots systems are present in some species of *Avicennia*, *Lumnitizera racemosa*, *X. australasicum*, and *X. moluccensis* Roem. For an extensive study of anatomy of respiratory roots of mangroves, see Ernould (1921).

Chapman (1944c) showed that the composition of gas in the roots of *A. nitida* was similar to air and that there was no fundamental difference between composition of gas in the pneumatophores and in the horizontal roots. He stated that the large cortical air spaces allowed longitudinal gas flow between organs. Scholander et al. (1955) studied respiratory gas exchange in the roots of *A. nitida* and *R. mangle*. The radial roots of *A. nitida* send numerous pneumatophores up to 30 cm above the ground. There is a direct gas connection between the radial roots and the pneumatophores. When the tide covered the pneumatophores, there was a decrease in the oxygen content of the whole root system. At low tide, oxygen comprised between 15 and 18% of the gas content. At high tide, oxygen content was about 7%. At high tide, the oxygen content dropped until the pneumatophores were again exposed to air at low tide. There was little change in carbon dioxide content of the roots over the tidal cycle.

Gas in the subterranean roots oof *R. mangle* contained 15 to 18% oxygen and there was always a direct gas connection between these roots and lenticels on the prop roots. The high oxygen tensions in the roots were maintained by means of ventilation through the lenticels on the prop roots (Scholander 1955).

Macnae (1968) gave diagrams of the cross sections of leaves of *Rhizophora*, *Avicennia*, and *Sonneratia*. Schimper (1891, 1898) showed that the leaves of most mangroves contain water storage tissue. This is initially in the form of a hypodermis in *Rhizophora* and *Avicennia* and a centrally located layer of cells in *Sonneratia*. Stace (1966) made a detailed study of leaf anatomy of seven genera (Tables 5 and 6) and also the epidermal characteristics of *Bruguiera* spp. and *Avicennia* spp. He concluded that the leaf and epidermal characteristics of mangroves are similar to most xeromorphs. All species had common epidermal features, notably a thick cuticular membrane, straight epidermal cell walls, and the presence of water-storage tissue, hydathodes, cork warts, and water stomata. See Artz (1936) for descriptions of the cuticula of *S. alba*, *C. candolleana*, *R. mucronata*, *B. gymnorhiza*, *Lumnitizera racemosa*, *X. obovatus*, and *A. officinalis*. In the study of Stace, almost all of the species studied had sunken stomata or stomata surrounded

Table 5. Characteristics of the leaves of rhizophoraceous mangroves (Stace 1966).

	Rhizophora	Ceriops	Bruguiera and Kandelia
Venous system on upper epidermis	Midrib only, very broad and conspicuous; cells broader than long	Midrib only, narrow and inconspicuous; cells broader than long	Midrib only, narrow but conspicuous; cells broader than long
Venous system on lower epidermis	Midrib only, very broad and conspicuous, or lateral veins also discernible	Midrib only, very broad and conspicuous	Midrib only, very broad and conspicuous
Epidermal cells of non-venuous areas	Straight- or curved-walled, not second divided, mostly ca. 11-25 μ across	Straight- or curved-walled, not second divided, mostly ca. 15-35 μ across	Mostly straight- or curved-walled, not second divided, mostly ca. 15-40 μ across
Stomata	Sunken, ca. 30-55 x 20-35 μ; outer stomatal ledge conspicuous, single or with minute second lip	Sunken, ca. 36-46 x 22-34 μ; outer stomatal ledge conspicuous, conspicuously two-lipped	Sunken, ca. 30-44 x 16-28 μ; outer stomatal ledge conspicuous in some spp. conspicuously two-lipped
Subsidary cells	5-8, cyclocytic	6-8, cyclocytic	4-6(8), cyclocytic

Con't on next page

Table 5 con't

Water stomata, hydathodes and cork-warts	Large conspicuous cork-warts on lower epidermis, sometimes also on upper epidermis; water-like structures on both epidermides	Cork-warts ± absent; frequent water-stomata-like structures on both epidermides	All apparently absent
Hypodermis, including extra epidermal layers	Upper three-to five-layered, sparsely chloroplasted; lower usually absent	Upper two-layered, sparsely chloroplasted; lower usually absent	Upper and lower one-layered, densely chloroplasted
Mesophyll	One to three layers of palisade and ca. eight to ten layers of spongy below upper hypodermis	Usually one layer of palisade and ca. eight to ten layers of spongy below upper hypodermis	Usually one layer of palisade and ca. eight to ten layers of spongy below upper hypodermis
Water-storage tissue	Upper hypodermis ?	Upper hypodermis ?	Absent, or ? sometimes in spongy mesophyll

Table 6. Characteristics of leaves of combretaceous mangroves (Stace 1966).

	Lumnitzera	Laguncularia	Conocarpus
Venous system on upper epidermis	Absent, or midrib only very inconspicuous; cells longer than broad	Midrib only, broad and conspicuous to very inconspicuous; cells longer than broad	At least midrib and lateral veins distinct; cells longer than broad
Venous system on lower epidermis	Midrib only, broad and conspicuous	Midrib only, broad and conspicuous	Midrib, lateral, secondary and lesser veins distinct
Epidermal cells of non-venous areas	Straight-walled, not second divided, mostly ca. 25-40 μ across	Straight- or slightly curved-walled, many second divided, mostly ca. 15-30 μ across	Mostly curved or straight-walled, not second divided, mostly ca. 15-35 μ across
Margin	Of several regular rows of rectangular cells with angular lumina	Of small cells with rounded lumina not arranged in rows	Of several regular rows of rectangular cells with angular lumina
Stomata	Sunken or not, not protected by hairs, always more frequent on upper epidermis, absent only from margins, randomly	Scarcely sunken, not protected by hairs, usually more frequent on upper epidermis, absent only from margins, orien-	Not sunken, protected by dense hairs or not, usually slightly more frequent on lower epidermis,

Con't on next page

Table 6 con't

	oriented, ca. 24-32 x 19.5-24.5 μ; outer stomatal ledge conspicuous, single	tated at right angles to midrib on upper epidermis, ca. 25-35 x 20-26 μ; outer stomatal ledge conspicuous, single	absent from margin and lower epidermal midrib, randomly orientated, ca. 25-30 x 17-25 μ; outer stomatal ledge fairly conspicuous, single
Subsidiary cells	(3)4-5(6), cyclocytic	(3-)4(-5), cyclocytic	3-6, not differentiated
Trichomes	Compartmented hairs only, often extremely sparse to absent on both epidermides	Compartmented hairs and apparently sessile deeply sunken glands on both epidermides	Compartmented hairs and stalked superficial glands on both epidermides
Water stomata, hydathodes and cork-warts	Large water-stomata present on both epid.; hydathode-like areas present, mostly on midrib of lower epidermis and on margin	As in Lumnitzera but very sparse	Usually apparently absent, rarely a few water-stomata present
Domatia	Shallow pits along margins may be rudimentary domatia	Absent	Large, primary-axillary lebetiform domatia on lower epidermis

Con't next page

Table 6 con't

Mesophyll	Two layers of palisade below each epidermis; spongy absent	Two layers of palisade below upper epidermis; one to two layers of spongy, palisade or mixed above lower epidermis	One or two layers of palisade below upper epidermis, one layer of palisade above lower epidermis; spongy absent
Water-storage tissue	ca. six to twelve layers of centrally placed ± isodiametric cells, not chloroplasted	ca. six to twelve layers of centrally placed ± isodiametric cells, very sparsely chloroplasted	ca. four to six layers of centrally placed ± vertically elongated cells, sparsely chloroplasted

by dense trichomes. All genera, except *Avicennia* and *Conocarpus*, lacked lateral and lesser epidermal veins, a condition associated with development of water-storage tissue. Stack also gave a key to the genera based on epidermal characters of the leaves.

Bowman (1921) observed that the water-storing hypodermis and tannin-containing cells of *R. mangle* were much larger in trees that grew in seawater than in trees from brackish water. Possible reasons for this will be discussed under "Physiological" in this report.

Sidhu (1962, 1968) reported chromosome numbers of mangrove species from India (Table 7). He concluded that most species from the mangrove habitat possess higher chromosome numbers than other species of the same genera from mesic habitats. However, among the mangroves, the size and number of chromosomes did not show any correlation to habitat conditions.

Physiological

Most research on physiology of mangroves has stressed halophytic adaptations. Halophytes are ordinarily distinguished from other plants by their ability to grow in high concentrations of salt. They complete their entire life cycles and compete successfully with other plants in saline environments. Genkel' and Shakone (1946) classified halophytes as (1) Euhalophytes (salt accumulating), (2) Crynohalophytes (salt excreting), (3) Glycohalophytes

Table 7. Chromosome numbers (n) of mangroves and related species from India (Sidhu 1962, 1968).

Family and Species	Chromosome Number	Family and Species	Chromosome Number
Rhizophoraceae		Chenopodiaceae	
R. mucronata	18	*Suaeda nudiflora*	18
R. conjugata	18	*S. monoica*	9
B. parviflora	18	*S. maritima*	9
B. gymnorhiza	18	Euphorbiaceae	
C. candolleana	18	*Exocoearia agallocha*	65
C. roxburghiana	18	Palmae	
Sonneratiaceae		*Nypa fruiticans*	8
S. apetala	12	Sterculiaceae	
Duabanga sonneratioides	12	*Heritiera littoralis*	19
Myrsinaceae		Meliaceae	
Aegiceras corniculatum	24	*Xylocarpus moluccensis*	21
Salvadoraceae		*Xylocarpus granatum*	21
Salvadora persica	13	Papilionaceae	
Acanthaceae		*Derris uliginosa*	10
Acanthus ilicifolius	24	Rubiaceae	
Verbenaceae		*Ixora parviflora*	11
Avicennia alba	16		

(salt impermeable), and (4) those in which salt is localized in special structures. Depending on the species, mangroves may be placed in (1), (2), or (4). It is doubtful that mangroves are intolerant or obligate halophytes, although Stern and Voight (1959) and Connor (1969) have shown that *R. mangle* and *A. marina* grow best when salt is present in the soil water. Some mangroves (e.g. *R. mangle, C. tagal, N. fruiticans*) adapt to glycophytic conditions and may be considered to be faculative halophytes. Mangroves species have been reared in fresh water in the laboratory, and Stocker (1924, 1925) proposed the term "miohalophytes" for such plants.

Barbour (1970) suggested that ability to reproduce, rather than short-term growth, should be the ultimate criterion of salt tolerance, but this has not been studied with regard to mangroves. In the field, Bowman (1917), Davis (1940), and Stern and Voight (1959) in Florida, and Pannier (1959) in Venezuela reported that *R. mangle* grew and reproduced in fresh water, but height of trees and area covered were greatest in brackish water.

In the laboratory, Winkler (1931) reported that *Bruguiera eriopetala* and *R. mangle* grew and flowered in pots of sand watered only with fresh water. Davis (1940) grew *R. mangle* in fresh water in the laboratory. Pannier (1959) grew the same species in rain water and in salinities up to full strength seawater. Although seedlings grew in the rain water, root growth was optimal at 50% seawater and shoot growth was optimal in 25% seawater. Stern and Voight (1959) reported that height, dry weight, and survival of *R. mangal* increased with increasing salinity in the laboratory. They used artificial seawater, and plant dry weight was approximately three times as great in the highest salinity than in the lowest. Maximum growth occurred at salt concentrations equivalent to seawater. Patil (1964) grew *R. mucronata, K. candel, B. parviflora, C. tagal, A. ilicifolius, X. moluccensis, E. agallocha,* and *Heritiera fomes* in salt concentrations between 0.3 and 1.2%. All species grew at all salinities, but growth was best at 1.2%. Clarke and Hannon (1970) found that *A. marina* at the 0-2 leaf stage maintained optimal growth in 20% seawater. Concentrations above 60% seawater retarded growth. Seedlings at the 2-4 leaf stage were more tolerant, and optimal growth occurred at 40% seawater. Connor (1969) reported that the optimal concentration for laboratory growth of *A. marina* from Australia was approximately 1.5%, or half the concentration of seawater. Connor reported that suppression of height by higher salt concentrations was more marked than suppression of dry weight production. An important aspect of Connor's work was that while growth appeared normal when sodium chloride was the main component of the salt mixture, potassium chloride and calcium choloride suppressed growth. Connor suggested that high concentrations of calcium caused nutrient imbalance leading to iron deficiency and speculated that the responses of mangroves to specific ions reflected the physiological ability of the plants to adapt to concentrations in the root environment.

Temperature as a factor in seedling establishment of *A. germinans* was shown by McMillan (1971). Exposure to temperatures of 39-40°C for 48

hours was lethal to stemless seedlings, but not to seedlings with stems and roots.

Bharucha and Navalkar (1942) reported the chloride content of leaf cell sap of *A. alba* in relation to that of seawater and soisalinity (Table 9). They concluded that seasonal variations in the chloride content of leaf cell sap were dependent directly upon climatic conditions of temperature, rainfall, and humidity. It will be shown later that such high sap concentrations are common in mangroves that possess glands for salt excretion.

Table 9. Chloride content of seawater, soil water, and leaf cell sap of *A. alba* (Bharucha and Navalkar 1942).

	Percent Chloride
Seawater	0.77-3.24
Soil Water	0.55-3.47
Leaf cell sap	1.59-5.05

Blum (1941) reported osmotic pressures in the leaves of several mangrove species from Java (Table 10). Avicennia had the highest osmotic pressure, whereas *Rhizophora*, *Bruguiera*, and *Sonneratia*, genera which possess mechanisms for salt exclusions and/or dilution, had relatively low osmotic pressures.

Bole and Bharucha (1954) reported data on osmotic relationships in leaves of *A. alba* (Table 11) and concluded that higher rates of transpiration brought about higher accumulation of osmotically active substances in the older leaves. The osmotic pressure did not vary directly with the water content, but older leaves always had higher water contents and higher osmotic pressures than younger leaves.

Chapman (1968) stressed that research on saline vegetation must emphasize the roles of different ions upon plant metabolism. He stated that the interrelations of sodium and potassium are particularly important because the amount of potassium absorbed is influenced greatly by the presence of sodium. There is evidence that temperature and light affect the responses of halophytes to salinity (Tsopa 1939), but tolerance of plants depends mainly upon the type of soil salinity (Cl^-, $SO_4^=$, etc.), the species or variety of plant, and the stage of plant development (Chapman 1966).

Table 10. Osmotic pressures in the leaves and soil of mangroves from Java (Blum 1941).

Species	Osmotic Pressure, Atmos.		
	Leaf	Soil	Diff.
A. officinalis, High tide	45	23	22
A. officinalis, Low tide	40	18	22
Sonneratia acida, High tide	27	6	21
Sonneratia acida, Freshwater	20	0.3	20
Rhizophora conjugata, Seawater	31	23	8
Bruguiera gymnorhiza, Freshwater	25	0.3	25

Table 11. Osmotic relationships in young and old leaves of *A. alba* (Bole and Bharucha 1954).

	Leaf	
	Young	Old
Osmotic pressure, atmos.	38.8-47.7	51.5-57.4
Water content, percent	69.5-72.9	57.5-62.7
Total carbon, percent	37.1-40.8	37.9-42.2
Total nitrogen, percent	1.1- 1.4	1.0- 1.6
Water loss/m^2/hr, grams	0.56-0.74	0.92-1.00

Jennings (1968) demonstrated positive correlations between the sodium and water contents and the phosphorus and water contents of halophytes. Potassium had no appreciable relationship to succulence. Jennings stated that increased succulence produced by high light intensity, aridity, and sodium ions was brought about by essentially the same mechanism. Jennings also suggested three mechanisms used by halophytes to cope with toxic concentrations of ions. The first was export of the ions from the shoots and leaves. This could occur in either of two ways: (a) transportation of ions through the phloem to the roots and extrusion back to the soil or (b) extrusion through specialized glands in the leaves. The former has not been reported for mangrove, but salt excretion through epidermal glands occurrs in *A. alba* (Walter and Steiner 1936), *A. nitida* (Biebl and Kinzel 1965), *Aegiceras* (Areschog 1902a, b; Schmidt 1940a), and *Aegiatilis* (Ruhland 1915), Mullan (1931a, b, c) reported salt-excreting glands on the petioles and upper and lower epidermis of *A. alba*, *A. ilicifolius*, and *A. corniculatum*. The glands were most numerous on plants from hypersaline areas. They were absent from *A. ilicifolius* from fresh water.

The xylem sap of salt-excreting species is composed of approximately 0.2-0.5% sodium chloride. This concentration exceeds that of non-secreting mangrove species by 10 times, and that of ordinary land plants by about 100 times (Scholander et. al. 1962)

Scholander et. al. (1962) also reported salt excretion by *A marina*, *A. corniculatum*, and *Aegiatilis annulata*. In *Aegitalis*, secretions from salt glands contained between 1.8 and 4.9% sodium chloride, with highest values during the day. Diel variation in rate of secretion occurred in *Aegiceras*, the average sodium chloride content of salt gland secretions being 2.9% during the day and 0.9% at night. The sodium chloride content of the xylem sap of *Avicennia* was very high, ranging from 4 to 8 mg/ml; one sample of gland secretion contained 4.1% sodium chloride. Jennings (1968) reported that the secretion process selects sodium over potassium, the Na/K ratio being 13 in the exudate, but only 3 in the leaves of *Aegialitis*. Atkinson et. al. (1967) reported that in *Aegiatilis*, input of chloride to a mature leaf was approximately 100 u-equiv./day and this was balanced by secretion, mainly of sodium chloride, from the salt glands. Secretions from the salt glands contained 450 u-equiv/ml of chloride, 355 u-equiv/ml of sodium, and 27 u-equiv/ml of potassium. Rate of secretion varied between 93 p-equiv/cm^2/sec during the day and 3 p-equiv/cm^2/sec in darkness. Atkinson et. al. suggested that because the water potential of the secretion is similar to that of the leaf, the secretory process involves active transport of salt and movement of water by local osmosis. Atkinson also presented light and electron microscope studies of the salt glands.

Rains and Epstein (1967) studied preferential absorption of potassium by leaves of *A. marina* in the presence of high concentrations of sodium chloride. They demonstrated that *A. marina* could (1) absorb and concentrate potassium within its tissues in excess of the concentration in the substratum and (2) preferentially select potassium when in the presence of high concentrations of sodium, a closely-related ion. The ability to select potassium over sodium is an extremely important adaptive character in the marine environment.

Another significant adaptation is the ability to tolerate, without injury, high internal concentrations of salt. *Avicennia marina*, unlike other mangroves such as *Rhizophora*, *Laguncularia*, and *Sonneratia*, absorbs salt in substantial amounts. The concentrations of ions in leaves examined by Rains and Epstein (1967) were 30 mM potassium, 210 mM sodium, and 245 mM chloride. The authors concluded that the effect of preferential absorption of potassium was not to exclude sodium, but rather to raise the concentration ratio of potassium to sodium from the value in seawater (1/40) to 1/7 within the tissue. The tissue did contain a high concentration of sodium chloride (1.8 mM/g dry weight), but excretion by salt glands prevented higher and possibly deleterious concentrations from developing.

Genkel' (1962) suggested that viviparous species of mangroves utilize seedlings for exclusion of salt. He found that the chloride content of seedlings

increased in proportion to size and were adapted to high salt content in the soil before dropping from the tree. This was shown to be true for *R. mangle* by Lotschert (1968). Excess chloride in soils delayed loss of seedlings from the tree and Genkel' concluded that vivipary is an adaptation to the salt regime in tidal areas.

The second mechanism suggested by Jennings (1968) was limitation of transfer of ions to the shoot by some mechanism located in the roots. Scholander et. al (1966) showed that *R. mucronata, Laguncularia racemosa,* and *S. alba* are efficient in salt exclusion. Atkinson et. al (1967) showed the same for *R. mucronata.* Scholander et. al. (1966) stated that the desalinization process in the root system produces a sap of fairly constant concentration that is independent of rate of transpiration. Salt glands, when present, eliminate salts left behind by transpiration.

Concentration of the soil solution, rainfall, tide, humidity, temperature, transpiration, nature of the organisms, leaf age, water content, nitrogen content, and carbon content have effects upon osmotic relationships of mangroves (Blatter 1909; Cooper and Pasha 1935; Navalkar 1940, 1942, 1948; Bharucha and Navalkar 1942; Bole and Bharucha 1954). Gessner (1967), however, found that water which passed from the stems to the leaves of *R. mangle* was nearly salt-free.

Scholander et. al. (1965) reported that halophytes such as *Rhizophora, Osbornia, Salicornia,* and *Batis* have strong negative sap pressures, ranging from -35 to -60 atmospheres, whereas the osmotic potential of seawater is approximately -25 atmospheres. The activity of water in the marine environment is always higher than that of water in the roots, xylem sap, and leaves. In *R. mangle, Laguncularia racemosa,* and *C. erectus,* the xylem sap content of sodium chloride was only 1.2-1.5 mg/ml. At night, when transpiration by *Rhizophora* and *Osbornia* was nil, sap tension was the same as the osmotic potential of seawater but the solute pressure in the leaves was 10 to 20 atmospheres higher than seawater (Scholander 1971). In *Rhizophora, Laguncularia,* and *Conocarpus* only about 50-70% of the freezing point depression in leaf cells was produced by sodium and chloride ions, most of the remaining solutes being organic (Scholander et. al 1966). Benecke and Arnold (1931) demonstrated that osmotic pressure of *S. alba* was lower under glycophytic than halophytic conditions. These pressure differences give mangrove such as *Rhizophora, Laguncularia, Sonneratia,* and *Conocarpus,* which do not possess salt-excreting organs, the ability to obtain fresh water osmotically from seawater by transpiration and by diffusion at the roots. Scholander (1968) concluded that *Rhizophora, Sonneratia, Avicennia, Osbornia, Bruguiera, Ceriops, Exocoecaria, Acrostichum, Aegiceras,* and *Aegialitis* separate fresh water from the sea by simple nonmetabolic ultrafiltration of the seawater combined with ion transport. The negative xylem pressure is produced by high salt concentration in the cells, resulting in a solute pressure which exceeds that of seawater.

The third mechanism proposed by Jennings (1968) for coping with toxic concentrations of ions was production of increased succulence. High concentrations of ions in the leaf may be prevented because of the dilution effect brought about by increased water content of cells. Bowman (1921) reported greater succulence in *R. mangle* from seawater than from fresh water, a phenomenon which gives support to the third mechanism. Reinders-Gouwentak (1953) found that succulent leaves were common in *Sonneratia* and were due to the presence of a distinct hypodermal aqueous tissue layer. In leaves that were immersed in tidal water, the hypodermal layer was three to five times as thick as leaves at higher levels of the same tree. The same author stated that the hypodermal layer was almost absent in trees grown in fresh water in botanical gardens. Reinders-Gouwentak believed that succulence in *Sonneratia* was related to the chloride content of the water.

Jennings (1968) related succulence in halophytes to sodium metabolism. He postulated that an outwardly-directed sodium pump exists in halophytes and that this pump is related to cation-activated ATPase in the cell wall. This same pump would drive potassium ions into the cell against an electrochemical potential gradient. Jennings admitted that the evidence for such a pump must be viewed with caution, but stated that there are no reasonable arguments against its existence and suggested a relationship to ATPases. In relation to succulence, Jennings proposed that sodium-activated ATPases might be involved in the synthesis of new wall material or in increasing cell wall extensibility. In a similar way, succulence is also induced by increased amount of light which increases the rate of photo-phosphorylation and production of ATP. Also, aridity causes succulence because increased rate of transpiration causes the ration of potassium to sodium in the shoot to change in favor of sodium. The concentration of sodium in the xylem sap reaches such a level that the sodium-activated ATPases in the plasmalemma bring about synthesis of ATP. It should be stated that these proposals of Jennings are highly theoretical and have yet to be tested.

Kylin and Gee (1970) presented evidence that the leaves of *A. nitida* possess ATPases that are dependent upon the ratio of sodium to potassium. Enzyme activities were directly related to ionic strength of the growth medium. Unlike animal systems in which synergistic effects of sodium and potassium yield a peak at only one ratio, *A. nitida* yielded three peaks. At 50 mM total concentration (NaCl + KCl), activity peaks occurred at Na:K ratios of 2:8, 5:5, and between 8:2 and 9:1. These results were interpreted as indicating that either several enzymes functioned in the membrane system, or else structural changes allowed more than one ion to activate a transport site. Whatever the mechanism, the report of Kylin and Gee gives credence to the hypothesis of Jennings that sodium-activated ATPases are present in halophytes. Their role in succulence has yet to be established.

Salt exclusion, salt excretion, and succulence are not the only physiological mechanisms whereby mangroves adapt to their saline environment. Metabolic

processes of photosynthesis, growth, and respiration are also important in adaptation of mangrove, but little work has been reported. Chapman (1966) pointed out that little is known about respiration and photosynthesis by mature mangroves, but speculated that high concentration of salt in the soil would cause slower rate of water uptake, slower rate of upward water movement in the trunk, and slower transpirational loss as compared with many other tropical trees. He suggested that the net result would contribute toward a slow growth rate as compared with trees from mesophytic habitats.

Bharucha and Shirke (1947) stated that the respiratory activity of a plant is influenced by food reserves. In the case of *A. officinalis*, the intensity of respiration of seedlings increased from a minimal to a high rate and then gradually declined. As the seedlings grew, there was an increase in water content and fresh weight, but dry weight decreased, indicating that the growing plant utilized reserve material for growth. Also, the authors showed that in germinating seeds, respiration rate increased during the period of absorption of water and gain in fresh weight.

Bharucha and Shirke also studied the respiration of seedlings of *A. officinalis* from germination to the eighth day of growth in both air and under seawater. Their data, some of which are given in Table 12, showed that the rate of respiration increased with growth under both aerial and submerged conditions. However, respiration rate was much slower under water and this was ascribed to the limiting influence of oxygen in water. Chapman (1962b) reported minimal respiration rates in "medium sized" seedlings of *R. mangle*. He also reported that the cotyledonary body of *R. mangle* and the fruit wall of *A. marina* (both structures function in transport of food from parent to embryo) had very high respiration rates. On a dry weight basis, seedlings of *Avicennia* had a higher respiration rate than those of *Rhizophora*. Chapman suggested that this was related to differential development of aerenchyma.

Table 12. Respiration rates of *A. officinalis* in air and submerged in seawater (after Bharucha and Shirke 1947).

	Respiratory Index	
Stage of Growth	Air	Submerged
1-Day seedlings	2.79	1.21
2-Day seedlings	2.85	1.48
3-Day seedlings	2.94	1.45
4-Day seedlings	3.09	1.46
5-Day seedlings	3.34	1.50
6-Day seedlings	3.67	1.51
7-Day seedlings	3.84	1.62
8-Day seedlings	3.92	1.66

Bharucha and Shirke (1947) also reported increase in the respiration rate of seedlings when the fruit wall was removed. Chapman (1962a, b; 1966b) reported that the fruit wall caused a marked inhibition of respiration in *A. marina*, *A. nitida*, *R. mangle*, *R. apiculata*, and *B. gymnorhiza* and speculated that mangroves are capable of anaerobiosis, although the amount of energy released under anaerobic conditions was inadequate for growth. Anaerobiosis is apparently important during the periods when seedlings float in seawater and no growth occurs. Brown et al. (1969) demonstrated anaerobic respiration in *A. marina*, *B. gymnorhiza*, and *R. apiculata*.

Lotschert (1968) showed that chloride accumulates in the seedlings of *R. mangle* before they fall from the tree. Conversely, Chapman (1944c) reported that a salt exclusion mechanism operated in *A. nitida*. He stated that when the seedlings fell from the tree, there was an immediate uptake of salt and a sudden reduction in respiration rate. Successful colonization of *Avicennia* was related, therefore, to the capacity of seedlings to respond to sudden changes in internal salt content, whereas this was not necessary for *Rhixophora* seedlings.

Arnold (1955) showed that the transpiration rates of mangroves are very much lower than those of mesophytes. Because of this, Chapman (1962a) suggested that mangroves are lacking in dry tropical areas, such as the west coast of South America, because low humidity reduces respiration rate to the point where seedlings cannot grow.

Lewis and Naidoo (1970) reported that the apparent transpiration rate of *A. marina* in South Africa rose in the morning as light intensity increased and humidity decreased. Maximum transpiration occurred at mid-morning, after which the rate progressively decreased, regardless of atmospheric conditions. Tidal inundation after the mid-morning maximum caused increase in transpiration rate and a second maximum. The authors speculated that decrease in rate at mid-morning was caused by incipient wilting following excessive transpiration.

Chemical Composition

The chemical composition of mangrove trees has been studied by Sokoloff et. al. (1950), Sidhu (1963), Morton (1965), and Golley (1969), but little is known in relation to environmental factors and age of the trees. Sidhu (1963) stated that concentrations of ash and sodium in leaves of species of *Rhizophora*, *Avicennia*, and *Aegiceras* which grew near the sea were lower than from species which inland, but gave data only for *Avicennia* (Table 13).

Table 13. Ash and sodium contents of three species of *Avicennia* (Sidhu 1963).

	Species	Ash, percent	Sodium, percent
Sea	*A. officinalis*	14.8	2.3
	A. alba	15.8	3.3
	A. alba	19.4	3.7
Inland	*A. marina*	30.4	5.0

Sidhu (1963) also divided species into three catagories based on sodium content of the leaf:

A. Species with more than 5% sodium: *A. marina, Salvadora persica.*

B. Species with 3-5% sodium: *A. alba, Lumnitizera racemosa, Ceriops candolleana,* and *A. ilicifolius.*

C. Species with 1-3% sodium: *A. officinalis, R. mucronata, S. Apetala, B. Caryophylloides, A. Corniculatum, Eleopodendron inerme, Exocoecaria agallocha, D. uliginosa.*

Atkinson et. al (1967) reported concentrations of some ions in leaves of various ages in *R. mucronata* and *Aegiatilis annulata* R.Br. Measurements were made on successive leaf pairs of shoots (Tables 14 and 15). Concentrations of sodium and chloride in the leaves of *R. mucronata* increased with age, but in relation to amount of leaf water, chloride content was constant, sodium concentration increased, and potassium concentration deceased.

In contrast, there was a decrease in concentrations of sodium, potassium, and chloride with age of *A. annulata.* Atkinson et. al. ascribed this to excretion of salt by epidermal glands, an adaptation lacking in *Rhizophora.*

Table 14. Concentrations of Na^+, K^+, and Cl^- in the leaves of R. mucronata (after Atkinson et al. 1967). Leaves in Sample 1 were youngest in the sequence of increasing age.

	Sample Number					
	1	2	3	4	5	6
Dry weight (g)	0.16	0.50	0.50	0.61	0.57	0.63
Water (% fresh weight)	56	65	66	65	67	69
Na^+ (μ-equiv/leaf)	61	290	420	480	520	645
Na^+ (μ-equiv/ml H_2O)	305	313	431	435	461	461
K^+ (μ-equiv/leaf)	25	81	57	48	69	45
K^+ (μ-equiv/ml H_2O)	124	88	59	44	61	32
Cl^- (μ-equiv/leaf)	74	520	510	585	580	730
Cl^- (μ-equiv/ml H_2O)	370	562	522	530	515	522

Table 15. Concentrations of Na^+, K^+, Mg^{++}, and Cl^- in the leaves of A. annulata (after Atkinson et al. 1967). Leaves in Sample 1 were youngest in the sequence of increasing age.

	Sample Number				
	1	2	3	4	5
Dry weight (g)	0.32	0.45	0.45	0.50	0.44
Water (% fresh weight)	72	60	60	59	60
Na^+ (μ-equiv/leaf)	420	325	275	280	235
Na^+ (μ-equiv/ml H_2O)	518	480	411	388	356
K^+ (μ-equiv/leaf)	155	106	87	93	70
K^+ (μ-equiv/ml H_2O)	191	157	130	129	106
Mg^{++} (μ-equiv/leaf)	108	330	440	590	530
Mg^{++} (μ-equiv/ml H_2O)	133	488	659	819	802
Cl^- (μ-equiv/leaf)	415	290	270	361	386
Cl^- (μ-equiv/ml H_2O)	512	429	405	361	386

There is a paucity of literature data concerning elemental composition of mangroves. Most studies of which I am aware have not been published. Values given in Tables 16 and 17 were personal communications from F.B. Golley (Univeristy of Georgia, Athens), S. S. Sidhu (University of Western Ontario, London, Canada), and T. F. Hollister (U. S. Environmental Protection Agency, Gulf Breeze, Florida). The samples were collected in Panama (Golley), India (Sidhu), and Florida (Hollister).

There are wide differences in concentrations of each element between species. For example, *Avicennia* species contain relatively high concentrations of sodium and potassium in all organs. Also, the roots of *Laguncularia racemosa* contained very high concentrations of all elements, except magnesium.

Sidhu (personal communication) found no correlation between the mineral status of soils and the elemental content of 16 mangrove species. It is clear that research which relates species and habitat to elemental composition is needed.

Table 16. Concentrations of various elements in mangrove leaves (personal communications: + Siddhu, * Golley, # Hollister).

Species	Concentration, ppm dry weight									
	Ca	Co	Fe	K	Mg	Mn	Na	P	Si	Zn
Acanthus ilicifolius (+)	7800	-	-	8200	8600	-	30000	3200	30000	-
Aegiceras corniculatum (+)	6800	-	-	8400	10200	-	23100	2500	4600	-
Avicennia alba (+)	10400	-	-	12100	10000	-	35000	2300	4000	-
Avicennia marina (+)	11200	-	-	15200	13500	-	50600	2300	5000	-
Avicennia nitida (#)										
Overstory	12430	-	147	21000	3600	-	4590	-	-	154
Understory	6680	-	300	29400	2320	53	6500	-	-	24
Avicennia officinalis (#)	11600	-	-	19100	12200	-	23300	4000	20800	-
Bruguiera caryophylloides (+)	24800	-	-	6100	10000	-	27700	2400	17200	-
Ceriops candolleana (+)	20400	-	-	5300	11700	-	36600	2500	11000	-
Conocarpus erectus (#)										
Overstory	13350	22	305	4100	-	50	5500	-	-	160
Understory	7600	24	251	21600	-	88	4110	-	-	142
Derris ulginosa (+)	17600	-	-	25800	12400	-	12300	2600	18000	-
Exocaria agallocha (+)	14200	-	-	12900	14700	-	4800	4100	18800	-
Laguncularia racemosa (#)										
Overstory	6510	12	125	17700	4440	74	10520	-	-	92
Understory	7600	32	149	25000	4740	116	8620	-	-	84
Lumnitizera racemosa										
India (+)	24200	-	-	22400	15600	-	43100	2500	5800	-
Florida (#)	9100	-	169	19900	3940	-	9370	-	-	170
Rhizophora brevistyla (*)										
Overstory	12200	46	82	8400	4700	387	9800	9000	-	11
Understory	7800	56	672	8500	5000	125	8300	8000	-	15
Rhizophora mangle										
Hollister	10760	9	132	16400	4320	92	11130	-	-	146
Morton (1965)	13500	52	152	6500	8000	30	-	1400	-	43
Rhizophora mucronata (+)	19800	-	-	21600	14500	-	22800	3300	6000	-
Sonneratia apetala (+)	11200	-	-	3100	10500	-	14900	3100	4800	-

Table 17. Concentrations of various elements in organs, other than leaves, of mangroves (personal communications: * Golley, # Hollister).

Species	Concentration, ppm dry weight								
	Ca	Co	Cu	Fe	K	Mg	Mn	Na	Zn
Avicennia nitida (#)									
Overstory stems	7430	-	-	282	14700	3080	-	3730	227
Understory stems	7100	16	10	580	27800	3650	48	9340	28
Roots	3930	40	13	465	29200	3700	34	9000	121
Conocarpus erectus (#)									
Overstory stems	3930	15	13	329	4100	3400	47	412	180
Understory stems	5600	36	15	648	27300	2400	83	1260	112
Roots	21200	25	40	979	-	7160	75	3560	162
Laguncularia racemosa (#)									
Overstory stems	3100	18	9	49	10000	1880	92	4590	89
Understory stems	6850	16	-	516	9200	1220	46	4120	38
Fruit .	8590	-	15	332	35000	2920	123	4120	79
Roots of seedlings	19390	54	-	1000	81000	1990	-	14100	514
Lumnitizera racemosa (#)									
Stems	11000	25	10	99	13200	3405	-	618	25
Rhizophora brevistyla (*)									
Overstory stems	12900	52	7	36	3000	1000	168	5500	11
Understory stems	5700	83	8	1000	3900	2900	255	9500	12
Overstory fruits	5900	81	6	82	10100	2900	191	9600	11
Understory fruits	6900	56	4	45	7100	3400	164	9700	7
Rhizophora mangle (#)									
Overstory stems	8936	-	-	252	2200	2820	74	8370	120
Fruit (2-10 cm long)	2350	58	12	155	4000	3050	121	8890	2
Fruit (11-20 cm long)	1260	-	15	113	2990	2460	69	10250	152
Roots of seedlings	600	36	12	422	13800	1070	71	12840	12
Prop roots	4850	-	11	253	13800	2540	-	10660	74
Rhizophora mucronata (#)									
Fruit	932	20	-	346	13860	2620	46	8620	6

Sokoloff et. al (1950) and Morton (1965) gave data from chemical analyses of leaves of *R. mangle* from Florida (Table 18.) Their values for vitamin content vary greatly but cannot be compared because methods of treatment of leaves and assay procedures were not given. Sokoloff et. al. suggested that leaf meal could replace alfalfa in chicken rations and Morton recommended its use as cattle feed.

Golley (1969) compared the caloric content of *R. brevistyla* in Panama with trees from tropical moist, premontane, and gallery forests. The energy values of the mangrove (Table 19) were generally greater than those of the other tropical trees.

Tannin is an important constituent of all parts of mangrove trees. Bowman (1921) showed that tannin is usually stored in solid masses in cells, but is frequently in solution in cytoplasm. He considered the tannin cells and water storage tissue of *R. mangle* to constitute a true hypodermis.

The function of tannin in mangrove is unknown. Tannins have a strong protein-binding capacity and, therefore, are able to inhibit enzymes. In the living plant it is possible that tannins aid in resistance to fungi, as fungi have been shown to occur in large numbers in mangals (Swart 1958; Kohlmeyer 1965, 1966, 1968a, b, 1969a, b; Kohlmeyer and Kohlmeyer 1971; Ahern et al. 1968; Rai et al. 1969, and Ulken 1970). Lee and Baker (1972a, b) identified 52 species of soil fungi from a Hawaiian swamp. Swain (1965) suggested that the presence of tannin causes resistance of dead organic matter to attack by fungi and other decomposers. Crossland (1903) stated that the Arabs of Zanzibar used mangrove wood for houses and furniture because it was not attacked by termites, and suggested that the high tannin content repelled the termites. The ability of tannins to inhibit enzymes probably affects the rate of decay of plant detritus and, therefore, is important in relationships within the detritus based food web. In most plants, hydrolyzable tannins are usually present in leaves and fruit, whereas condensed tannins occur in the bark or heartwood. This implies that leaves and fruit of mangroves are less persistant as particulate detritus than woody parts.

Most research on mangrove tannin has been done on samples of bark. Drabble (1908) illustrated distribution of tannin in *R. mangle* and *Laguncularia racemosa.* Trimble (1892) reported the empirical formula a $C_{25}H_{25}0_{11}$ for tannin from the bark of *R. mangle.* Baillaud (1912) found that 30% of the dry weight of bark from *Rhizophora* and *Bruguiera* in Africa was comprised of tannin. Dry bark of *Xylocarpus* contained 26% tannin. Dried bark of *R. mucronata* from Africa contained between 41.3 and 42.8% tannin (Anon. 1904). Brown and Fisher (1918) pointed out that tannin content varied greatly between species from the Indo-West-Pacific region. In Malaya, Buckley (1929) reported the following percentages of tannin in fresh bark: *R. Mucronata* 20.7-30.8, *R. congugata* 7.9-17.6, *B. gymnorhiza* 14.5-25.6, *B. eriopetala* 17.3-23.0, *B. caryophylloides* 15.8, *B. parviflora* 4.7-7.6, *C. candolleana* 19.0-30.8, *Carapa obovata* 29.8-41.6. The fruits and

Table 18. Chemical analysis of dry leaves of *R. mangle* from Florida (after Sokoloff et al. 1950 and Morton 1965).

Total protein, percent	12.1-14.3	7.5
Crude fiber, percent	13.9	13.9
Crude fat, percent	2.9	3.6
Calcium, percent	1.6	1.4
Sulphur, percent	0.6	–
Ash, percent	6.7	10.1
Iodine, percent	0.8	0.5
Manganese, mg/kg	0.3	0.03
Thiamin, mg/kg	1.56-2.03	130
Riboflavin, mg/kg	4.5-5.6	190
Folic acid, mg/kg	0.60-0.67	320
Niacin, mg/kg	20.3-28.0	2,400
Pantothenic acid, mg/kg	4.0-4.5	53

Table 19. Mean caloric values and standard errors of *R. brevistyla* from Panama (Golley 1969).

	Energy, g cal/g dry weight	
Compartment	Mean	SE
Canopy leaves	4182	22
Canopy stems	4337	11
Understory leaves	4299	132
Understory stems	4204	12
Canopy fruit	4298	29
Understory fruit	4360	20
Epiphytes	4585	11
Litter	4141	13
Roots	4034	48

leaves contained least tannin. Buckley concluded that *R. mucronata* was the best source of tannin because the yield of bark per tree was good. *Carapa obovata* contained the highest concentration of tannin, but its bark was thin and the yield per tree was small. Drabble and Nierenstein (1907) reported that older *R. mangle* trees contained more tannin than young trees.

Although mangroves are not used extensively as a source of dyes, when the bark of *R. mangle* is treated with copper or iron salt, brown, olive, rust, and slate-colored dyes are obtained (Fanshaws 1950, Morton 1965). According to Morton, a boiled concentrate of the bark may be used for staining wood in floors and furniture, dyes for textiles may be obtained from the roots, and a red dye from the shoots may be used for coloring leather.

Energy Relationships

Except for a few reports on yield of wood (see "silviculture" section of this report), little is known about production of organic matter in mangrove swamps. The first detailed study of photosynthesis, respiration, biomass, and export of organic matter was made by Golley et al. (1962) in a Puerto Rican *R. mangle* forest in May. Gross photosynthesis was 8.23 g C/m^2/day; total respiration was 9.16 g C/m^2/day. The greatest rates of photosynthesis (7.33 g C/m^2/dayO and respiration (4.31 g C/m^2/day) occurred in the upper canopy of leaves. Shaded leaves accounted for gross photo synthesis of only 0.40 g C/m^2/day and respiration of 0.48 g C/m^2/day. Seedling photosynthesis was 0.12 g C/m^2/day and respiration was 0.36 g C/m^2/day. At the soil surface, respiration by prop roots was 2.03 g C/m^2/day. At and below the soil surface respiration was 1.64 g C/m^2/day. Gross photosynthesis and respiration above ground was related to dry leaf biomass (1017 gm/m^2), leaf area (4.4 m^2/m^2), and chlorophyll *a* content of the leaves (1.19 g/m^2). The trees were approximately 8 m tall, and the factors measured attained their greatest values at between 4 and 6 m height. Unfortunately, the subterranean algal flora was not studied. This might have been important as Marathe (1965) showed 12 algal species in the soil of mangals near Bombay. Another source of primary production was algae on the roots and mud. Dawson (1954) described many attached algal species from roots and mud in Vietnam. Golley et al. stated the *R. mangle* community was more fertile than most marine and terrestrial communities. It was not, however, as efficient as the montane rain forest or coral reefs of Puerto Rico in conversion of sunlight into organic matter under similar light regimes.

Miller (1972), using a model, calculated gross photosynthesis, net photosynthesis, and respiration of *R. mangle* in Florida (Table 8). He contrasted these data with those of Golley et al. (1962). Using Miller's model, the estimates of Golley et al. corresponded to 9.4 g organic matter/m^2/day for gross photosynthesis, 3.4g organic matter/m^2/day for net photosynthesis, and 5.9 g organic matter/m^2/day for respiration. Miller ascribed differences between his data and Golley's to different leaf areas.

Miller's model predicted that maximum photosynthesis occurrs at a leaf area index of approximately 2.5 if no acclimation to shade within the canopy

Table 8. Gross primary production (P_g), net primary production (P_n), and respiration (R) of R. mangle leaves in Florida (after Miller 1972). Data are expressed as grams organic matter/m^2/day.

Height (m)	Sunny			Cloudy		
	P_g	P_n	R	P_g	P_n	R
			June			
1.75-2.00	0.08	0.03	0.05	0.07	0.03	0.04
1.50-1.75	0.31	0.12	0.19	0.29	0.12	0.17
1.25-1.50	2.17	0.93	1.24	2.02	0.92	1.09
1.00-1.25	30.8	1.48	1.61	2.59	1.13	1.46
0.75-1.00	3.39	1.64	1.75	2.48	0.84	1.65
0.50-0.75	2.16	0.87	1.33	1.61	0.35	1.26
0.25-0.50	0.97	0.31	0.66	0.73	0.10	0.63
0.00-0.25	0.67	0.17	0.51	0.51	0.02	0.49
Total	12.83	5.55	7.34	10.30	3.51	6.79
			January			
1.75-2.00	0.09	0.05	0.03	0.09	0.05	0.03
1.50-1.75	0.34	0.21	0.13	0.34	0.21	0.12
1.25-1.50	2.14	1.32	0.82	2.08	1.28	0.80
1.00-1.25	2.43	1.33	1.10	2.52	1.45	1.07
0.75-1.00	2.15	0.91	1.24	2.41	1.19	1.21
0.50-0.75	1.31	0.36	0.95	1.55	0.62	0.93
0.25-0.50	0.57	0.10	0.47	0.70	0.23	0.46
0.00-0.25	0.39	0.03	0.37	0.49	0.13	0.36
Total	9.42	4.31	5.10	10.18	5.16	4.98

is present and predicted that production decreased with increase in leaf area index and leaf width. Also, the environmental variables with the greatest influence on primary production were air temperature and humidity. Increase in solar radiation up to a point, increased primary production as did increasing amounts of diffuse energy. Infrared variation decreased production.

Gill and Tomlinson (1971) reviewed phenological phenomena associated with growth of *R. mangle* in Florida. Although the general progression of development appeared to be mediated endogenously, climatic factors were a strong governing influence. Environmental control of growth was through effects on development of the apical bud. Vegetative branches, inflorescences, and axillary buds are developed within the apical bud of *R. mangle.* The rates of leaf expansion and fall were highest in the summer when temperature and radiation were maximal. Throughout the year, leaf fall was closely correlated with leaf expansion so there was a fairly constant number of leaves on a shoot. Flower buds appeared in greatest abundance between May and July, open flowers between June and September, and fruit between September and March. The hypocotyl appeared in March and greatest fall of propagules was between June and October.

In some swamps, phytoplankton in the water contribute appreciably to synthesis of organic matter. This was the subject of extensive studies by Teixeira and Kutner (1962), Teixeira et al. (1965, 1967, 1969), Watanable and Kutner (1965), Tundusi and Tundusi (1968), and Tundusi and Teixeira (1964, 1968) in Brazil. Tundusi (1969) summarized the work in a Brazilian mangal. Gross primary production of surface water ranged between 2.10 and 91.3 mg $C/m^3/hr$. Respiration values were between 1.0 and 21.3 mg $C/m^3/hr$. Nannophytoplankton (size range 5-65 u) accounted for 61.8% of the total carbon uptake. Diatoms were the numerically dominant unicells, a phenomenon also reported by Mattox (1949) in Puerto Rico, Walsh (1967) in Hawaii, and Bacon (1971) in Trinidad.

An important finding in the work of Golley et al. (1962) was that tidal export of particulate matter was 1.1 g $C/m^2/day$. Heald and Odum (1971) reported production, consumption, and export of organic detritus in a *R. mangle* stand in southern Florida. Heald and Odum pointed out that many commercially important finfish and shellfish live in the mangrove environment and that vascular plant detritus is the primary source of food for many estuarine organisms.

Heald (1971) estimated that production of mangrove debris averaged 2.4 g $C/m^2/day$, oven dry weight. This was equivalent to almost nine tons/ha/yr. Annually, plants other than mangrove accounted for less than 15% of the total organic debris. Rate of degredation of mangrove detritus was related to conditions of the environment. Breakdown was most rapid in brackish water. The amphipods *Melita nitida* Smith and *Corophium lacustre* and the crab *Rithropanopeus harrisii* Gould were important consumers of detritus in brackish water.

The actively photosynthesizing leaf of *R. mangle* was reported by Heald to contain 6.1% protein, 1.2% fat, 67.8% carbohydrate, 15.7% crude fiber, and 9.2% ash. During abscission, protein and carbohydrate contents were 3.1 and 59.6%, respectively. After falling into brackish water, the carbohydrate content of leaf detritus,fell to approximately 36%, but protein content rose to approximately 22%. Heald speculated that increase in protein was due to growth of bacteria and fungi on the detritus particles, and stated that the food value of detritus, which was in the water for one year, was more nutritious than that in the water for one or two months.

Odum (1971) studied the use of mangrove detritus as food by many animal species. He concluded that vascular plant detritus which originated from *R. mangle* leaves was the main source of food for the aquatic animal community. As the detritus decomposed into finer particles, it became covered with bacteria, fungi, and protozoans. The caloric content rose from 4.742 Kcal/g in ash-free fresh leaves to 5.302 Kcal/g in ash-free remains of leaves which were submerged for two months.

There were at least four pathways by which mangrove leaves were utilized by heterotrophs: (1) dissolved organic substances from the leaves → microorganisms → higher consumers, (2) dissolved organic substances → sorption on sediment and aged detritus particles → higher consumers, (3) particulate leaf material → higher consumers, and (4) particulate leaf material →→ bacteria, fungi, and protozoans → higher consumers. Odum believed the last pathway to be the most important. He speculated that microorganisms convert compunds such as cellulose and lignin into digestible protein utilized by invertebrates and fishes.

SILVICULTURE

Mangrove is one of the most important sources of timber, fuel, posts, poles, railroad ties, and tannin in the tropics. It also has resins which are used as plywood adhesives, and the bark, leaves, shoots, and roots contain dyes. Chatterjee (1958) gave the following uses for mangrove in India: *Heritiera* (boat building, planking, fuel), *Amoora cuculata* (wooden pipes for hookahs and wooden toys), *Aegiatilis rotundiflora* (extraction of high-grade salt after burning), *Avicennia* (fuel wood for brick burning), *Exocoecaria agallocha* (match boxes), *Xylocarpus granatum* (pencils), and *Salicornia brachiata* (source of sodium carbonate).

Because of their many uses, silviculture of mangroves has been practiced for many years in southeastern Asia. Banerji (1958) reported that *R. mucronata* and *B. gymnorhiza* were grown successfully on a plantation of 685 acres in the Andaman Islands between 1898 and 1908. Banerji stated that *B. gymnorhiza* was an excellent source of poles for transmission lines and that *Rhizophora* produced 30 cords of fuel wood per acre, whereas *Bruguiera* produced 11 cords. The annual yield of firewood was estimated at 130,000 tons.

In the Andamans, clearfelling and planting of *B. gymnorhiza* was the best silviculture method. Because this species grows slowly, a rotation to 100 years was recommended for an exploitable breast-height girth of 27 inches. On some plantations, *Bruguiera* attains a height of 30-35 feet (9.2-11.7 m) and a girth of 9-12 inches (23-30 cm) in 15 years. The tree provides one of the strongest timbers in India, and has a durability life of 10 years after treatment with creosote.

In Thailand, Walker (1937, 1939) reported that *R. conjugata* and *R. mucronata* were the mangrove of choice for poles and firewood and have been used on a large scale for planting. There was an abundance of seedlings at all times of the year. Seedlings of *R. mucronata* were less susceptible to attack by crabs, and their long radicle was an advantage in competition with growth of *A. aureum* and *B. parvifloraa.* Seedlings were deep-planted at six-foot (1.8 m) intervals and the maximum felling girth was eight inches (20 cm). The prescribed time for planting was two years after felling. Unfortunately, Walker did not give production figures for the Thai mangroves. He did point out that *B. gymnorhiza* was used for fuel, *S. griffithii* for fishing stakes, and *C. candolleana* as fuel and tanbark.

Becking et al. (1922) divided the mangrove of southeastern Asia into three classes based upon the diameters of mature trees: Class A, less than 20 cm, *A. corniculatum,, Scyphiphora hydrophyllacease* Gaert., *Ceriops* spp.; Class B, 20-40 cm, *A. marinaa, Lumnitzera racemosa, Bruguiera* spp.; Class C, greater than 40 cm., *A. officinalis, S. alba, Rhizophora* spp., *Bruguiera* spp., *Xylocarpus* spp. The authors showed that production of wood per unit area by trees of larger diameter was greatest. Although *Sonneratia* was one of the more productive genera, Backer and van Steenis (1954) stated that in Malaya its economic usage was small. Small amounts were used for fuel and in boats and houses. The young berries of *Sonneratia* can be consumed by humans and pectin can be extracted from them.

According to Banijbatana (1958), approximately 133,400 ha of mangrove forests were available for silviculture in Thailand. The shelterwood system was judged best, and for young forest with trees of 20 cm girth and under, clearing and thinning was recommended. For forests in which the majority of trees were 20-50 cm, heavier thinning was recommended with seedling felling for control of *C. roxburghiana* and *B. cylindricaca.* The *Rhizophoras* reached 65-70 cm in girth between the ages of 39 to 43 years and a rotation system of 40 years was adopted. Yield was calculated at 50-60 cm per acre.

Approximate rates of growth in girth of several mangrove species in Malaya were given by Durant (1941 (Table 22). The volume of wood per acre of mixed *Rhizophora* forest increased from 1,375 cubic feet at 10 years to 5,600 cubic feet at 50 years. Durant recommended harvesting at 22-23 years when the mean annual volume increment was at its maximum of 147.7. The volume of wood at 22 years was 3,250 cubic feet per acre.

Noakes (1955) stated that the total area of mangrove forest in Malaya was approximately 760 square miles. Of this, 460 square miles were under

Table 22. Growth in girth of several mangrove species in Malaya (Durant 1941)

Age Years	Girth, inches R. conjugata	R. mucronata	B. gymnorhiza	B. hainesii	B. parviflora	B. caryophylloides	C. candolleana
5	2	5	3	2	2	1	2
10	6	10	7	5	5	5	5
15	9	13	9	8	8	7	8
20	12	16	12	11	11	9	10
25	16	18	15	14	13	11	13
30	19	20	18	17	16	13	15
35	21	22	20	19	18	15	18
40	24	24	23	21	20	17	20
45	27	26	25	24	23	18	22
50	29	28	28	27	25	20	24
55	32	30	30	30	27	22	
60	35	32	32	33	29	24	
65	37	34	35	36		26	
70	39	36	37	40		27	
75	41		39	45		29	
80	42		41			30	

sustained yield management. *Rhizophora mucronata* and *R. conjugata* were the most important Malayan species, covering two-thirds or more of the total area. Fuel was the main product of the mangrove forest, but poles for houses and fish traps were also important.

According to Noakes, fruiting of *Rhizophora* occurred at the age of four years, was annual and highly prolific. Full stocking by water-borne seedlings occurred rapidly after clearcutting. As in Thailand, *Rhizophora* grew slowly. The annual growth increment of boles was slightly greater than one inch in the early stages of growth to just over one-half inch above 12 inches diameter. The trees grew to a maximum height of 70-120 feet. Normal felling size was 50-60 feet in height and 1.5-2.5 feet in girth. Felling size was achieved in 20-30 years. The mean annual volume increment of mixed *Rhizophora* forest culminated at approximately 25 years, allowing for a three-year regeneration period. The yield was approximately 3,106 cubic feet of wood per acre.

Wadsworth (1959) reported silviculture of *Laguncularia racemosa* in Puerto Rico. Undisturbed stands 22 years old attained an average diameter at breast height of 5.0 inches and 2,680 cubic feet of wood per acre. Natural regeneration by water-borne seedlings occurred within two years after clearfelling. Holdridge (1940) recommended a cutting cycle of five years and a rotation cycle of 25 years for exploitation of mangroves in Puerto Rico.

Golley et al. (1962) reported that in a *R. mangle* forest in Puerto Rico, annual production of wood was 0.84 g/m^2/day (0.42 g C/m^2/day). This was much less than that reported by Noakes in Malaya (14 g C/m^2/day).

It should be pointed out here that in some areas, silviculture of mangrove is practiced in the belief that the wood is resitant to marine boring organisms. Southwell and Boltman (1971) tested resistance to marine borers by *R. mangle*, *R. brevistyla*, *A. marina*, *C. erectus*, and *Laguncularia racemosa*. Only *C. erectus* showed natural resistance to teredo, pholad, and limnorid borers. The *Rhizophora* species were almost completely destroyed after immersion in Pacific Ocean water for 14 months.

HERBICIDES

Herbicides have been used for almost 20 years for control of mangrove. In Africa (Sierra Leone), Ivens (1957) reported that application of the auxin-type herbicides 2, 4-dichlorophenoxyacetic acid (2, 4-D), 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T), and 2-methyl, 4-chlorophenoxy-acetic acid (MCPA) were effective in eradication of both *R. racemosa* and *A. nitida* when applied to the bases of trunks at concentrations of 4-20% in diesel oil. Frilled *R. racemosa* were killed by the butyl ester of 2, 4-D at concentrations between 0.5 and 1.0%, whereas 4% was required to kill unfrilled trees. *Avicennia nitida* was slightly more resistant to 2, 4-D than *R. racemosa*, concentrations between 2 and 4% being required to kill frilled trees. 2, 4, 5-T and MCPA were not as effective as 2, 4-D. Recovery of trees after treatment with 2, 4, 5-T was reported.

The first signs of herbicidal effect were noted approximately three weeks after application, when the leaves turned yellow. Extensive defoliation occurred by seven months after treatment, at which time many trees of both genera were dead. Seedlings and young trees were more resistant than old trees.

Ivens also reported that 3-(4-chlorophenyl)-1, 1-dimethylurea (CMU) killed all trees when applied to the pneumatophores of *A. nitida* at the rate of 20 lb/acre. Dalapon (2, 2-dichloropropionic acid) caused complete kill with no regrowth at 40 and 80 lb/acre. There was a small amount of regrowth after application of dalopon at 20 lb/acre.

Truman (1961) reported total kill of treated *A. marina* in Australia by 1% 2, 4-D applied to the basal bark. Only 54% were killed by treatment with 1% 2, 4, 5-T. Spotted gum (*Eucalyptus maculata* Hook.), an upland tree, was only slightly affected by the same treatment. Truman concluded that *A. Marinna* was very susceptible to auxin-type herbicides.

The concept of high susceptibility of mangrove to auxin-type herbicides was extended by Tschirley (1969), Orians and Pfeiffer (1970), and Westing (1971a, b), who stated that mangrove forests in Vietnam were destroyed after a single application of 6.72 kg/ha of the triisopropanolamine salt of 2, 4-D in combination with 0.61 kg/ha of the triisopropanolamine salt of 4-amino-3, 5, 6-trichlorophcolinic acid (picloram). The forests were composed mainly of *S.*

alba, *B. parviflora*, *B. gymnorhiza*, *A. marina*, *A. intermedia*, *R. conjugata*, *C. candolleana*, and *N. fruiticans*. Westing (1971a) reported that treated areas in Vietnam remained uncolonized by mangrove six years after treatment. Westing (1971c) also published a list of references to effects of herbicides in Vietnam. For reviews of herbicidal effects in Vietnam, see Boffey (1971) and Aaronson (1971).

Westing (1971a) pointed out that application of 2, 4-D in combination with picloram both defoliated and killed nearly all trees in the sprayed areas. He also stated that herbicides seemed to prevent recolonization by mangrove, although he observed rapid recolonization of an area cleared by cutting. It is true, however, that a large portion of the denuded mud flats of Vietnam are only occasionally inundated by tidal water and that sufficient numbers of seedlings for regeneration are not carried in. Another possible reason for lack of recolonization may be related to texture of the denuded soil. Natural regeneration of mangrove is greatly retarded when soil becomes too stiff or hardens after exposure to the sun (Banijbatana 1958).

We (Walsh et al., in press) have studied affects of Tordon® 101 on seedlings of *R. mangle* from Florida. Tordon 101 is a mixture of the triisopropanolamine salts of 2, 4-D (39.6%) and picloram (14.3%). Seedlings that had no leaves and one or two pairs of leaves were treated with 1.12, 11.2, and 112.0 kg/ha (1, 10, and 100 lb/acre). These rates were equivalent to 0.44, 4.40, and 44.0 kg/ha 2, 4-D, and 0.16, and 16.0 kg/ha picloram. A combination of 0.44 kg/ha 2, 4-D and 0.16 kg/ha picloram caused stunted growth of seedlings without leaves, but had no permanent effects upon seedlings with one or two pairs of leaves. Higher concentrations caused death of all treated seedlings by 50 days after treatment.

We were never able to quantify tissue residues in seedlings without leaves that had been treated at the lowest concentrations. The limits of quantification were 0.02 ppm (parts per million) 2, 4-D and 0.01 ppm picloram. Even though tissue residues were very low, seedling development was greatly inhibited. In seedlings with leaves, greatest herbicidal residues occurred in the highest leaves and hypocotyl. Table 20 shows distribution of 2, 4-D and picolinic acid in the organs of seedlings treated when two pairs of leaves were present.

At the tissue level, symptoms of herbicide poisoning were desiccation of leaves, plugging of vessel elements, and destruction of root cortex. Root destruction probably impared the ability of seedlings to regulate salt and water balance. For example, concentrations of sodium and potassium in seedlings were directly related to application rate and time (Table 21). No changes were found in concentrations of magnesium, manganese, calcium, iron, or zinc. Strogonov (1964) said that symptoms of salt poisoning in plants include bleaching of chlorophyll accompanied by browning of the leaves. Both symptoms were observed in our treated seedlings.

Table 20. Concentrations of 2,4-D and picolinic acid, in parts per million (± 20%), of wet tissue in organs of R. mangle seedlings treated with Tordon 101 when two pairs of leaves were present. Residues were detected in every analysis of seedlings treated with 1.12 kg/ha but were below quantifiable levels (0.01 for picolinic acid, 0.02 for 2,4-D) (Walsh et al. in press).

Treatment kg/ha	Day	Roots		Hypocotyl		Stem		1st leaves		2nd leaves	
		2,4-D	PA	2,4-D	PA	2,4-D	PA	2,4-D	PA	2,4-D	PA
11.2	30	0.02	0.01	0.10	0.03	0.02	0.01	0.02	0.01	0.13	0.06
	40	0.02	0.01	0.23	0.10	0.23	0.10	0.29	0.10	0.35	0.10
112.0	10	1.23	0.39	1.68	0.49	1.02	0.43	0.63	0.24	0.87	0.41

Table 21. Concentrations of sodium and potassium in the stems of *R. mangle* seedlings treated with Tordon 101 when two pairs of leaves were present; 50 days after treatment (Walsh, unpubl.)

Treatment	ppm Dry Weight	
	Na	K
Control	37,500	4,375
1.12 kg/ha	49,800	4,821
11.2 kg/ha	64,900	5,295
112.0 kg/ha	96,200	6,321

Acknowledgements

I thank Drs. Frank B. Golley and S. S. Sidhu and Mr. Terry A. Hollister for data on elemental composition. Special thanks are given to Mrs. Ann Valmus, Librarian at the Gulf Breeze Laboratory, for aid in obtaining copies of many publications and for her patience in checking the references. The quotation from "The Night Country" was made with permission of Charles Scribner's Sons, Publishers, New York. Thanks are also given to Mrs. Steven Foss for making the illustrations.

® Registered trademark, Dow Chemical Co., Midland, Michigan. Reference to trade names in this publication does not constitute endorsement by the Environmental Protection Agency.

REFERENCES

Aaronson, T. 1971. A tour of Vietnam. Environment 13: 34-43.

Abe, N. 1937. Ecological survey of Iwayama Bay, Palao. Palao Trop. Biol. Stn. Stud. (Tokyo) 1: 217-324.

Abe, N. 1942. Ecological observations on *Melaraphe (Littorinopsis) scabra* (Linnaeus) inhabiting the mangrove tree. Palao Trop. Biol. stn. Stud. (Tokyo) 2: 391-435.

Abel, O. 1926. Amerikafahrt: Eindrucke, Beobachtungen und Studien eines Naturforschers auf einer Reise nach Nordamerika und Westindien. Gustav Fischer, Jena, 462 pp.

Abel, O. 1927. Fossile Mangrovesumpfe. Palaont. Z. 8: 130-140.

Abeywickrama, B. A. 1964. The estuarine vegetation of Ceylon. *in* Les problemes scientifiques des deltas de la zone tropicale humide et leurs implications. Colloque de Dacca. UNESCO, Paris, pp. 207-209.

Abreu, S. F. 1926. Notes on the tanning materials of Brazil. Am. Leather Chem. Assoc. J. 21: 357-358.

Acosta-Solis, M. 1947. Commercial possibilities of the forests of Ecuador – mainly Esmeraldas Province. Trop. Woods Yale Univ. Sch. For. 89: 1-47.

Acosta Solis, M. 1959. Los manglares del Equador. Contrnes Inst. Ecuat. Cienc. Nat. No. 29, 82 pp.

Acuna, R. A. 1953. Mangrove swamps. For. Leaves 6: 35-41.

Adriani, M. J. 1937. Sur la transpiration de quelques halophytes cultivies dans les milieux different en comparison avec celle de quelques nonhalophytes. Proc. K. ned. Akad. Wet. 40: 524-529.

Ahmad, N. 1967. Seasonal changes and availability of phosphorus in swamp-rice soils of north Trinidad. Trop. Agric. Trinidad 44: 21-32.

Alexander, T. R. 1967. Effect of hurricane Betsy on the southeastern Everglades. Q. J. Fla. Acad. Sci. 30: 10-24.

Almodovar, L. R. and R. Biebl. 1962. Osmotic resistance of mangrove algae around La Parguera, Puerto Rico. Rev. Algol. 3: 203-208.

Almodovar, L. R. and F. Pagan. Notes on a mangrove lagoon and mangrove channels at La Parguera, Puerto Rico. Nova Hedwigia *in press*.

Alston, A. H. G. 1925. Revision of the genus *Cassipourea.* Kew Bull. 6: 241-276.

Altrock, A. von. 1897. Uber Mangrove-Nutzung in St. Catharina Brasilien. Tropenpflanzer 1: 263.

Altevogt, R. 1955. Beobachten und Untersuchungen an Indischen Winkerkrabben. Z. Morph. Oekol. Tiere. 43: 501.

Altevogt, R. 1957. Untersuchungen zur Biologie und Physiologie Indischer Winkerkrabben. Z. Morph. Oekol. Tiere 46:1.

Anderson, J. A. R. 1964. The structure and development of the peat swamps of Sarawak and Brunei. Malay. J. Trop. Geogr. 18: 7-16.

Anderson, J. A. R. 1964. Observations on climatic changes in peat swamp forests in Sarawak. Comm. For. Rev. 43: 145-158.

Anon. 1904. Mangrove barks, and leather tanned with these barks, from Pemba and Zanzibar. Bull. Imper. Instit., London, Sept., pp. 163-166.
Anon. 1951. Three hundred homes blackened by mangrove root gas. Miami Herald, Nov. 14.
Anon. 1957. Establishment of mangroves. Rep. For. Admin., Malaya, No. 34.
Anon. 1958. Poisoning of *Exoecaria agallocha*. Rep. For. Dept., Fiji, No. 7.
Anon. 1961. Mangrove gas kills two men in Fort Lauderdale ditch. Miami News, July 28.
Anon. 1965. New fruit-fly found in mangroves. Aust. Nat. Hist. 15: 60.
Arbelaez, E. P. 1956. Plantas utiles de Colombia. Libreria Colombiana, Bogota, pp. 642-643.
Arenes, J. 1954. Rhizophoraceae Madagascarienses nova. Not. Syst. 15: 1-4.
Areschoug, F. W. C. 1902a. Untersuchungen uber den Blattbau der Mangrovepflanzen. Bibl. Bot. 56: 1-90.
Areschoug, F. W. C. 1902b. Om bladbyggnaden hos Mangrove-vaxterna. Bot. Notiser, 1902, pp. 129-140.
Areschoug, F. W. C. 1904. Zur Frage der Salzausscheidung der Mangrovepflanzen und anderer mit ihnen zusammen wachsender Strandpflanzen. Flora, Marburg 93: 155-160.
Argo, V. N. 1953. Root growth claims soil from the sea: mangrove (*Rhizophora* and *Avicennia*) spreads by unique adaptation. Nat. Hist. 72: 52-55.
Armstrong, P. W. 1954. Shorelines and coasts of the Gulf of Mexico. U. S. Dept. Int. Fish. Bull. 89: 39-65.
Arnott, G. W. 1838. On the Rhizophorae. Ann. Nat. Hist. 1: 359-374.
Artz, T. 1936. Die Kutikula einiger Afrikanischer Mangrove Pflanzen. Ber. Dtsch. bot. Ges. 54: 247-260.
Ascherson, P. 1903. Der nordlichste Fundort der Mangrove in Agypten. Bot. Z. 61, Abt. S, pp. 235-238.
Asenjo, C. F. and J. A. Goyco. 1942. El aceite del almendro tropical. Puerto Rico Dept. Agr. Com. Bol. Mens. 1: 5-7.
Ashby, W. C. and N. C. Beadle. 1957. Studies in Halophytes, III. Salinity factors in the growth of Australian saltbushes. Ecology 38: 344-352.
Ashton, P. S. 1969. Speciation among tropical forest trees: some deductions in the light of recent evidence. Biol. J. Linn. Soc. London 1: 155-196.
Asprey, G. F. 1959. Vegetation in the Caribbean area. Caribb. Q. 5: 245-263.
Asprey, G. F. and R. G. Robbins. 1953. The vegetation of Jamaica. Ecol. Monogr. 23: 359-412.
Atkinson, M. R., G. P. Findlay, A. B. Hope, M. G. Pitman, H. D. W. Saddler, and K. R. West. 1967. Salt regulation in the mangroves *Rhizophora mucronata* Lam. and *Aegialitis annulata* R. Br. Aust. J. Biol. Sci. 589-599.
Aubert, G. 1954. Les sols hydromorphes d'Afrique Occidental Francaise. Trans. 5th Int. Congr. Soil Sci., pp. 447-450.
Aubreville, A. 1964. Problems de la mangrove d'hier et d'aujourd'hui. Addisonia 4: 19-23.

Ayers, A. D. 1951. Seed germination as affected by soil moisture and salinity. Agron. J. 44: 82-84.

Axelrod, D. I. 1952. A theory of angiosperm evolution. Evolution 6: 29-60.

Backer, G. A. and C. G. G. J. van Steenis. 1951. Sonneratiaceae. Fl. Males. 1: 280-289.

Bacon, P. R. 1967. Life in the estuaries of the Caroni Swamp. J. Biol. Univ. West Indies, Trinidad 2: 10-13.

Bacon, P. R. 1970. The ecology of Caroni Swamp, Trinidad. Spec. Publ. Central Statistical Off., Trinidad, 68 pp.

Bacon, P. R. 1971c. Plankton studies in a Caribbean estuarine environment. Caribb. J. Sci. ½: 81-89.

Bacon, P. R. 1971b. The maintenance of a resident population of *Balanus eburneus* (Gould) in relation to salinity fluctuations in a Trinidad mangrove swamp. J. Exp. Mar. Biol. Ecol. 6: 189-198.

Bacon, P. R. 1971C. Studies on the biology and cultivation of the mangrove oyster in Trinidad with notes on other shellfish resources. Trop. Sci. 12: 265-278.

Baillaud, E. 1912. La situation et la produktion des matieres tannates tripicals. J. d'Agric. Trop. 12: 105-107.

Baillon, H. 1875. Combretacees. Hist. Pl. 6: 260-283.

Baillon, H. 1875. Rhizophoracees. Hist. Pl. 6: 284-304.

Baker, R. T. 1915. The australian grey mangrove. J. R. Soc. N.S.W. 49: 257.

Bakhuizen van der Brink, R. C. 1921. Revisio generis Avicenniae. Bull. Jard. bot. Buitenz., Ser. 3, 3: 199-226.

Baltzer, F. 1969. Les formations vegetales associees au delta de la Dumbea (Nouvelle Caledonie). Cah. ORSTOM, Ser. Geol. 1, 1: 59-84.

Banerji, J. 1958a. The mangrove forests of the Andamans. Trop. Silvic. 20: 319-324.

Banerji, J. 1958b. The mangrove forests of the Andamans. World For. Congr. 3: 425-430.

Banijbatana, D. 1957. Mangrove forest in Thailand. Proc. 9th Pac. Sci. Congr., Bangkok, pp. 22-34.

Barbour, M. G. 1970. Is any angiosperm an obligate halophyte? Am. Midl. Nat. 84: 105-120.

Barbour, W. R. 1942. Forest types of tropical America. Carrib. For. 3: 137-150.

Barnard, R. C. and G. G. K. Setten. 1947. Investigation scheme for growth and increment studies in Jahore mangrove forest. Malay. For. 16: 40.

Barroweliff, M. 1912. Chemical notes on some malayan economic products. Malay. Agric. J. 1: 178.

Barry, J. P., L. C. Kiet, and V. V. Cuong. 1961. La vegetation des plages vasosablonneuses de la presquile de Cam Ranli. Ann. Fac. Sci. 1961, pp. 129-140.

Bascope, F., A. L. Bernardi, R. N. Jorgenson, K. Huek, H. Lamprecht, and P.

Martinez. 1959. Descripciones de arboles forestales No. 5. Los manglares en America. Inst. for. lat. Invest. Capacit. Merida 5: 1-53.
Baxter, P. H. 1968. Vegetation notes on Fraser Island. Queensland Nat. 19: 11-20.
Baylis, G. T. S. 1940. Leaf anatomy of the New Zealand mangrove. Trans. R. Soc. New Zealand 70: 164-170.
Beadle, N. C. W. 1952. Studies in halophytes. I. The germination and establishment of the seedlings of five species of *Atriplex* in Australia. Ecology 33: 49-62.
Beard, J. S. 1944. The natural vegetation of the island of Tabago, British West Indies. Ecol. Monogr. 14: 135-163.
Beard, J. S. 1944. Climax vegetation in tropical America. Ecology 25: 127-158.
Beard, J. S. 1946. The Natural Vegitation of Trinidad. Clarendon Press, Oxford, 152 pp.
Beard, J. S. 1955. The classification of tropical American vegetation types. Ecology 36: 89-100.
Beard, J. S. 1967a. An inland occurrence of mangrove. West Austr. Nat. 10: 112-115.
Beard, J. S. 1967b. Some vegetation types of tropical Australia in relation to those of Africa and America. J. Ecol. 55: 271-290.
Becking, J. H., L. G. den Berger, and H. W. Meindersma. 1922. Vloed-of mangrovebosschen in Ned.-Indie. Tectona. 15: 561-611.
Behrens Motta, A. 1960. Estudios preliminares para la produccion de extracto curtiente de corteza de mangle (*Rhizophora mangle*). La Escuela de Farmacia 20: 28-32.
Belcher, C. and G. P. Smooker. 1934. Birds of the colony of Trinidad and Tobago. Ibis 4: 572-595.
Benecke, W. and A. Arnold. 1931. Kulturversuche mit Keimlingen von Mangrovepflanzen. Planta (Berl.) 14: 471-481.
Bennett, H. and R. D. Coveney. 1959. Mangrove bark from Sarawak. Trop. Sci. 1: 116-130.
Benson, A. A. and M. R. Atkinson. 1967. Choline sulphate and phosphate in salt secreting plants. Fed. Proc. 26: 394.
Bentham, G. 1859. Synopsis of Legnotideae, a tribe of Rhizophoraceae. Proc. Linnean Soc. Bot. 3: 65-80.
Bentham, G. and J. D. Hooker. 1865a. Rhizophorae. Gen. Pl. 1: 677-683.
Bentham, G. and J. D. Hooker. 1865b. Combretaceae. Gen. Pl. 1: 683-690.
Bergman, H. F. 1954. Oxygen deficiency as a cause of disease in plants. Bot. Rev. 25: 417-485.
Bernstein, L. 1964. Salt tolerance of plants. U.S.D.A. Bull. 283. Govt. Print. Off., Washington, D. C.
Bernstein, L. and H. E. Hayward. 1958. Physiology of salt tolerance. Ann. Rev. Plant Physiol. 19: 25-46.
Berry, A. J. 1963. Faunal zonation in mangrove swamps. Bull. Singapore

Natl. Mus. 32: 90-98.
Berry, A. J. 1968. Fluctuations in the reproductive condition of *Cassidula auris felis*, a malayan mangrove ellobiid snail. (Pulmonata, Gastropoda). J. Zool. 154: 377-390.
Berry, A. J., S. C. Loong, and H. H. Thum. 1967. Genital systems of *Pythia*, *Cassidula* and *Auricula* (Ellobiidae, Pulmonata) from malayan mangrove swamps. Malacol. Soc. London Proc. 375: 325.
Berry, E. W. 1913. A fossil flower from the Eocene. Proc. U. S. Natl. Mus. 45: 261-263.
Berry, E. W. 1924. The Middle and Upper Eocene floras of southeastern North America. U. S. Geol. Surv. Prof. Pap. 92: 85-87 and 189-190.
Berry, E. W. 1930. Revision of the Lower Eocene Wilcox Flora of the southeastern states. U. S. Geol. Surv. Prof. Pap. 156: 1-196.
Berry E. W. 1936. Miocene plants from Colombia, South America. Bull. Torrey Bot. Club 63: 53-66.
Beschel, R. E. and P. J. Webber. 1962. Gradient analysis in swamp forests. Nature 194: 207-209.
Bews, J. W. 1916. The vegetation of Natal. Ann. Natal Mus. 2: 253-331.
Bews, J. W. 1920. The plant ecology of the coast belt of Natal. Ann. Natal Mus. 4: 368-469.
Bharucha, F. R. and B. S. Navalkar. 1942. Studies in the ecology of mangroves. 3. The chloride-content of sea-water, soil-solution and the leaf cell-sap of mangroves. J. Univ. Bombay 10: 97-106.
Bharucha, F. R. and V. S. Shirke. 1947. A study of the important katabolic changes in the seedlings of *Avicennia officinalis* Linn. J. Univ. Bombay 15: 1-14.
Biebl, R. 1962. Protoplasmatisch-okologische Untersuchungen an Mangrovealgen von Puerto Rico. Protoplasma 55: 572-606.
Biebl, R. 1964. Temperaturresistenz Tropischer Pflanzen auf Puerto Rico. Protoplasma 59: 133-156.
Biebl, R. and H. Kinzel. 1965. Blattbau und Salzhaushalt von *Laguncularia racemosa* (L) Gaertn. f. und anderer Mangrovenbaume anf Puerto Rico. Ost. bot. Z. 112: 56-93.
Bigarella, J. J. 1946. Contribuicao do estudo da planicie littornea do Estado do Parana. Agric. de Biol. Tecnol. 1: 75-111.
Birch, W. R. 1963. Observations on the littoral and coral vegetation of the Kenya coast. J. Ecol. 51: 603-615.
Biswas, K. 1927. Flora of the saltmarshes. J. Dept. Sci. Univ. Calcutta 8: 1-47.
Biswas, K. 1934. A comparative study of Indian species of *Avicennia*. Notes Bot. Gard. Edinburgh 18: 159-166.
Blake, S. T. 1940. The vegetation of Goat Island and Bird Island in Mareton Bay. Old. Nat. 11: 94-101.
Blake, S. T. 1968. The plants and plant communities of Fraser, Moreton, and Stradbroke Islands. Old Nat. 19: 23-30.

Blatter, E. 1905. The mangrove of Bombay presidency and its biology. J. Bombay Nat. Hist. Soc. 16: 644-656.

Blatter, E. 1908. On the flora of Cutch. I. J. Bombay Nat. Hist. Soc. 18: 756-777.

Blatter, E. 1909. On the flora of Cutch. II. Bombay Nat. Hist. Soc. 19: 157-176.

Blomquist, H. L. and L. R. Almodovar. 1961. The occurrence of *Gelidiella tenuissima* Feldm. et Hamel in Puerto Rico. Nova Hedwiga 111: 67-69.

Blum, G. 1941. Uber osmotische Untersuchungen in der Mangrove. Ber. Schweiz. bot. Ges. 51: 401-420.

Boaler, S. B. 1959. *Conocarpus lancifolius* Engler in Somaliland Protectorate. Empire For. Rev. 38: 371-379.

Boer, N. P. de, Th. van der Hammen, and T. A. Wijmstra. 1965. A palynological study on the age of some borehole smaples from the Amazonas delta area, N. W. Brazil. Geol. Mijnb. 44: 254-258.

Boergesen, F. 1909. Notes on the shore vegetation of the Danish West Indian Islands. Bot. Tidsskrift. 29: 201-259.

Boergesen, F. and O. Poulsen. 1900. La vegetation des Antilles danoises. Rev. gen. de Bot. 12: 99.

Boffey, P. M. 1971. Herbicides in Veitnam: AAAS study finds widespread devastation. Science 171: 43-47.

Bole, P. V. and F. R. Bharucha. 1954. Osmotic relations of the leaves of *Avicennia alba* Bl. J. Univ. Bombay 22: 50-54.

Bond, G. 1956. A feature of the root nodules of *Casuarina.* Nature 177: 192.

Booberg, G. 1933. Die malayische Strandflora-ein Revision der Schimperschen Artenliste. Bot. Jb. 66: 1-38.

Bonfils, P. and J. Faure. 1961. Etude des sols due Bao Bolon. Agron. Trop. (Maracay) 16: 127-147.

Boone, R. S., M. Chudnoff, and E. Goytia. 1969. Chemical control of Bostrichidae during air drying of fence posts. U. S. For. Ser. Res. Pap. IFT, No. 8: 1-8.

Boughey, A. S. 1957. Ecological studies of tropical coast-lines. I. The Gold Coast, West Africa. J. Ecol. 45: 665-687.

Boughey, A. S. 1963. Dating of tropical coastal regression (based on roots of mangrove (*Rhizophora mucronata*). Nature 200: 600.

Bouillene, R. 1930. Un voyage botanique dans le Bas-Amazone. In Une Mission Biologique Belge au Bresil 2: 13-185. Imprimerie Medicale et Scientifique, Bruxelles.

Bouillene, R. and F. Went. 1933. Reserches experimentales sur la neoformation des racines les plantules et les boutures de plantes superecures. Ann. Jard. Bot. Buitz. 43: 25.

Bournot, K. 1913. Gewinnung von Lapachol aus dem Kernholz von *Avicennia tomentosa.* Archiv der Pharm. 1913: 351-356.

Bower, C. A. and C. H. Wadleight. 1948. Growth and cationic accumulation by four species of plants as influenced by various levels of exchangeable

sodium. Soil Sci. Am. Proc. 13: 218-223.
Bowman, H. H. M. 1915. Report on botanical work at the Tortugas Laboratory for the season of 1915. Carnegie Inst. Wash. Yearb. No. 14, p. 200.
Bowman, H. H. M. 1916a. Report on botanical investigation at the Tortugas Laboratory, season 1916. Carnegie Inst. Wash. Yearb. No. 15, pp. 188-192.
Bowman, H. H. M. 1916b. Physiological studies on *Rhizophora.* Proc. Natl. Acad. U. S. 2: 685-688.
Bowman, H. H. M. 1917. Ecology and physiology of the Red Mangrove. Proc. Am. Philos. Soc. 56: 589-672.
Bowman, H. H. M. 1918. Botanical ecology of the Dry Tortugas. Carnegie Inst. Wash., Pub. No. 252, Pap. Dept. Mar. Biol., vol. 12, paper V, pp. 109-138.
Bowman, H. H. M. 1921. Histological variations in *Rhizophora mangle.* Pap. Michigan Acad. Sci. 22: 129-134.
Brandis, D. 1898. Combretaceae. Nat. Pflanzenfam. III. 7: 106-130.
Brass, L. J. 1938. Botanical results of the Archibold Expeditions. IX. Notes on the vegetaion of the Fly and Wassi Kussa Rivers, British New Guinea. J. Arnold Arb. 19: 175-190.
Breen, C. M. and B. J. Hill. 1969. A mass mortality of mangroves in the Kosi estuary. Trans. R. Soc. S. Africa 38: 285-303.
Breen, C. M. and I. D. Jones. 1969. Observations on the anatomy of foliar nodes of young *Bruguiera gymnorrhiza.* J. S. Afr. Bot. 35: 211-218.
Brelie, G. v. d. and M. Teichmuller. 1953. Beitrage zur Geologie El Salvadors. III. Mikroskopische Beobachtungen an Mangrove – Sedimenten aus El Salvador. Neues Jb. Geol. Paleontol., Mh., No. 6, pp. 244-251.
Brenner, W. 1902. Ueber die Luftwurzeln von *Avicennia tomentosa.* Ber. Dtsch. bot. Ges. 20: 175-189.
Briolle, C. E. 1969. Le cocotier sur les terres de mangrove au Cambodge: amenagement de plantations familiales. Oleagineaux 24: 545-549.
Broekhuysen, G. J. and H. Taylor. 1959. The ecology of South African Estuaries. Part viii: Kosi Bay system. Ann. S. Afr. Mus. 44: 279-296.
Bronnimann, P. and L. Zaninetti. 1965. Notes sur *Lituola salsa* (Cushman et Bronnimann 1948), un Foraminifere da la mangrove de l'ile de la Trinitie, W. I. Arch. Sci. 18: 608-615.
Brown, D. S. 1971. Ecology of Gastropoda in a south African mangrove swamp. Proc. Malacol. Soc. London. 39: 263-279.
Brown, J. M. A., H. A. Outred, and C. F. Hill. 1969. Respiratory metabolism in mangrove seedlings. Plant Physiol. 44: 287-294.
Brown, R. 1814. *In* Matthew Flinders' A Voyage to Terra Australis, Vol. II, Appendix No. III. G. and W. Nicol, London.
Brown, W. H. and A. F. Fischer. 1918. Philippine mangrove swamps. Philippines Dept. Agric. Nat. Resources Bull. No. 17, pp. 1-132.
Brown, W. H. and A. F. Fischer. 1920. Philippine mangrove swamps.

Philippines Bur. For. Bull. No. 22, pp. 9-125.
Browne, F. G. 1955. Forest trees of Sarawak and Brunei and their products. Sarawak Gov. Printer, Kuching.
Brownell, P. F. and J. G. Wood. 1957. Sodium, an essential micro-nutrient for *Atriplex vesicaria* Heward. Nature. 179: 635-636.
Brunnich, J. C. and F. Smith. 1911. Some Queensland mangrove barks and other tanning materials. Old Agric. 27: 86.
Buchanan, J. 1874. On the flowering plants and ferns of Chatham Islands. Trans. N. Z. Inst. 7: 333-341.
Buckley, T. A. 1929. Mangrove bark as a tanning material. Malay. For. Rec. 7, 40 pp.
Bunning, E. 1944. Botanische Beobachtungen in Sumatra. Flora 137: 334-344.
Burkill, I. H. 1935. A dictionary of the Economic products of the Malay Peninsula. 2 vols., 2402 pp. Publ. by Governments of the Straits Settlements and the Federated Malay States, London.
Burkholder, P. R. and L. M. Burkholder. 1958. Studies on B. vitamins in relation to productivity of the Bahia Fosforescente, Puerto Rico. Bull. Mar. Sci. Gulf Caribb. 8: 201-223.
Burkholder, P. R. and L. R. Almodovar. Species composition and productivity of algal mangrove communities in Puerto Rico. *In press Q.* J. Fla. Acad. Sci.
Burtt, Davy, J. 1938. The classification of woody vegetation types. Imperial For. Inst. Pap. No. 13, Oxford.
Calderon, S. E. and P. C. Stanley. 1941. Flora Salvadorena, 2nd. Ed., El Salvador.
Candolle, A. P. de. 1828a. Combretaceae, Prodr. Syst. Nat. 3: 9-24.
Candolle, A. P. de 1828b. Rhizophoraceae, Prodr. Syst. Nat. 3: 31-34.
Carey, G. 1934. Further investigations on the embryology of viviparous seeds. Proc. Linn. Soc. N. S. W. 59: 392-410.
Carey, G. and L. Fraser. 1932. The embryology and seedling development of *Aegiceras majus* Gaertn. Proc. Linn. Soc. N. S. W. 57: 341-360.
Carlquist, S. 1967. The biota of long distance dispersal. V. Plant dispersal to Pacific islands Bull. Torrey Bot. Club 94: 129-162.
Carter, G. S. 1930. The fauna of the swamps of Paraguayan Chaco in relation to its environment. I. Physicochemical nature of the environment. J. Linn. Soc. London, Zool. 37: 206-258.
Carter, J. 1959. Mangrove succession and coastal change in South-West Malaya. Trans. Inst. Br. Geogr. 26: 79-88.
Case, G. O. 1938. The use of vegetation for coast protection. Agric. J. Br. Guiana 9: 4-11.
Catala, R. L. A. 1957. Report on the Gilbert Islands: some aspects of human ecology. Atoll Res. Bull. 59: Oct. 31.
Cawkell, E. M. 1964. The utilization of mangroves by African birds. Ibis 106: 251-253.

Chandler, M. E. J. 1951. Notes on the occurrence of mangroves in the London clay. Proc. Geol. Assoc. 62: 271-272.

Champion, H. G. 1936. A preliminary survey of the forest types of india and Burma. Indian Forest Rev. Bot. 1, 265 pp.

Chang, Hung-ta, Chao-Chang Chang, and Paisun Wang. 1957. The mangrove vegetation of the Liochow Peninsula. Sunyatsenia Nat. Sci. Ed. 1957: 122-143 (in Chinese).

Chapman, V. J. 1936. The halophyte problem in the light of recent investigations. Q. Rev. Biol. 11: 209-220.

Chapman, V. J. 1940. The botany of the Jamaica shoreline. Geogr. J. 96: 312-323.

Chapman, V. J. 1942. A new perspective in halophytes. Q. Rev. Biol. 17: 291-373.

Chapman, V. J. 1944a. 1939 Cambridge University Expedition to Jamaica. I. A study of the botanical processes concerned in the development of the Jamaican shore-line. J. Linn. Soc. London Bot. 52: 407-447.

Chapman, V. J. 1944b. 1939 Cambridge University Expedition to Jamaica. II. A study of the environment of *Avicennia nitida* Jacq. in Jamica. J. Linn. London Soc. Bot. 52: 448-486.

Chapman, V. J. 1944c. 1939 Cambridge University Expedition to Jamica. III. The morphology of *Avicennia nitida* Jacq. and the function of its pneumatophores. J. Linn. Soc. London Bot. 52: 487-533.

Chapman, V. J. 1962a. Respiration studies of mangrove seedlings. I. Material and some preliminary experiments. Bull. Mar. Sci. Gulf Caribb. 12: 137-167.

Chapman, V. J. 1962b. Respiration studies of mangrove seedlings. II. Respiration in air. Bull. Mar. Sci. Gulf Caribb. 12: 245-263.

Chapman, V. J. 1966a. Some factors involved in mangrove establishment. *In* Les problemes scientifiques des deltas de la zone tropicale humide et leurs implications. Colloque de Dacca. UNESCO, Paris, pp. 219-225.

Chapman, V. J. 1966b. Vegetation and salinity. *In* Salinity and Aridity, H. Boyko (ed.), Junk Publishers, The Hague, Netherlands, pp. 23-42.

Chapman, V. J. 1969. Lagoons and mangrove vegetation. *in* Lagunas Costeras, un Simposio, Mem. Simp. Intern. Lagunas Costeras. UNAM-UNESCO, Nov. 28-30, 1967. Mexico, D. F., pp. 505-514.

Chapman, V. J. 1970. Mangrove phytosociology. Trop. Ecol. 11: 1-19.

Chapman, V. J. and J. W. Ronaldson. 1958. The mangrove and salt-grass flats of the Auckland Isthmus. N. Z. Dept. Sci. Ind. Res., Bull. 125, 79 pp.

Chapman, V. J. and C. B. Trevarthen. 1953. General schemes of classification in relation to marine coastal zonation. J. Ecol. 41: 198-204.

Chatelain, G. 1932. L'exploitation forestiere a la Guayane Francaise. Actes e Comp. Rendus de l'Association Colonies-Sciences, Paris 8: 217-222.

Chattaway, M. M. 1932. The wood of the Sterculiaceae. 1. Specialization of the vertical wood parenchyma within the sub-family Sterculieae. New

Phytol. 31: 119-132.
Chattaway, M. M. 1938. The wood anatomy of the family Sterculiaceae. Phil. Trans. R. Soc. B. 228: 313-365.
Chatterjee, D. 1958. Symposium on mangrove vegetation. Sci. Cult. 23: 329-335.
Chaudhri, I. I. 1967. The vegetation of Karachi. Vegetatio 10: 229-246.
Chayapongse, C. 1955. Yield of mangrove forest. Vanasarn For. J. 2, April 1955.
Cherrie, G. K. 1891. Notes on Costa Rican birds. Proc. U. S. Natl. Mus. 14: 517-537.
Chevalier, A. 1924. Exploitation des Paletuviers a tanin et leur valeur d'apres quelques travaux recents. Rev. Bot. Appl. Agric. Colon 4: 320.
Chevalier, A. 1929. Possibilite de cultiver le Bananier nain sur les terrains a Paletuviers ou Bresil et en Afrique occidentale. Rev. Bot. Appl. Agric. Colon 9: 334-337.
Chevalier, A. 1931. Graines d'Avicennia
comme aliment de famine. Rev. Bot. Appl. Agric. Colon 11: 1000.
Chidester, G. H. and E. R. Schafer. 1959. Pulping of Latin American woods. Products Lab. Rep. No. 2012, Madison, Wisconsin, 11 pp.
Chiovenda, E. 1929. Flora Somala. Rome, 436 pp.
Chipp, C. G. 1921. A list of the fungi of the Malay Peninsula. Bull. Straights Settlements 11: 311-418.
Chipp, T. F. 1931. The vegetation of northern tropical Africa. Scot. Geog. Mag. 47: 193-214.
Chowdhury, K. A. 1934. The so-called terminal parenchyma cells of the wood of *Terminalia tomentosa* W. and A. Nature 133: 215.
Christian, C. S. and G. A. Stewart. 1953. General report on a survey of the Katherine-Darwin region, 1946. CSIRO Austr. Land Res. Ser. 1.
Christophersen, E. 1935. Flowering plants of Samoa. B. P. Bishop Museum Bull. No. 128, Honolulu, Hawaii.
Ciferri, R. 1939. La associazioni de litorale marino della Somalia meridionale. Riv. Biol. Colon No. 2.
Clarke, L. D. and N. J. Hannon. 1967. The mangrove swamp and salt marsh communities of the Sydney district. I. Vegetation, soils and climate. J. Ecol. 55: 753-771.
Clarke, L. D. and N. J. Hannon. 1969. The mangrove swamp and salt marsh communities of the Sydney district. II. The holocoenotic complex with particular reference to physiography. J. Ecol. 57: 213-234.
Clarke, L. D. and N. J. Hannon. 1970. The mangrove swamp and salt marsh communities of the Sydney district. III. Plant growth in relation to salinity and waterlogging. J. Ecol. 58: 351-369.
Clarke, L. D. and N. J. Hannon. 1971. The mangrove swamp and salt marsh communities of the Sydney district. IV. The significance of species interaction. J. Ecol. 59: 535-553.
Coaldrake, J. E. 1961. The ecosystem of the coastal lowlands (Wallum) of

southern Queesland. CSIRO Austr. Bull. No. 28, 138 pp.
Cockayne, L. 1958. The vegetation of New Zealand. Hafner Pub. Co., New York, 456 ppb.
Coelho, P. A. 1965-1966. Os crustaceos decapodos de alguns manguezais pernambucanos. Trab. Inst. Oceanogr., Univ. Fed. Pernambuco, Recief (7/8): 71-89.
Cogger, H. G. 1959. Australain goannas. Aust. Mus. Mag. 13: 71-75.
Cohen, E. 1939. The marine angiosperms of Inhaca Island. S. Afr. J. Sci. 36: 246.
Collins, M. I. 1921. On the mangrove and salt marsh vegetation near Sydney, N. S. W., with special reference to Cabbage Creek, Port Hacking. Proc. Linn. Soc. N. S. W. 46: 376-392.
Colman, J. 1940. Zoology of the Jamaican shoreline. Geogr. J. 96: 323-327.
Compere, P. 1963. The correct name of the Afro-American black mangrove (*Avicennia germinans* L., Verbenaceae). Taxonomy 13: 150-152.
Connor, D. J. 1969. Growth of grey mangrove (*Avicennia marina*) in nutrient culture, Biotropica 1: 36-40.
Cook, E. P. and R. C. Collins. 1903. Economic plants of Puerto Rico. Cont. U. S. Natl. Herb. 8: 2. 229-230.
Cook, M. T. 1907. The embryology of *Rhizophora mangle.* Bull. Torrey Bot. Club 34: 271-277.
Cook, M. T. 1908. The hypertrophied fruit of *Bucida buceras.* Bull. Torrey Bot. Club 35: 305-306.
Cooper, R. E. and S. A. Pasha. 1935a. Osmotic and suction pressure of some species of mangrove vegetation. J. Indian Bot. Soc. 14: 109-120.
Cooper, R. E. and S. A. Pasha. 1935b. Osmotic pressure and the H-ion concentration of sea weeds in relation to those of sea-water. J. Indian Bot. Soc. 14: 3.
Corner, E. J. H. 1940. Wayside trees of Malaya, Vol. 1. Government Printer, Singapore, 772 pp.
Cotton, B. C. 1943. Australian Gastropoda of the families Hydrobiidae, Assimineidae, and Acmaeidae. Trans. R. Soc. Austr. 66: 124-129.
Cotton, B. C. 1950. An old mangrove mud-flat exposed by wave scouring at Glenelg, South Australia. Trans. R. Soc. S. Austr. 73: 59-61.
Craighead, F. C. 1964. Land, mangroves and hurricanes. Fairchild Trop. Gard. Bull. 19: 5-32.
Craighead, F. C. 1968. The role of the alligator in shaping plant communities and maintaining wildlife in southern Everglades. Florida Nat. 41: 2-7.
Craighead, F. C. 1969. Vegetation and recent sedimentation in Everglades National Park. Fla. Nat. 42: 157-166.
Craighead, F. C. 1971. The Trees of South Florida. Vol. 1. The Natural Environments and Their Succession. Univ. Miami Press, Coral Gables, 212 pp.
Craighead, F. C. and V. C. Gilbert. 1962. The effects of hurricane Donna on

the vegetation of southern Florida. Q. J. Fla. Acad. Sci. 25: 1-28.
Crane, J. 1941. Eastern Pacific expeditions of the New York Zoological Society. XXVI. Crabs of the genus *Uca* from the west coast of Central America. Zoologia, N. Y. 26: 145-208.
Crane, J. 1947. Intertidal brachygnathous crabs from the west coast of tropical America with special reference to ecology. Zoologia, N. Y. 31: 69-95.
Creager, D. B. 1962. A new Cercospora of *Rhizophora mangle*. Mycologia 54: 536-539.
Crossland, C. 1903. Note on the dispersal of mangrove seedlings Ann. Bot. Fenn. 17: 267-270.
Cruz, A. A. de la and J. F Banaag. 1967. The ecology of a small mangrove patch in Matabung Kay Beach, Batangas Province. Univ. Philipp. Nat. Appl. Sci. Bull. 20: 486-494.
Cuatrecasas, J. 1947. Vistazo a la vegetacion natural del calima. Acad. Colombiana de Cient. Exact. Fis. y Nat. Rer. 7: 306-312.
Cuatrecasas, J. 1952. Mangroves of the Pacific coast of South America. Abstract of a series of lectures presented before the Department of Botany Seminar, Northwestern University, by the botanical staff of the Chicago Natural History Museum (cited in West, 1956).
Cuatrecasas, J. 1958a. Introduction al estudia de los manglares. Boln. Soc. Bot. Mex. 23: 84-98.
Cuatrecasas, J. 1958b. Aspectos de la vegetacion natural de Colombia. Rev. Acad. Colombiana Cienc. Exactas, Fis., Nat. 10: 221-264.
Curtis, A. H. 1888. How the mangrove forms islands. Gard. For. 1: 100.
Daiber, F. C. 1960. Mangroves: The tidal marshes of the tropics. Univ. Delaware Estuarine Bull. 5: 10-15.
Daiber, F. C. 1963. Tidal creeks and fish eggs. Univ. Delaware Estuarine Bull. 7: 6-14.
Dale, I. R. 1938. Kenya mangroves. Z. Weltforstwirtsch. 5: 413-421.
Danhof, G. N. 1946. Rotation and management of mangrove in Riouw-Singga Archipelago. Tectona 36: 59-72.
Dabsereau, P. 1947. Zonation et succesion sur la restinga de Rio de Janeiro. I. La holosere. Rev. Can. Biol. 6: 448-477.
Dansereau, P. 1957. Biogeography, an Ecological Perspective. Ronald Press. New York, 394 pp.
Darteville, E. 1949. Les mangroves d'Afrique equatorial. Atti. Acad. Ligure. Sci. Lett. 6: 1-48.
Darteville, E. 1950. Les mangroves de Congo et les autres mangroves d'Afrique occidentale. Bull. Seances Inst. R. Coll. Belge 21: 946-971.
Davies, J. B. 1967. The distribution of sand flies (*Culicoides* supp.) breeding in a tidal mangrove swamp in Jamaica and the effects of tides on the emergence of *C. furens* Poey and *C. barbosai* (Wirth and Blanton). West Indies Med. J. 16: 39-48.
Davies, J. B. 1969. Effect of felling mangroves on emergence of *Culicoides*

spp. in Jamaica. Mosq. News 29: 566-571.
Davis, C. C. 1966. Notes on the ecology and reproduction of *Trichocorixa reticulata* in a Jamaican salt-water pool. Ecology 47: 850-852.
Davis, C. C. and R. H. Williams. 1950. Brackish water plankton of mangrove areas in southern Florida. Ecology 31: 519-531.
Davis, J. H., Jr. 1938. Mangroves, makers of land. Nat. Mag. 31: 551-553.
Davis, J. H. 1939a. Vivipary and dispersal of mangrove seeds. J. Tennessee Acad. Sci. 15: 415.
Davis, J. H. 1939b. The role of mangrove vegetation in land building in southern Florida. Am. Phil. Soc. Yearb., 1938, pp. 162-164.
Davis, J. H. 1940a. Peat deposits of Florida. Fla. Geol. Sur. Bull. 30: 1-247.
Davis, J. H. 1940b. The ecology and geologic role of mangroves in Florida. Pap. from the Tortugas Lab. Vol. 32. Carnegie Inst. Wash. Pub. No. 517, pp. 303-412.
Davis, J. H. 1942. The ecology of the vegetation and topography of the sand keys of Florida. Pap. from the Tortugas Lab. Vol. 33. Carnegie Inst. Wash. Pub. No. 524, pp. 113-195.
Davis, J. H. 1943. The natural features of southern Florida. Florida Dept. Conserv. Geol. Bull. No. 25. 311 pp.
Davis, J. H. 1945. Jamaican shoreline ecology. Ecology 26: 312.
Davy, J. B. 1938. The classification of tropical woody vegetation-types. Imperial For. Inst., Univ. Oxford, Inst. Pap. No. 13. 85 pp.
Dawson, E. Y. 1954. Marine plants in the vicinity of the Institut Oceanographique de Nha Trang, Viet Nam. Pac. Sci. 8: 372-481.
Day, J. H. 1951. The ecology of South African estuaries, Part I. General considerations. Trans. R. Soc. S. Afr. 33: 53-91.
Day, J. H., N. A. H. Millard, and A. D. Harrison. 1952. The ecology of South African estuaries. Part VII. The biology of Durban Bay. Ann. Natal Mus. 13: 259.
Day, J. H., N. A. H. Millard, and G. J. Broekhuysen. 1953. The ecology of South African estuaries. Part IV. The St. Lucia system. Trans. R. Soc. South Africa 34: 129.
Day, J. H. and J. F. C. Morgans. 1956. The ecology of South African estuaries. Part VIII. The biology of Durban Bay. Ann. Natal Mus. 13: 259.
Decaisne, J. 1835. Observations sur quelque nouveau generes et especes des plantes de l'Arabie-Hereuse. Rhizophoraceae. Ann. des Sci. Nat., Bot., Paris, pp. 75-77.
Degener, O. 1946. Flora Hawaiiensis, Books 1-4. The Patton Col., Honolulu.
Deignan, H. G. 1961. Type specimens of birds in the United States National Museum. U. S. Natl. Mus. Bull. 221, 718 pp.
Delevoy, G. 1945. Les mangroves africaines. Bull. Soc. Cient. For. Belg. 52: 84-89.
Delf, E. M. 1912. Transpiration in succulent plants. Ann. Bot. 26: 409-412.
Dent, J. M. 1947. Some soil problems in empoldered rice lands in Sierra Leone. Empire J. Exp. Agric. 15: 206-212.

Derijard, R. 1965. Contribution a l'etude du peuplement des sedments sable-vaseux, et vaseux intertidaux, compactes ou fixes par la vegetation de la region de Tulear (Madagascar). Rec. Trav. Stat. Mar. Endoume. Fasc. h. s. suppl. No. 43in-8, 94 pp.

Detwiler, S. B. 1948. Some basic information regarding tannin and tannin-yielding crops, prepared for use by tannin-research workers. U. S. Off. For. Agric. Washington, D. C. 112 pp.

D'Hoore, J. 1963. Soil Map of Africa (5th revision). C.C.T.A./C.S.A., Joint Project No. 11, Leopoldville.

Dickerman, R. W. and G. Gavino T. 1970. Studies of a nesting colony of green herons at San Blas, Mexico. Living Bird 8: 95-111.

Dickerman, R. W. and C. Juarez L. 1971. Nesting studies of the boat-billed heron *Cochlearius cochlearius* at San Blas, Nayarit, Mexico. Ardea 59: 1-16.

Dickey, D. R. and A. J. van Rossem. 1938. The birds of El Salvador. Field Mus. Nat. Hist. Publ. Zool. Ser. 23.

Diels, L. 1915. Vegetationstypen vom untersten Kongo. Vegetations-bilder 12, VIII, 16 pp.

Ding Hou. 1957. A conspectus of the genus *Bruguiera* (Rhizophoraceae). Nova Guinea (n.s.) 8: 163-171.

Ding Hou. 1958. Rhizophoraceae. F. Males. I. 5: 429-493.

Ding Hou. 1960. A review of the genus *Rhizophora* with special reference to the Pacific species. Blumea 10: 625-634.

Ding Hou. 1965. Studies in the Flora of Thailand. Rhizophoraceae. Dansk. Bot. Arkiv. 23: 187-190.

Dodd, J. R. and C. T. Siemers. 1971. Effect of Late Pleistocene karst topography on Holocene sedimentation and biota, lower Florida Keys. Geol. Soc. Am. Bull. 82: 211-218.

Doderlein, L. 1881. Botanische Mitteilungen aus Japan. Bot. Centrabl. 8: 27-31.

Dolianiti, E. 1955. Frutos de Nipa no Palaeocene de Pernambuco. Div. Geol. e Mineral Brasil Bol. 158: 1-36.

Doyne, H. C. 1937. A note on the acidity of mangrove swamp soils. Trop. Agric. Trinidad 14: 236-237.

Doyne, H. C. and R. R. Glanville. 1933. Some swamp rice growing soils Sierra Leone. Trop. Agric. Trinidad 10: 132-138.

Drabble, E. 1908. The bark of the red and the white mangroves. Q. J. Instit. Comm. Res. Tropics 3: 33-39.

Drabble, E. and L. Hilda. 1905. The osmotic strength of cell-sap in plants growing under different conditions. New Phytol. 4: 8.

Drabble, E. and M. Nierenstein. 1907. A note on the West-African mangroves. Q. J. Instit. Comm. Res. Tropics 2.

Drakenstein, H. A. Rheede tot. 1678-1703. Hortus indicus malabaricus. 12 vols. (*Sonneratiaa*, vol. 3; *Aegiceras*, *Rhizohora*, and *Bruguiera*, vol. 6, *Avicennia*, vol. 4). Van Someren and van Dijk, Amsterdam.

Drar, M. 1933. A note on some plants of Gebel Elba: *Avicennia officinalis.* Hart. Rev. Egypt. Hart. Soc. 21: 7-10.

Dubois, J. 1954. Sur une classification de sols de delta soumis a des influences salines appliquee au bas-Senegal. Proc. 2nd. Int. Afr. Soil Conf. 2: 1119-1124.

Dugros, M. 1937. Le domaine forestier inonde de la Cochine. Bull. Econ. de l'Indochine 40: 283-314.

Durand, J. H. 1964. La mise en valeur des mangroves de la cote N. W. de Madagascar. Bull. AFES Versailles 5: 200-206.

Durant, C. C. L. 1941. The growth of mangrove species in Malaya. Malay. For. 10: 3-15.

Eddy, W. H. and B. Sokoloff. 1953. Mangrove meal as a cattle food ingredient. Southern Bio-Research Lab., Fla. Southern Col., Lakeland, Florida.

Edwall, P. W. 1877. Enseio para una synonimia dos nomes populares das plantas indigenas do estado de Sao Paulo. Sao Paulo, Boletin No. 16.

Eggers, H. von. 1877. Rhizophora mangle L. Vidensk. Meddel. Dansk Naturhist. Foren. Kjobenhaven 28: 177-181.

Eggers, H. von. 1892. Die Manglares in Ecuador. Bot. Centralb. 52: 49-52.

Eggers, H. von. 1894. Das Kustenbebiet von Ecuador. Geog. Gesell. Bremen 17: 265-289.

Egler, F. E. 1948. The dispersal and establishment of red mangrove in Florida. Caribb. For. 9: 299-310.

Egler, F. E. 1952. Southeast saline Everglades vegetation, Florida, and its management. Veg. Acta Geobot. 3: 213-265.

Egler, F. E. 1961. A cartographic guide to selected regional vegetation literature – where plant communities have been described. II. Southeastern United States. Sarracenia 6: 1-87.

Eisley, L. 1971. The Night Country, Charles Scribner's Sons, New York, 240 pp.

Ekman, S. 1953. Zoogeography of the Sea. Sidgewick and Jackson Ltd., London, 417 pp.

Emery, K. O. and R. E. Stephenson. 1957. Estuaries and lagoons. *in* Treatise on Marine Ecology and Paleoecology. Vol. 1, J. S. Hedgepeth (ed.). Geol. Soc. Am. Mem. 67: 673-750.

Engler, A. 1876. Rhizophoraceae in Martius. Flora Brazil. 12: 426.

Engler, A. 1921. Die Pflanzenwelt Afrikas. Erster Band, 1 Heft; Driter Band, II Heft. Leipzig.

Engler, A. and L. Diels. 1899. Monographieen Afrikanisher Pflanzenfamilien und - gattungen. III. Combretaceae-*Combretum.* 116 pp., Leipzig.

Engler, A. and L. Diels. 1900. Monographieen Afrikanisher Pflanzenfamilien und - gattungen. IV. Combretaceae excl. *Combretum.* 44 pp. Leipzig.

Enns, T. 1965. Tracer studies of water and sodium transport in mangroves (*Laguncularia racemosa*). Havalradets Dkr. 48: 161-163.

Epstein, E., W. E. Schmid, and D. W. Rains. 1963. Significance and technique

of short-term experiments on solute absorption by plant tissue. Plant Cell Physiol 4: 79-84.
Erichson, R. 1921. Die Mangrove-vegetation ihre Verbreitung und ihre Bedeuting fur Schwemmland-bildungen. Ungerdrukte Diss. (Maschinenschrift), Halle.
Erichson, R. 1925. Die Mangrove-Vegetation. Z. f. Naturfr. 16: 190-199.
Ernould, M. 1921. Recherches anatomiques et physiologiques sur les racines respiratoires. Mem. Acad. R. de Bel. 6: 1-52.
Exell, A. W. 1931. The genera of Combretaceae. J. Bot. 69: 113-128.
Exell, A. W. 1954. Combretaceae. Fl. Males 1: 533-589.
Exell, A. W. 1958. Combretaceae. Ann. Mo. Bot. Gar. 45: 143-164.
Exell, A. W. and C. A. Stace. 1966. Revision of the Combretaceae. Bol. Soc. Broteriana 40: 5-25.
Faber, F. C. von. 1913. Uber Transpiration und osmotischen Druch bei den Mangroven. Ber. Dtsch. bot Ges. 31: 277-286.
Faber, F. C. von. 1923. Zur Physiologie der Mangroven. Ber. Dtsch. bot. Ges. 41: 227-234.
Fairbridge, R. W. and C. Treichert. 1947. The rampart system at Low Isles. Rep. Great Barrier Reef Comm. 6: 1016.
Fanshawe, D. 1948. Forest products of British Guiana. Part I. Principal timbers. For. Bull. 1 NS, For. Dept. Brit. Guiana, 39 pp.
Fanshaw, D. 1950. Forest products of British Guiana. Part II. Minor forest products. For. Bull. 2 NS, For. Dept. Brit. Guiana, pp. 16-18, 49.
Fanshaw, D. 1952. The Vegetation of British Guiana, A Preliminary Review. Pap. For. Inst., Oxford, 96 pp.
Feldman, J. and R. Lami. 1936. Sur la vegetation de la mangrove a la Guadelope. C. R. Acad. Sci. (Paris) 203: 883-885.
Feliciano, C. 1962. Notes on the biology and economic importance of the land crab *Cardiosoma guanhumi* Latreille of Puerto Rico. Contrib. Inst. Mar. Biol. Univ. Puerto Rico, Mayaguez, 1-29.
Fenner, C. and J. B. Cleland. 1932. The geography and botany of the Adelaide coast. S. Aust. Nat. 14: 45-48, 55-56, 109-120, 128-133.
Fernandos, D. A. 1934. Ueber Mangroven Cultuven. Tectona 27: 299-303.
Ferns, G. W. 1955a. Tidal forests in southeast Kalimantan. Indian J. For. 4: 3-5.
Ferns, G. W. 1955b. Hutan pajan sekitar Kalimantantenggara (Tidal forests of S. E. Borneo). Rimba Indonesia Penerb. pop. 4: 90-98.
Ffrench, R. P. 1966. The utilization of mangroves by birds in Trinidad. Ibis 108: 423-424.
Ffrench, R. P. 1970. The scarlet Ibis in Surinam and Trinidad. Living Bird 9: 147-165.
Field, G. D. 1968. Utilization of mangroves by birds on the Freetown peninsula, Sierra Leone. Ibis 110: 354-357.
Finucane, J. H. 1965. Threadfish in Tampa Bay, Florida. Q. J. Fla. Acad. Sci. 28: 267-270.

Fischer, P. H. 1940. Notes sur les peuplements littoraux d'Australia. III. Sur le faune de la Mangrove australienne. Mem. Soc. Biogeogr. 7: 315-329.
Fisher, C. E. C. 1927. Contributions to the flora of Burma, 2. Kew Bull. 1927: 81-94.
Flamm, B. R. and J. H. Cravens. 1971. Effects of war damage on the forest resources of South Vietnam. J. For. 69: 784-790.
Fly, L. B. 1952. Preliminary pollen analysis of the Miami, Florida, area. J. Allergy 23: 48-57.
Forattini, O. P. 1957. *Culicoides* daaregiao neotropical (Diptera, Ceratopogonidae). Arch. Hig. Sao Paulo 11, 526 pp.
Forattini, O. P., E. X. Rabello, and D. Pattoli. 1958. *Culicoides* de regiao neotropical. 2. Observacoes sobre biologia em conicoes naturais. Arch. Hig. Sao Paulo 12: 1-52.
Fosberg, F. R. 1947. Micronesian mangroves. J. N. Y. Bot. Gard. 48: 128-138.
Fosberg, F. R. 1953. Vegetation of central Pacific atolls. Atoll Res. Bull. 23, Sept. 30.
Fosberg, F. R. 1960. The vegetation of Micronesia: 1. General description of the vegetation of Guam. Bull. Am. Mus. Nat. Hist. 119: 1-75.
Fosberg, F. R. 1961. Vegetation-free zone on dry mangrove coasts. U. S. Geol. Soc. Prof. Pap. No. 424D, pp. 216-218.
Fosberg, F. R. 1964. Vegetation as a geological agent in tropical deltas. *In* Les problems scientifiques des deltas de la zone tropicale humide et leurs implications. Colloque de Dacca. UNESCO, Paris, pp. 227-233.
Fourmanoir, P. 1953. Notes sur la faune de la mangrove dans la region de Majunga: crabs, crevettes, poissons. Nat. Malgache 5: 87-92.
Foxworthy, F. W. 1910. Distribution and utilization of the mangrove-swamp of Malaya. Ann. Jar. Bot. de Buitz., Suppl. 3, Part I, pp. 319-344.
Foxworthy, F. W. and D. M. Matthews. 1916. Mangrove and nipa swamps of British North Borneo. For. Bull. British N. Borneo 1: 1-67.
Freise, F. W. 1932. Brasilianische Gerbstoffpflanzen und ihre Ausbeutung. Tropenpflanzen 35: 70-74.
Freise, F. W. 1935. A importancia de conversazao dos mangues como viveiros de peixes. Anais do 1º Congr. Nac. de Presca, Rio de Janeiro, 1934. pp. 315-319.
Freise, F. W. 1938. Untersuchungen am Schlick der Mangrovekuste Brasiliens. Chemie der Erde 2: 333-355.
Freyberg, B. von. 1930. Zerstorung und Sedimentation and der Mangrovekuste Brasiliens. Leopoldina 6: 69-118.
Fryckberg, M. 1945. Mangrove bark. Agric. Am. 5: 199.
Fuchs, H. P. 1970. Ecological and palynological notes on *Pelliciera rhizophoraee.* Acta Bot. Neerl. 19: 884-894.
Ganapati, P. N. and M. V. L. Rao. 1959. Incidence of marine borers in the mangroves of the Godavari estuary. Curr. Sci. 28: 332.
Gardner, C. A. 1923. Botanical notes from the Kimberly division of Western

Australia. W. Aust. For. Dept., Bull. No. 32, Government Printer, Perth, Australia.
Gardner, C. A. 1942. The vegetation of Western Australia with special reference to the climate and soils. J. Proc. R. Soc. West Austral. 28: xi-lxxxvii.
Gehrmann, K. 1911. Zur Blutenbiologie der Rhizophoraceae. Ber. Dtsch. bot. Ges. 29: 308-318.
Genkel, P. A. 1963. K. ecologii restanii mangrov. *In* Fizioligiya drevesnykh rastenii. Akad. Nauk SSR, Moscow. Referat. Zh. Biol. No. 7V151, pp. 223-232.
Genkel, P. A. and I. S. Fan. 1958. On the physiological significance of viviparity in mangrove plants. (In Chinese: Russian summary). Acta Bot. Sin. 7: 51-71.
Gerlach, S. A. 1958. Die Mangroveregion tropischer Kusten als Lebensraum. Z. Morph. Okol. Tiere 46: 636-731.
Gerlach, S. A. 1963. Okologische Bedeutung der Kuste als Grenzraum zwischen Land und Meer. Naturw. Rdsch. 16: 219-227.
Germeraad, J. H., C. A. Hopping, and J. Muller. 1965. Palynology of Tertiary sediments from tropical areas. Rev. Palaeobotan. Palynol. 6: 139-348.
Gessner, F. 1955. Die Mangroven. Hydrobotanik, Band I. Veb Deutscher Verlag der Wissenchaften, Berlin, 517 pp., pp. 249-262.
Gessner, F. 1967. Untersuchungen an der Mangrove in Ost-Venezuela. Int. Rev. Ges. Hydrobiol. 52: 769-781.
Giglioli, M. E. C. and I. Thornton. 1965a. The mangrove swamps of Keneba, Lower Gambia River basin. I. Descriptive notes on the climate, the mangrove swamps and the physical composition of their soils. J. appl. Ecol. 2; 81-103.
Giglioli, M. E. C. and I. Thornton. 1965b. The mangrove swamps of Keneba, Lower Gambia River basin. II. Sulphur and pH in the profiles of swamp soils. J. appl. Ecol. 2: 257-269.
Giglioli, M. E. C. and D. F. King. 1966. The mangrove swamps of Keneba, Lower Gambia River basin. III. Seasonal variations in the chloride and water content of swamp soils, with observations on the water level and chloride concentration of free soil water under a barren mud flat during the dry season. J. appl. Ecol. 3: 1-19.
Gill, A. M. 1969. Tidal trees: orient and occident. Bull. Fairchild Trop. Gard. July 1969, pp. 7-10.
Gill, A. M. 1970. The mangrove fringe of the Eastern Pacific. Bull. Fairchild Trop. Gard. July 1970, pp. 7-11.
Gill, A. M. Endogenous control of growth-ring development in *Avicennia*. For. Sci. *in press*.
Gill, A. M. and P. B. Tomlinson. 1969. Studies on the growth of red mangrove (*Rhizophora mangle* L.). I. Habit and general morphology. Biotropica 1: 1-9.
Gill, A. M. and P. B. Tomlinson. 1971. Studies on the growth of red

mangrove (*Rhizophora mangle* L.). II. Growth and differentiation of aerial roots. Biotropica 3: 63-77.

Gill, A. M. and P. B. Tomlinson. 1971. Studies on the growth of red mangrove. (*Rhizophora mangle* L.). III. Phenology of the shoot. Biotropica, 3: 109-124.

Gilmour, A. J. 1965. The implication of industrial development on the ecology of a marine estuary. Victoria Fish. Wildl. Dept. Fish Contrib. 20: 1-12.

Gilroy, A. B. and L. J. Chwatt. 1945. Mosquito-control by swamp drainage in the coastal belt of Nigeria. Ann. Trop. Med. Parasitol. 39: 19-40.

Glanville, R. R. and H. C. Doyne. 1933. Some swamp soils of Sierra Leone. Trop. Agric. 10: 132.

Glassman, S. R. 1952. Flora of Ponape. B. P. Bishop Mus. Bull. No. 209, Honolulu, Hawaii.

Gleason, H. A. and M. T. Cook. 1927. Plant ecology of Puerto Rico: Scientific survey of Puerto Rico and the Virgin Islands. Proc. N. Y. Acad. Sci. 7: 1-96.

Gledhill, D. 1963. The ecology of the Aberdeen Creek mangrove swamp. J. Ecol. 51: 693-703.

Glynn, P. W. 1964. Common marine invertebrate animals of the shallow waters of Puerto Rico. Historia Natural de Puerto Rico. Univ. Puerto Rico, Mayaguez, pp. 12-20.

Glynn, P. W., L. R. Almodovar, and J. C. Gonzalez. 1964. Effects of Hurricane Edith on marine life in La Parguera, Puerto Rico. Caribb. J. Sci. 4: 335-345.

Goebel, K. von. 1886. Ueber die Rhizophoren-vegetation. Sitz. ber. d. Naturf. Ges. Rostock. December 1886.

Goebel, K. von. 1889. Ueber die Luftwurzeln von *Sonneratia.* Ber. Dtsch. bot. Ges. 5: 249-255.

Gokhale, A. V. 1962. Control of mangroves. Poona Agric. Col. Mag. 53: 30.

Gola, G. 1905. Studi sui rapporti de la distribuzione della piante e la constituzione fisico-chimica del suolo. Ann. di Bot. 3: 455-512.

Golley, F. B. 1969. Caloric value of wet tropical forest vegetation. Ecology 50: 517-519.

Golley, F., H. T. Odum, and R. F. Wilson. 1962. The structure and metabolism of a Puerto Rican red mangrove forest in May. Ecology 43: 9-19.

Gomez-Pompa, A. 1966. Estudios botanicos en la region de Misantla, Veracruz, Publ. Inst. Mex. Rec. Nat. Renov. Mexico, 173 pp.

Gonzales, J. G. and T. E. Bowman. 1965. Planktonic copepods from Bahia Fosforescente, Puerto Rico, and adjacent waters. Proc. U. S. Natl. Mus. 117: 241-304.

Gonzalez Ortega and J. Candelon. 1934. *Rhizophora mangle* L. Bol. Pro-Cultura Regional, Mazatlan, Mexico 1: 14-16.

Gooch, W. L. 1944. Survey of tannin resources in Mexico. J. For. 43: 56-58.

Good, R. 1953. The Geography of Flowering Plants. 2nd Ed. Longmans, Green and Co., London. xiv, 452 pp.
Gooding, E. G. B., A. R. Loveless, and G. R. Proctor. 1965. Flora of Barbados. H.M.S.O., London, 486 pp.
Goossens, H. J. 1936. Tambakherverkaveling in het ressort Tegal de Binnenvisserij. Landbouw 12: 191-200.
Gosh, D. 1967. Mangrove: A potential Indian tanning material. Tanner (Bombay) 21. 379-383.
Graham, B. M. 1929. Notes on the mangrove swamp of Kenya. J. E. Afr. and Uganda Nat. Hist. Soc. 29: 157-164.
Graham, C. L. 1934. Flora of the Kratabo region, British Guiana. Ann. Carnegie Mus. 22: 204.
Graham, J. 1929. Mangroves of East Africa. J. East Afr. Nat. Hist. Soc. 29: 157.
Graham, S. A. 1964. The genera of Rhizophoraceae and Combretaceae in the southeastern United States. J. Arnold Arbor. 45: 285-301.
Grant, D. K. S. 1938. Mangrove woods of Tanganyika territory, their silviculture and dependent industries. Tanganyika Notes and Rec. 1938 (April), pp. 5-15.
Grass, T. H. 1904. Forstatatistik fur die Waldungen des Rufiyideltas, angefangen im Jahre 1902. Ber. uber Land-u. Forstwirtsch (Deutsch Ostafrika) 2: 165-186.
Gray, J. 1960. Temperate pollen genera in the Eocene (Clairborne) flora, Alabana. Science 132: 808-810.
Greenway, H. 1962. Plant responses to saline substrates. III. The offsets of nutrient concentration on the growth and ion uptake of *Hordeum vulgare* during a sodium stress. Aust. J. Biol. Sci. 16: 616-628.
Greenway, H. and A. Rogers. 1963. Growth and ion uptake of *Agrophyron elongatum* on saline substrates, as compared with a salt-tolerant variety of *Hordeum vulgare*. Plant and Soil 18: 21-30.
Gregory, D. P. 1958. Rhixophoraceae. Ann. Miss. Bot. Gard. 45: 136-140.
Grewe, F. 1941. Afrikanische Mangrovelandschaften, Verbreitung und wirtschaftsgeographische Bedeutung. Wiss. Veroff. D. Mus. F. Landerk. N. F. 9: 103-177.
Griffith, A. L. 1950. Working scheme for the mangroves of the Zanzibar Protectorate. Gov. Printer, Zanzibar, 42 pp.
Groom, P. and S. E. Wilson. 1925. On the pneumatophores of paludal species of *Amoora*, *Goaurapa* and *Heritiera*. Ann. Bot. 39: 9-24.
Guillaumin, A. 1964a. Rhizophoraceae. Scientific Results of the French and Swiss Botanical Mission to New Caldonia (1950-1952). III. Mem. Mus. Nat. Hist. Natur. Nouv. Ser., Ser. B. Bot. 15: 76-77.
Guillaumin, A. 1964b. Revision des Rhizophoracees. Not. Syst. 3: 55.
Guppy, H. B. 1906. Observations of a Naturalist in the Pacific Between 1896 and 1899. Vol. II, Plant Dispersal. Macmillan and Co., Ltd., London. 627 pp.

Guppy, H. B. 1917. Plants, Seeds and Currents in the West Indies and Azores. Macmillian and Co., Ltd., London. 531 pp.

Gurke, A. N. 1895/1897. Notizen uber die Verwertung der Mangroverinde als Gerbmaterial. Not. Konigl. Bot. Gard. Mus. Berlin, 1895/1897, No. 1.

Haan, J. H. De. 1931. Het een en auder over de Tjilatjapsche vloedbosschen. Tectona 24: 39-76.

Haberlandt, G. 1895. Uber die Ernahrung der Keimlange und die Bedeutung des Endosperms bei viviparen Mangrovepflanzen. Ann. Jar. Botan. Buiten. 12: 105-114.

Haberlandt, G. 1910. Botanische Tropenreise; Indo-Malayische Vegetationsbilder und Reiseskizzen. Wilhelm Engelman, Leipzig, 296 pp.

Haden-Guest, S. J. K. Wright, and E. M. Teclaff. 1956. A World Geography of Forest Resources. The Ronald Press Co., New York, 736 pp.

Hagen, B. 1890. Die Pflanzen-und Thierwalt von Deli auf der Ostkuste Sumatra. Tidj. van het Kon. Nederl. Aardijks. Gen. 7: 1-240.

Hager, L. C. 1959. Anatomical study of *Avicennia marina* (Forsk.) Vieih. (Formerly called *A. officinalis*). M. S. Botany Dept., Univ. Witwatersrand, Johannesburg.

Hamilton, A. A. 1919. An ecological study of the salt marsh vegetation of the Port Jackson District. Proc. Linn. Soc., N.S.W. 44: 463-513.

Hammen, T. van der. 1962. A palynological study on the Quaternary of British Guiana. Leidse Geol. Meded. 29: 125-180.

Hammen, T. van der and T. A. Wijmstra. 1964. A palynological study on the Tertiary and Upper Cretaceous of British Buiana. Leidse Geol. Meded. 30: 183-241.

Hammer, L. 1961. Sobre la ecologia de aqua de lus mangles. Bol. Inst. Oceanogr. 1: 249-261.

Hammer, H. E. 1929. The chemical composition of Florida Everglades peat soils, with special reference to their inorganic constituents. Soil Sci. 28: 1-14.

Hammond, W. 1971. A case for mangroves. Littoral Drift. 1: 2-5.

Harms, J. W. 1941. Beobachtungen in der Mangrove Sumatras, insbesondere uber Lebensweise und Entwicklung der Solzlandturbellarie *Stylochus* sp. und der *Oncis stuxbergi.* Z. wiss. Zool. 154: 389-408.

Harms, J. W. and O. Dragendorff. 1933. Die Realisation von Genen und die consecutive Adaption. 3. Mitt. Osmotische Untersuchungen an Phycosoma lurco Sel. und DeMan aus den Mangrove-Vorl andern der Sumatra Inseln. Z. wiss. Zool. 143: 263-322.

Harper, R. M. 1917. Geography of central Florida. Fla. St. Geol. Surv., 18th Ann. Rep., pp. 27-276.

Harper, R. M. 1927. Natural resources of southern Florida. Fla. Geo. Surv. Ann. Rep. 18: 27-192.

Harrer, F. 1939. Die Mangroven des Rufiji-Deltas in Deutsch-Ostafrika. Kolonialforstl. Mitt. 2: 160.

Harris, J. A. and J. V. Lawrence. 1917. The osmotic concentration of the sap

of the leaves of mangrove trees. Biol. Bull. 32: 202-211.
Harrison, S. G. 1961. Notes on the vegetation of the coastal region of British Guiana. J. R. Agric. Comm. Soc. B. G. 40: 93-104.
Harrisson, T. 1965. Some quantitative effects of vertebrates on the Borneo flora. *in* Symp. Ecolo. Res. Humid Trop. Veg. UNESCO Sci. Coop. Of. Southeast Asia, Kuching, Sarawak, pp. 164-169.
Harshberger, J. W. 1908. The comparative leaf structure of the sand dune plants of Bermuda. Proc. Am. Phil. Soc. 47: 97-110.
Harshberger, J. W. 1914. The vegetation of south Florida, south of 27°30' North, exclusive of the Florida Keys. Wagner Free Inst. Sci. Philadelphia Trans. 7: 49-189.
Hart, M. G. R. 1959. Sulphur oxidation in tidal mangrove soils of Sierra Leone. Plant and Soil 11: 215-236.
Hart, M. G. R. 1962. Observations on the source of acid in empoldered mangrove soils. I. Formation of elemental sulphur. Plant and Soil 17: 87-98.
Hart, M. G. R. 1963. Observations on the source of acid in empoldered mangrove soils. II. Oxidation of soil polysulphides. Plant and Soil 19: 106-114.
Hartmann, G. 1956a. Zur Kenntnis des Mangrove-Estero-Gebietes von El Salvador und seiner Ostracoden-Fauna I. Meeresforsch. 12: 219-248.
Hartmann, G. 1956b. Zur Kenntnis des Mangrove-Estero-Gebietes von El Salvador und seiner Ostracoden-Fauna. II. Meeresforsch. 13: 134-159.
Hartman-Schroder, G. 1959. Zur Oekologie der Polychaete des Mangrove-Estero-Gebietes von El Salvador. Beitr. z. neotrop. Fauna 1: 69-183.
Hartnoll, R. G. 1965. Notes on the marine grapsoid crabs of Jamaica. Proc. Linn. Soc. London 176: 113-147.
Hatheway, W. H. 1953. The land vegetation of Arno Atoll, Marshall Islands. Attoll Res. Bull. 16, April 30.
Hanheway, W. H. 1955. The natural vegetation of Canton Island, an equatorial Pacific atoll. Res. Bull. 43, Aug. 15.
Haug, T. A. 1909. Die Mangrove Deutsch-Ostafrikas. Naturw. Z. Forst-u. Landwirt. 7: 413-425.
Haverschmidt, F. 1962. Notes on some Surinam breeding birds. II. Ardea 50: 173-179.
Haverschmidt, T. 1965. The utilization of mangroves by South American birds. Ibis 107: 540-542.
Haverschmidt, F. 1969. Notes on the Boat-billed Heron in Surinam. Auk 86: 130-131.
Hayata, B. 1912. Rhizophoraceae. Icones Plant. Formosarum 2: 15-16.
Heald, E. 1971. The production of organic detritus in a south Florida estuary. Univ. Miami, Sea Grant Tech. Bull. No. 6, 110 pp.
Heald, E. 1970. The contribution of mangrove swamps to Florida Fisheries. Proc. Gulf Caribb. Fish. Inst., 22nd Ann. Session, pp. 130-135.

Heiden, H. 1893. Anatomische Characteristik der Combretaceen. Bot. Centralbl. 55: 353-391.

Heiden, H. 1893. Anatomische Characteristik der Combretaceen. Bot. Centralbl. 56: 1-12, 65-75, 129-136, 163-170, 193-200, 225-230, 355-360, 385-391.

Hellmayer, C. E. and B Conover. 1948. Catalogue of birds of the Americas. Field Mus. Nat. Hist. Publ. Zool. Ser. 8, Part 1, No. 2.

Hemsley, W. B. 1894. The flora of the Tonga or Friendly Islands. J. Linn. Soc. London Bot. 30: 176.

Henckel, P. A. 1963. On the ecology of the mangrove vegetation. Mitteil. d. forist.-soziol. Srbeitsgem., N.F. 10: 201-206.

Henkel, J. S., S. St. C. Ballenden, and A. W. Bayer. 1936. Account of the plant ecology of the Dukuduku forest reserve and adjoining areas of the Zululand coast belt. Ann. Natal Mus. 8: 95-123.

Herbert, D. A. 1932. The relationships of the Queensland Flora. Proc. R. Soc. Queensland 44: 2-22.

Herklots, G. A. C. 1961. The Birds of Trinidad and Tobago. Collins Press, London, 287 pp.

Hesse, P. R. 1961a. Some differences between the soils of *Rhizophora* and *Avicennia* mangrove swamps in Sierra Leone. Plant and Soil 14: 335-346.

Hesse, P. R. 1961b. The decomposition of organic matter is a mangrove swamp soil. Plant and soil 14: 249-263.

Hesse, P. R. 1962. Phosphorus fixation in mangrove swamp muds. Nature 193: 295-296.

Hesse, P. R. 1963. Phosphorus in mangrove swamp mud with particular reference to aluminum toxicity. Plant and Soil 19: 205-218.

Heylighers, P. C. 1965. Lands of the Port Moresby-Kairuka area, Papua, New Guinea. VIII. Vegetation and ecology of the Port Moresby-Kairuka area. C.S.I.R.O. Austr. Land. Res. Ser. 14: 146-173.

Hicks, G. H. 1894. Nourishment of the embryo and the importance of the endosperm in viviparous mangrove plants. Bot. Gaz. 19: 327-330.

Hill, B. J. 1966. A contribution to the ecology of the Umalalazi estuary. Zool. Afr. 2: 1-24.

Hill, T. G. 1908. Observations on the osmotic properties of the root ahirs of certain salt marsh plants. New Phytol. 7: 133-142.

Hillier, J. M. 1907. Anti-opium plants. Kew Bull. 1907: 198-199.

Hillier, J. M. 1908. The malayan anti-opium plant. Kew Bull. 1908: 235-236.

Hillis, W. E. 1956. The production of mangrove extract in the delta region of Papua. Empire For. Rev. 35: 220-236.

Hirakawa, Y. 1933. Synoekologische Studien uber die Strandflora der Insel Formosa. I. Rep. Taihoku Bot. Gar. 3: 1-47.

Hirakawa, Y. 1936. Mangrove vegetation in Takao. J. Taiwan Mus. Ass. 4: 1-5 (in Japanese).

Ho, Ching 1957. Ecology of mangrove plants. Biol. News 8: 1-5.

Ho, R. 1962. Physical geography of the Indo-Australian tropics. *in*, Impact of

Man on Humid Tropics Vegetation. Goroka, New Guinea, pp. 19-35.
Ho, T. 1899. Rhizophorae in Japan. Ann. Bot. 456-468.
Hodge, W. H. 1956. The trees that walk to the sea. Nat. Mag. 49: 456.
Hoffmeister, J. E. and H. G. Multer. 1965. Fossil mangrove reefs of Key Biscayne, Florida. Geol. Assoc. Am. Bull. 76: 845-852.
Hofmeister, W. 1859. Neuere Beobachtungen uber Embryobildung bei Phanerogamen. Pring. Jahrb. 1: 82.
Holdridge, L. R. 1940. Some notes on the mangrove swamps of Puerto Rico. Caribb. For. 1: 19-29.
Holdridge, L. R. 1942. Trees of Puerto Rico, USDA, For. Serv., Trop. For. Experiment. Stat. Occas. Pap. No. 1.
Holdridge, L. R., L. V. Teesdale, J. E. Meyer, E. Z. Little, E. F. Horn, and J. Marrero. 1947. The forests of Central Equador. U. S. For. Ser. Bull. 1947, 134 pp.
Honel, Fr. von. 1882. Beitrage zur Pflanzenanatomie und Physiologie V. Zur Anatomie der Combretaceen. Bot. Z. 40: 177-182.
Hooker, W. J. 1830. *Terminalia catappa* L. Bot. Mag. 57.
Horn, C. L. 1945. Plant resources of Puerto Rico. *in* Plants and Plant Science in Latin America, F. Verdoorn (ed.), Chronica Botanica Co., Waltham, Mass., pp. 83-84.
Horn, E. F. 1946. Brazilian tanning materials. Trop. Woods 88: 33.
Horn, E. F. 1948. Durability of brazilian corssties. Trop. Woods 93: 30-35.
Hosakawa, T. 1957. Outline of the mangrove and strand forests of the micronesian islands. Mem. Fac. Sci. Kyushu Uni. Ser. E, 2: 101-118.
How Koon-Chew and Ho Chun-Nien. 1953. Rhizophoraceae in the Chinese Flora. Acta Phytotaxon. Sin., Vol. 2.
Howard, R. A. 1950. Vegetation of the Bimini Group, Bahamas. Ecol. Monogr. 20: 317-349.
Howe, M. A. 1911. A little-known mangrove of Panama. J. New York Bot. Gard. 12: 61-72.
Howes, F. N. 1953. Vegetable tanning materials. Butterworth's Sci. Publ., London, pp. 77-79.
Howmiller, R. and A. Weiner. 1968. A limnological study of a mangrove lagoon in the Galapagos. Ecology 49: 1184-1186.
Huberman, M. A. 1957. Mangrove silviculture. Trop. Silvic. 13: 188-195.
Huek, K. 1969. Los mangalares de America. Description de arboles forstales, No. 5. Bol Inst. For. Lat. Amer., Merida, Venezuela.
Huggard, E. R. 1960. Logging road construction. Proc. 5th World For. Congr. 3: 1921-1926.
Hummel, K. 1930. Tierfahrtenbilder vom Tropenstrand. Natur und Mus. 60.
Hunter, J. B. 1970. A survey of the oyster population of the Freetown estuary, Sierra Leone, with notes on the ecology, cultivation, and possible utilization of mangrove oysters. Trop. Sci. 11: 276-285.
Ito, T. 1899a. Rhizophoraceae in Japan. Ann. Bot. 13: 465-466.
Ito, T. 1899b. Mangroves growing in Japan. Nature 60: 79.

Ivens, G. W. 1957. Arboricides for killing mangroves. W. Afr. Rice Res. Stat. Per. Sci. Rep. No. 8, 6 pp.
Jackson, C. R. 1952. Some topographic and edaphic factors affecting distribution in a tidal marsh. Q. J. Fla. Acad. Sci. 15: 137-146.
Jackson, G. 1964. Notes on west African vegetation: I. Mangrove vegetation at Ikorodu, western Nigeria. West Afr. Sci. Assoc. J. 9: 98-111.
Jackson, M. L. 1958. Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, N. J. 498 pp.
Jacques-Felix, H. 1957. Les*Rhizophora* de la mangrove atlantique d'Afrique. J. Agric. Trop. Bot. Appl. 4: 343-347.
Jakobi, H. 1955. O genero Enhydrosoma no manguezal da costa S. Paulo-Parana (Harpacticoidea-Crustacea). Dusenia 6: 89-96.
Janssonius, H. H. 1950. Vessels in the wood of Javan mangrove trees. Blumea 6: 465-469.
Jardine, F. 1928. The topography of the Townsville littoral. Rep. Great Barrier Reef Comm. 2: 70-87.
Jenik, J. 1967. Root adaptations in west African trees. J. Linn. Soc. London Bot. 60: 136-140.
Jenik, J. and G. W. Lawson. 1968. Zonation of microclimate and vegetation on a tropical shore in Ghana. Oilos 19: 198-205.
Jennings, D. H. 1968. Halophytes, succulence, and socium-a unified theory. New Phytol. 67: 899-911.
Jennings, J. N. and C. F. Bird. 1967. . Regional geomorphological characteristics of some Australian estuaries. *in* Estuaries, G. H. Lauff (ed.), AAAS, Washington, pp. 121-128.
Johnson, I. M. 1949. The botany of San Jose Island (Gulf of Panama). Sargentia 8: 1-306.
Johow, F. 1884. Vegetationsbilder aus West-Indien und Venezuela. I. Die Mangrove-Sumpfe. Kosmos, Z. ges. Entwicklungs. 14: 415-426.
Joly, A. B. and N. Yamaguishi-Tomita. 1967. *Dawsoniella bostrychiae:* a new parasite of mangrove algae. Sellowia 19: 63-70.
Jong, B. de. 1934. Ueber Mangrove Cultiven. Tectona 27: 258-298.
Jonker, F. P. 1942. Rhizophoraceae in Pulle. Fl. Suriname 3: 36.
Jonker, F. P. 1959. The genus *Rhizophora* in Suriname. Acta Bot. Neerl. 8: 58-60.
Jonker, H. A. J. 1933. De vloedbosschen van den Riouw lingga Archipel. Tectona 26: 717-741.
Jordan, H. D. 1954. The development of rice research in Sierra Leone. Trop. Agri. Trinidad 31: 27-32.
Jordan, H. D. 1959. The utilization of saline mangrove soils for rice growing. Trans. 3rd int. Afr. Soils Conf., Dalaba, pp. 327-331.
Jordan, H. D. 1964. The relation of vegetation and soil to the development of mangrove swamps for rice growing in Sierra Leone. J. appl. Ecol. 1: 209-212.
Jost, L. 1887. Kenntnis der Athmungsorgane der Pflanzen. Bot. Z. 45:

601-606, 617-628, 633-642.
Kalk, M. 1959. The zoogeographical composition of the intertidal fauna at Inhaca Island, Mozambique. S. Afr. J. Sci. 55: 178.
Kalk, M. 1959. The fauna of the shores of northern Moxambique. Rev. de Biol., Lisbon 2: 1.
Kanehira, R. 1938. On the genus *Sonneratia* in Japan. J. Jap. Bot. 14: 421-424.
Karny, H. 1928a. Signs of life in mangrove vegetation of Java. Palaeobiologica 1: 475-580.
Karny, H. 1928b. Lebensspuren in der mangroveformation Javas (Ein Beitrag zur Losung des Flyschproblems-Aus einum Briefan O. Abel). Paleobiologica 1: 475-480.
Karsten, G. 1891. Ueber die Mangrove-Vegetation im Malayschen Archipel. Eine Morphologische-biologische Studie. Bibl. Bot. 22: 1-71.
Karsten, G. 1905. Die Mangrovevegetation. Vegetationsbilder 2: 7-12.
Karyone, T. 1927. The mangrove of the South Sea Islands. J. Jap. Bot. 116-120.
Kassas, M. and M. A. Zahran. 1967. On the ecology of the Red Sea littoral salt marsh, Egypt. Ecol. Monogor. 37: 297-316.
Kato, K. 1966a. Geochemical studies on the mangrove region of Cananeia, Brazil. I. Tidal variations of water properties. Bol. Inst. Oceanogr. 15: 13-20.
Kato, K. 1966b. Geochemical studies on the mangrove region of Cannaneia, Brazil. II. Physico-chemical observations on the reduction states. Bol. Inst. Oceanogr. 15: 21-24.
Kawano, Y., H. Matsumoto, and R. Hamilton. 1961. Plant products of economic importance in Hawaii. II. Tannins. Tech. Prog. Rep. No. 130, Univ. Hawaii, Agric. Exper. Stn., Honolulu. pp. 1-14.
Keast, A. 1959. Australian birds, their zoogeography and adaptations to an arid climate. *in* Biogeogrphy and Ecology in Australia, A. Deast, R. L. Cracker, and C. S. Christian (eds.), pp. 89-114.
Keay, R. W. J. 1953. *Rhizophora* in west Africa. Kew Bull. 1: 121-127.
Kehar, N. D. and S. S. Negi. 1953. Mangrove leaves as cattle feed. Sci. Cult. 18: 382-382.
Kehar, N. D. and S. S. Negir. 1954. Mangrove leaves as cattle feed. Sci. Cult. 19: 556-557.
Keller, B. 1925. Halophyten und Zerophyten-studien. J. Ecol. 13: 224-261.
Kellett, J. 1969. Looking back on the Florida freeze of '62. Principle 13: 23-35.
Keng, H. 1952. Mangrove in Taiwan. Taiwan Agric. For. Month. 6: 38-39.
Kerr, A. 1928. Kaw Tao, its physical features and vegetation. J. Siam Soc. Nat. Hist. Suppl. 7: 137-149.
Khan, S. A. 1956. Mangrove forests of Andhra State. Proc. Silvic. Conf., Dehra Dun 9: 182-186.

Khan, S. A. 1961. Regeneration of *Avicennia officinalis* in the coastal forests of West Pakistan. Pak. J. For. 11: 43-45.

Kiener, A. 1966. Contributions a l'etude ecologique et biologique des eaux saumatres malagaches. Les poissons euryhalins et leur role dans le developpement des peches. Vie Milieu 16: 1013-1149.

Keinholz, R. 1928. Environmental factors of Philippine beaches with particular reference to the beach at Puerto Galera, Mindoro. Philipp. J. Sci. 36: 199-213.

Kint, A. 1934. De Luchtfoto en de topografische terreingsteldheid in de Mangrove. De Trop. Natuur 23: 173-189.

Kipp-Goller, T. 1940. Uber Bau and Entwicklung der viviparen Mangrovekeimlinge. Z. f. Bot. 35: 1-40.

Koerner, T. 1904. Encorces tannantes de mangliers. J. Agric. Trop. 4: 113.

Kohlmeyer, J. 1966. Neue Meirespilze an Mangroven. Ber. Dtsch. bot. Ges. 79: 27-37.

Kohlmeyer, J. 1968. A new *Trematosphaeria* from roots of *Rhizophorra racemosa*. Mycopathol. Mycol. Appl. 34: 1-5.

Kohlmeyer, J. 1969. Ecological notes on fungi in mangrove forests. Trans. Brit. Mycol. Soc. 53: 237-250.

Kohlmeyer, J. 1968. Marine fungi from the tropics. Mycologia 40: 252-270.

Kohlmeyer, J. 1969. Marine fungi of Hawaii including the new genus *Heliascus*. Can. J. Bot. 47: 1469-1487.

Kohlmeyer, J. and E. Kohlmeyer. 1965. New marine fungi from mangroves and trees along eroding shorelines. Nova Hedwiga 9: 89-104.

Kohlmeyer, J. and E. Kohlmeyer. 1971. Marine fungi from tropical America and Africa. Mycologia 63: 831-863.

Kolwitz, R. 1933. Zur Okologie der Pflanzenwelt Brasiliens. Ber. Dtsch. bot. Ges. 51: 396-406.

Koriba, K. 1958. On the periodicity of tree growth in the tropics. Gard. Bull. Singapore 17: 11-81.

Korner, A. 1900. Bericht uber weiter Untersuchungen von Mangrove-Extrakten. Jahresbericht der Deutschen Gerberschule Freiberg in Sachsen. Ferb. Freiberg, 1900.

Kostermans, A. J. G. H. 1959. Monograph of the genus *Heritera* Aitron (Stercul.). Reinwardtia 4: 465-583.

Kostermans, A. J. G. H. 1961. A monograph of the genus *Brownlowia* Roxb. (Tiliaceae). Communs For. Res. Inst. Bogor, No. 73, 62 pp.

Krejci-Graf, K. 1935. Beobachtungen am Tropenstrand I – IV. Senckenbergiana 17: 1-242.

Krejci-Graf, K. 1937. Uber Fahrten und Bauten tropischer Krabben. Geol. Meere und Binnengewasser 1: 39-46.

Kudo, Y. 1932. The mangrove of Formosa. Bot. Mag. Tokyo 46: 147-156.

Kuenzler, E. J. 1969. Mangrove swamp systems. *in* Coastal Ecological Systems of the United States. H. T. Odum, B. J. Copeland and E. A. McMahon (eds.), Inst. Mar. Sci. Univ. North Carolina, Vol. 1, pp. 353-383.

Kumar, L. S. S. and W. V. Joshi. 1942. False polyembryony in viviparous *Rhizophora mucronata* Lam. Curr. Sci. 11: 242.
Kunkel, G. 1965. Der Standort: Kompetenzfactor in der Stelzwurzelhildung. Biol. Centrablatt 84: 641-651.
Kunkel, G. 1966. Uber die Struktur und Sukzession der Mangrove Liberias und deren Randformationen. Ber. Schweiz. bot. Ges. 75: 20-40.
Kylin, A. and R. Gee. 1970. Adenosine triphosphatase activities in leaves of the mangrove *Avicennia nitida* Jacq. Pl. Physiol. 45: 169-172.
Laessle, A. M. and C. H. Wharton. 1959. Northern extensions in recorded ranges of plants on Seahorse and associated keys, Levy County, Florida. Q. J. Fla. Acad. Sci. 22: 105-113.
Lafond, J. 1957. Apercu sur la sedimentologie de l'estuaire de la Betsiboka (Madagascar). Rev. Inst. Fr. Ret. 12: 425-431.
Lagerwerff, J. V. 1958. Comparable effects of absorbed and dissolved cations on plant growth. Soil Sci. 86: 63-69.
Lakhanpal, R. W. 1952. *Nipa sahnii*, a palm fruit in the Tertiary of Assam. Paleobotanist 1: 289-294.
Lamb, F. B. 1959a. Prospects for forest land management in Panama. Trop. Woods 110: 16-28.
Lamb, F. B. 1959b. The coastal swamp forests of Navico, Colombia. Caribb. For. 20:79-89.
Lambert, F. and P. Polk. 1971. Observations sur l'ostreculture a Cuba. Hydrobiologia 38: 9-14.
Lamberti, A. 1969. Contribuicao ao Conhecimento de Ecologic das Plantas do Manguez al de Itanhaem. Bol. No. 317, Bot. No. 23, Fac. Fil., Cien. e Let. Univ. Sao Paulo, Brazil, 217 pp.
Landon, F. H. 1933. Planting in mangrove forests. Malay. For. 2: 131-133.
Landon, F. H. 1942. Mangrove volume tables. Malay. For. 11: 117-123.
Langenheim, J. H., B. L. Hackner, and A. Bartlett. 1967. Mangrove pollen at the depositional site of Oligo-Miocene amber from Chiapas, Mexico. Bot. Mus. Leafl. Harv. Univ. 21: 289-324.
LaRue, C. D. and T. J. Musik. 1951a. Mangrove vivipary. Rep. Fed. Exp. Stn. Puerto Rico, No. 13.
LaRue, C. D. and T. J. Musik. 1951b. Does the mangrove really plant its seedlings? Science 114: 661-662.
LaRue, C. D. and T. Jm Musik. 1954. Growth, regeneration, and prevocious rooting in *Rhizophora mangle*. Pap. Mich. Acad. Sci. Arts Lett. 39: 9-29.
Lawrence, D. B. 1949. Self-erecting habit of seedling red mangroves (*Rhizophora mangle* L.). Am. J. Bot. 36: 426-427.
Lawson, G. W. 1966. The littoral ecology of West Africa. Ocean et Mar. biol. Ann. Rev. 4: 405-448.
Le Cointe, P. 1947. Arvores e Plantas Uteis. Amazonia Brasiliera III. 2nd ed. Companhia Editora Nacional, Sao Paulo, Brazil, p. 283.
Lee, B. K. H. and G. E. Baker. 1972. An ecological study of the soil microfungi in a Hawaiian mangrove swamp. Pac. Sci. 26: 1-10.

Lee, B. K. H. and G. E. Baker. 1972. Environment and the distribution of microfungi in a Hawaiian mangrove swamp. Pac. Sci. 26: 11-19.

Leechman, A. 1908. The genus *Rhizophora* in British Guiana. Kew Bull. 1908: 4-8.

Leeuwen, W. D. von. 1911. Uber die Ursache der widerholten Verzweigung der Stutzwurzeln von *Rhizophora.* Ber. Dtsch. bot. Ges. 29: 476-478.

Lendrum, N. G. 1961. Problems of coastal development of Florida. Shore and Beach 29: 27-32.

Leopold, A. S. 1950. Vegetation zones of Mexico. Ecology 31: 507-518.

Lever, R. J. A. W. 1952. A malayan leaf tying caterpiller from mangroves periodically submerged in sea water. Entomol. Mon. Mag. 88: 95-98.

Lewin, R. A. 1970. Toxin secretion and tail autotomy by irritated *Oxynoe panamensis* (Opistobranchiata; Sacoglossa). Pac. Sci. 24: 356-358.

Lewis, J. 1956. Rhizophoraceae. Fl. Trop. E. Afr. Oct., 20 pp.

Lewis, O. A. M. and G. Naidoo. 1970. Tidal influence on the apparent transpirational rhythms of the white mangrove. S. Afr. J. Sci. 66: 268-270.

Li, Shang-Kuei. 1948. Distribution of mangroves in Taiwan. Taiwan For. Exp. Stn. Rep. 41-48: 337-339.

Liebau, O. 1914. Beitrage zur Anatomie und Morphologie der Mangrove-Pflanzen, insbesondere ihres Wurzelsystems. Beitr. Biol. Pfl. 12: 181-213.

Lindeman, J. C. 1953. The vegetation of Suriname. Vol 1, Part 1. The vegetation of the coastal region of Suriname. Paramaibo I - IV: 1-135.

Linke, O. 1939. Die Biota des Jadebusenwattes. Helgol. wiss. Meeres. 1: 201-348.

Little, E. L., Jr. 1945. Miscellaneous notes on nomenclaure of United States trees. Am. Midl. Nat. 33: 495-513.

Little, E. L., Jr. 1953. Check List of Native and Naturalized Trees of the United States (Including Alaska). Agric. Handbook 41, U.S.D.A. For. Serv., Washington, D. C. p. 365.

Little, E. L., Jr. 1961. *Avicennia nitida.* phytologia 8: 49-57.

Little, E. L., Jr. and F. H. Wadsworth. 1964. Common Trees of Puerto Rico and the Virgin Islands. Agric. Handb. 249, U.S.D.A. For. Serv., Washington, D. C.

Liu, T. 1955. Notes on mangroves in Taiwan. Taiwan For. 1: 1-4.

Liu, T. 1956. Mangrove forests. For. Bull. (Natl. Taiwan Univ.) 2: 1-25.

Loder, J. W. and G. B. Russell. 1969. Tumor inhibitory plants. The alkaloids of. *Bruguiera sexangula* and *Bruguiera exaristata.* Aust. J. Chem. 22: 1271-1276.

Long, R. W. and O. Lakela. 1971. A Flora of Tropical Florida. Univ. Miami Press, Coral Gables, Florida, 962 pp.

Lotschert, W. 1955. Die Mangrove von El Salvador. Unsch. wiss. Tech. 55: 47-50.

Lotschert, W. 1960. Die Mangrove von El Salvador. Ber. D. Senckenberg Naturforsch. Ges. 90: 213-224.

Lotschert, W. 1968. Speichern die keimlinge von Mangrovepflanzen Salz? Umsch. wiss. Tech. 68: 20-21.

Lotschert, W. and F. Liemann. 1967. Die Salzspeicherung im Keimling von *Rhizophora mangle* L. wahrend der Entwicklung auf den Mitterpflanze. Planta 77: 136-156.

Loveless, A. R. 1960. The vegetation of Antigua. J. Ecol. 48: 495-527.

Loveless, C. M. 1959. A study of the vegetation of the Florida Everglades. Ecology 40: 1-9.

Lowe-McConnell, R. H. 1967. Notes on the nesting of the Boat-bill, *Cochlearius cochlearius*. Ibis 109: 179.

Luederwaldt, H. 1919. Os manguesaes de Dantos. Rev. Mus. Paul. 11: 309-408.

Luytjes, A. 1923. De vloedbosschen van Atjeh. Tectonia 16: 575-601.

Macbride, J. F. 1960. Flora of Peru. Field Mus. Nat. Hist. Bot. Ser. 13: 72.

MacCaughey, V. 1917. The mangrove in the Hawaiian Islands. Hawaiian For. Agr. 14: 361-366.

MacCaughey, V. 1918. The strand flora of the Hawaiian archipelago. Bull. Torrey Bot. Club 45: 259-277.

MacKay, J. B. and V. J. Chapman. 19544. Some notes on *Suaeda australis* Moq. var. *nova zelandica* var. nov. and *Mesembryanthemum australe* Sol. ex Forst. Trans. R. Soc. N. Z. 82: 41-47.

Macluskie, H. 1952. The reclamation of mangrove swamp areas for rice cultiviation. World Crops 2: 129-132.

Macnae, W. 1956. Aspects of life on muddy shores in South Africa. S. Afr. J. Sci. 53: 40-43.

Macnae, W. 1957. The ecology of the plants and animals in the intertidal regions of the Zwartkops estuary, near Port Elizabeth, South Africa. J. Ecol. 45: 113-131 and 361-387.

Macnae, W 1962. The fauna and flora of the eastern coasts of southern Africa in relation to ocean currents. S. Afr. J. Sci. 58: 208-212.

Macnae, W. 1963. Mangrove swamps in South Africa. J. Ecol. 51: 1-25.

Macnae, W. 1966. Mangroves in eastern and southern Australia. Aust. J. Bot. 14: 67-104.

Macnae, W. 1967. Zonation within mangroves associated with estuaries in north Queesland. *in* Estuaries, G. H. Lauff (ed.), AAAS Publ. 83: 432-441.

Macnae, W. 1968a. A general account of the fauna and flora of mangrove swamps and forests in the Indo-West-Pacific region. Adv. Mar. Biol. 6: 73-270.

Macnae, W. 1968b. Mangroves and their fauna. Aust. nNat. Hist. 16: 17-21.

Macnae, W. and M. Kalk. 1962a. The mangrove swamps of Inhaca Island. J. Ecol. 50: 19-34.

Macnae, W. and M. Kalk. 1962b. The fauna and flora of sand flats at Inhaca Island, Mozambique. J. Anim. Ecol. 31: 93-128.

Mahabale, T. S. and J. V. Deshpande. 1959. The genus *Sonneratia* and its fossil allies. Palaeobotanist 6: 51-64.

Malaviya, M. 1963. On the distribution, structure and ontogeny of stone cells in *Avicennia officinalis* L. Proc. Indian Acad. Sci., Sect. B 58: 45-50.
Malcolm, C. V. 1964. Effects of salt, temperature, and seed scarification on germination of two varieties of *Arthrocnemum halocnemoides.* Jour. Proc. R. Soc. W. Aust. 47: 72-74.
Many authors. 1959. Los Manglares en America. Inst. For. Latinoamer., Merida, Venezuela, 52 pp.
Marathe, K. V. 1965. A study of the subterranean algal flora of some mangrove swamps. J. Indian Soc. Soil Sci. 13: 81084.
Marco, H. F. 1935. Systematic anatomy of the woods of the Rhizophoraceae. Trop. Woods 44: 1-26.
Marcus, B. 1941. Over den slibbodem van zoutwatervijvers. Landbouw 17: 891-910.
Margolef, F. 1962. Comunidades naturales. Inst. Biol. Mar. Univ. Puerto Rico, Spec. Pub., 499 pp.
Marshall, R. C. 1939. The Silviculture of the Trees of Trinidad and Tobago, British West Indies. Oxford Univ. Press, London, 247 pp.
Martinez, M. 1959a. Plantas Medicinales de Mexico, 4th ed., Ediciones Botas, Mexico, pp. 212-215.
Martinez, M. 1959b. Plantas Utiles de la Flora Mexicana. Revised edition, Ediciones Botas, Mexico, pp. 397-401.
Martyn, E. B. 1934. A note on the foreshore vegetation in the neighborhood of Georgetown, British Guiana, with special reference to *Spartina braziliensis.* J. Ecol. 22: 292-298.
Masamune, G. 1930. Contributions to be phytogeography of Japan. I. Trans. Nat. Hist. Soc. Formosa 20: 299-304 (in Japanese).
Mattox, N. T. 1949. Studies on the biology of the edible oyster, *Ostrea rhizophorae* Guilding, in Puerto Rico. Ecol. Monogr. 19: 339-356.
Mauritzon, J. 1939. Contributions to the embryology of the orders Rosales and Myratales. Lunds Univ. Arsskr. II. Sec. 2, 35: 1-120.
Mayr, E. 1944. Wallace's Line in the light of recent zoogeographical studies. Q. Rev. Biol. 19: 1-14.
MaAdam, J. B. 1952. Forestry in New Guinea. Sci. Soc. Ann. Rep. Proc., New Guinea, pp. 41-61.
McArthur, W. M. 1957. The plant ecology of Garden Island in relation to the neighboring islands and the adjacent mainland. Proc. R. Soc. W. Aust. 40: 46-64.
McGill, J. T. 1958. Map of coastal landforms of the world. Geog. Rev. 48: 402-405.
McMillan, C. 1971. Environmental factors affecting seedling establishment of the black mangrove on the central Texas Coast. Ecology 52: 927-930.
Mead, J. P. 1912. The mangrove forests of the west coast of the Federated Malay States. Govt. Print. Office, Kuala Lumpur.
Meer Mohr, J. C. 1929. Abnormal fruits in *Rhizophora conjugataa.* Rec. Trans. Bot. Neerl. 26: 15-18.

Meindersma, H. W., J. H. Becking, and L. G. den Berger. 1922. Vloed-of mangrovebouschen in Ned-Indie. Tectona 15: 561-611.
Mell, C. D. 1919. The mangroves of tropical America. Sci. Am. Suppl. 2292: 388-389.
Merrill, A. C. 1905. A review of the identification of the species described in Blanco's Flora de Filipinas. Dept. Int., Bur. Govt. Labs., Manila, Bull. No. 27.
Metcalf, C. R. 1931. The breathing roots of *Sonneratia* and *Bruguieraa*, a review of recent work by Troll and Dragendorff. Kew Bull. 11: 465-467.
Millard, N. A. H. and A. D. Harrison. 1953. The ecology of South African estuaries. Part V. Richards Bay. Trans. R. Soc. S. Afr. 34: 157-171.
Miller, A. H., H. Friedmann, L. Griscom, and R. T. Moore. 1957. Distributional check-list of the birds of Mexico. Part 2. Pacific Coast Avifauna, No. 33, 436 pp.
Miller, O. B. 1926. Mangroves at Sordivana Bay, N. Zululand. S. Afr. J. Nat. Hist. 6: 54-55.
Miller, P. C. 1972. Bioclimate, leaf temperature, and primary production in red mangrove canopies in south Florida. Ecology 53: 22-45.
Miller, R. C. 1957. Marine wood-boring molluscs of the Philippines. Pac. Sci. Congr. 3A: 1575-1577.
Millspaugh, C. F. 1907. Flora of the sand keys of Florida. Field Mus. Publ. No. 118. pp. 191-243.
Miquel, F. A. G. 1855-1859. Flora van Nederlandsch Indie. Flora Indiae Batavae, vols. 1 and 2. Leipzig.
Miranda, F. 1958. Estuadios acerea de la vegetacion. *in* Los Recursos Naturales del Surests y su Aprovechamiento, E. Beltran (ed.), Publ. Inst. Mex. Rec. Nat. Renov. 2: 215-271.
Miranda, M. 1952. La vegetacion de Chiapas. Part II. Dept. de Prensa y Turismo, Tuxtla Gutierrez, Chiapas, Mexico, pp. 119-120.
Miyata, I., N. Odani, and Y. Ono. 1963. An analysis of Jo-method of dispersion of the population of *Bruguiera conjugata* (L.) Merrill. Rep. Comm. For. Sci. Res., Kyushu Univ., No. 1, pp. 43-48.
Mogg, A. O. D. 1963. A preliminary investigation of the significance of salinity in the zonation of species in salt-marsh and mangrove swamp associations. S. Afr. J. Sci. 59: 81-86.
Mokievskii, O. B. 1969. Biogeotsenotischeskaya sistema litorali. Okeanologiya 9: 211-222.
Moldenke, H. N. 1958. Hybridity in the Verbenaceae. Am. Midl. Nat. 59: 333-370.
Moldenke, H. N. 1960a. Materials toward a monograph of the genus *Avicennia*. I. Phytologia 7: 123-168.
Moldenke, H. N. 1960b. Materials toward a monograph of the genus *Avicennis*. II. Phytologia 7: 179-232.
Moldenke, H. N. 1960c. Materials toward a monograph of the genus *Avicennia*. III. Phytologia 7: 259-292.

Moldenke, H. N. 1963. Avicenniaceae, Symphoremaceae and Verbenaceae. Studies on the flora of Thailand, 1963. Dansk Bot. Ark. 23: 83-92.
Moldenke, H. N. 1967a. Additional notes on the genus *Avicennia* I. Phytologia 14: 301-320.
Moldenke, H. N. 1967b. Additonal notes on the genus *Avicennia* II. Phytologia 14: 326-336.
Moldenke, H. N. 1968a. Additional notes on the genus *Avicennia.* III. Phytologia 15: 71-72.
Moldenke, H. N. 1968b. Additonal notes on the genus *Avicennia.* IV. Phytologia 15: 470-478.
Monod, T. and R. Schnell. 1952. Les groupements et les unites geobotaniques de la region Guineenne (Mangroves). Mem. De l'Instit. Franc. d'Afrique Noire 18: 197-218.
Montfort, C. and W. Brandrup. 1927. Physiologische und Pflanzen geographische See Salzwirkungen. II. Okologische Studien uber Keimung und erste Entwicklung bei Halophyten. Jahrb. Wiss. Bot. 66: 902-946.
Moore, H. B., L. T. Davies, T. H. Frazer, R. H. Gore, and N. R. Lopez. 1968. Some biomass figures from a tidal flat in Biscayne Bay, Florida. Bull. Mar. Sci. 18: 261-269.
Moreira Filho, H. and M. B. Kutner. 1962. Contribuicao para o conhecimento das diatomaceas do manguesae de Alexandra. Bol. Univ. Parana Bot. 4: 1-24.
Morton, J. F. 1965. Can the red mangrove provide food, feed and fertilizer? Econ. Bot. 19: 113-123.
Moul, E. T. 1957. Preliminary report on the flora of Onotoa Atoll. Atoll Res. Bull. 57, Sept. 15.
Moss, C. E. 1954. The species of *Arthrocnemum* and *Salicornia* in southern Africa. J. S. Afr. Bot. 20: 1-22.
Muir, J. 1937. The seed drift of South Africa, and some influences of ocean currents on the strand vegetation. Mem. Bot. Sur. S. Afr. 16: 7-14.
Mullan, D. P. 1931. On the occurrence of glandular hairs (slat glands) on the leaves of some Indian halophytes. J. Indian Bot. Soc. 10: 184-189.
Mullan, D. P. 1931. Observations on the water storing devices in the leaves of some Indian halophytes. J. Indian Bot. Soc. 10: 126-133 and 184-191.
Mullan, D. P. 1932. Observations on the biology and physiological anatomy of some Indian halophytes. I. J. Indian Bot. Soc. 11: 103-118 and 285-302.
Mullan, D. P. 1933a. Observations on the biology and physiological anatomy of some Indian halophytes. II. J. Indian Bot. Soc. 12: 165-182.
Mullan, D. P. 1933b. Observations on the biology and physiological anatomy of some Indian halophytes. III. J. Indian Bot. Soc. 12: 235-256.
Muller, J. 1961. A palynological contribution to the history of the mangrove vegetation. Pac. Sci. Congr., 1960, pp. 146-147.
Muller, J. 1964. A palynological contribution to the history of the mangrove vegetation in Borneo. *in* "Ancient Pacific Floras", Univ. Hawaii Press,

Honolulu, pp. 33-42.
Muller, J. 1968. Palynology of the Pedowan and Plateau sandstone formations (Cretaceous-Eocene) in Savanak, Malaysia. Micropalaentology 14: 1-37.
Muller, J. and S. Y. Hou-Liu. 1966. Hybrids and chromosomes in the genus *Sonneratia* (Sonneratiaceae). Blumea 14: 337-343.
Muller, J. and G. G. J. van Stennis. 1968. The genus *Sonneratia* in Australia. North Queensland Not. 35: 6-8.
Murphy, R. C. 1938. The littoral of the Pacific coasts of Colombia and Equador. Geogr. Rev. 29: 1-33.
Myers, J. G. 1933. Notes on the vegetation of the Venezuelan Llanos. J. Ecol. 21: 335-349.
Myers, J. G. 1935. Zonation along river courses. J. Ecol. 23: 356-360.
Nakanishi, S. 1964. An epiphytic community on the mangrove tree, *Kandelia kandel* (Rhizophoraceae). Hikobia 4: 124.
Nash, G. V. 1908. The story of mangrove. Torreya 8: 73-78.
Navalkar, B. S. 1940. Studies in the ecology of mangroves. I. Determination of the osmotic pressure of *Avicennia alba* Blume. J. Univ. Bombay 8: 1-19.
Navalkar, B. S. 1942. Studies in the ecology of mangroves. II. Physical factors of the mangrove soil. J. Univ. Bombay 9: 97-106.
Navalkar, B. S. 1948. Studies in the ecology of mangroves III. J. Univ. Bombay 16: 35-45.
Navalkar, B. S. 1952. Succession of the mangrove vegetation of Bombay and Salsette Island. J. Bombay Nat. Hist. Soc. 50: 157-161.
Navalkar, B. S. 1953. The analytical aspects of some of the marshy vegetation of Bombay and Salsette islands. J. Bombay Nat. Hist. Soc. 51: 636-652.
Navalkar, B. S. 1956. Geographical distribution of halophytic plants of Bombay and Salsette islands. J. Bombay Nat. Hist. Soc. 53: 335-343.
Navalkar, B. S. 1959. Studies in the ecology of mangroves. VII. Humus content of mangrove soils of Bombay and Salsette islands. J. Univ. Bombay 28: 6-10.
Navalkar, B. S. and F. R. Bharucha. 1948. Studies in the ecology of mangroves. IV. The hydrogen ion concentration of the seawater, soil solution, and the leaf cell sap of mangroves. J. Univ. Bombay 17: 35-45.
Navalkar, B. S. and F. R. Bharucha. 1949. Studies in the ecology of mangroves. V. Chemical factors of mangrove soils. J. Univ. Bombay 17: 17-35.
Navalkar, B. S. and F. R. Bharucha. 1950. Studies in the ecology of mangroves. VI. Exchangeable bases of mangrove soils. J. Univ. Bombay 18: 7-16.
Newell, N., J. Imbrie, E. G. Purdy, and D. Thurber. 1959. Organism communities and bottom facies, Great Bahama Bank. Bull. Am. Mus. Nat. Hist. 117: 181-228.
Nicolic, M. and S. A. Melendez. 1968. El ostion del mangle, *Crassostrea rhizophorae* Guilding 1928. Experimentos indicales en el cultivo. Nota

Invest Cent. Pesqueras Buta 7: 1-30.
Nisbet, I. C. T. 1968. The utilization of mangroves by malayan birds. Ibis 110: 348-352.
Noakes, D. S. P. 1950. The mangrove charcoal industry. Malay. For. 24: 201-203.
Noakes, D. S. P. 1951. Notes on the silviculture of the mangrove forests of Matang, Perak. Malay. For. 14: 183-196.
Noakes, D. S. P. 1955. Methods of increasing growth and obtaining natural regeneration of the mangrove tupe in Malaya. Malay. For. 18: 23-30.
Noakes, D. S. P. 1957. Mangrove. Trop. Silvic. 2: 309-318.
Noakes, D. S. P. 1958. Mangrove. Wld. For. Congr. 3: 415-424.
Noamesi, G. K. 1959. A revision of the Zylocarpeae (Meliaceae). Diss. Abstr. 19: 1531.
Noel, A. R. A. 1959. The vegetation of the fresh water swamps of Inhaca Island. S. Afr. J. Bot. 25: 189-201.
Nudaliar, C. R. and H. Sunandar. 1952. Distribution of *Rhizophora mucronata* Lam. in the "backwater" of the west coast and its economic importance. Madras Agric. J. 610-645.
Odani, N. 1964. An analysis of the mangrove communities of Iriomoti-jima, Yaeyama Group, the Tyukyus. Rep. Comm. For. Sci. Res., Kyushu Univ., No. 2, June.
Odum, E. P. 1971. Fundamentals of Ecology. 3rd ed., W. B. Saunders Co., Philadelphia, 574 pp.
Odum, H. T. 1971. Environment, Power and Society. John Wiley and Sons, Inc., New York, 331 pp.
Odum, W. E. 1971. Pathways of energy flow in a south Florida estuary. Univ. Miami, Sea Grant Tech. Bull. No. 7, 162 pp.
Ogura, Y. 1942. Mangroves in eastern Asia. Bot. and Zool. 10: 145-149 (in Japanese).
Ogura, Y. 1958. Some problems on the mangrove. Bull. Yokohama Municip. Univ. Soc. 10: 545-578.
Okuda, T., A. J. Garcia, and J. B. Alvarez. 1965. Variacion estacional de los elementos nutritivos en el agua de la Laguna Y el Rio Unare. Bol. Inst. Oceanogr. Univ. Oriente 4: 123-135.
Orians, G. H. And E. W. Pfeiffer. 1970. Ecological effects of the war in Vietnam. Science 168: 544-554.
Orr, A. P. and F. W. Newhouse. 1933. Physical and chemical conditions in mangrove swamps. Br. Mus. Nat. Hist. Sci. Rep., Great Barrier Reef Exped. 1928-1929. 2: 102-110.
Ostenfeld, C. H. 1918. Stray notes from tropical west Australia. Dan. Bot. Ark. 2: 1-29.
Outred, H. A. 1966. The effects of low oxygen tensions upon the intensity and pattern of respiration in certain mangrove seedlings. M. Sc. Thesis, Auckland Univ., Auckland, N. Z.

Oyama, K. 1950. Studies of fossil biocoenosis. No. 1. Biocoenological studies on the mangrove swamps, with descriptions of new species from Yatuo Group. Rep. Geol. Surv. Japan 132: 1-14.

Padmanabhan, D. 1960. The embryology of *Avicennia officinalis* L. I. Floral Morphology and gametophytes. Proc Indian Acad. Sci. Sect. B, 52: 131-145.

Padmanabhan, D. 1962a. The embryology of *Avicennis officinalis* L. III. The embryo. J. Madras Univ. 32B: 1-19.

Padmanabhan, D. 1962b. The embryology of *Avicennia officinalis* L. The seedling. Proc. Indian Acad. Sci. 56: 114-122.

Paijmans, K. 1967. Lands of the Sofia-Pourgani area, Territory of Papua and New Guinea. VI. Vegetation of the Sofia-Pourgani area. CSIRO Aust. Land Res. Ser. 17: 142-167.

Paijmans, K. 1969. Lands of the Kerema-Vailala area, Papua-New Guinea. VIII. Vegetation and ecology of the Kerema-Vailala area. CSIRO Aust. Land Res. Ser. 23: 95-116.

Pal, N. 1951. On the embryology of Terminalia catappa. Sci. Cult. 17: 178-179.

Pammel, L. H. and C. N. King. 1948. Gerination studies of some trees and shrubs. Proc. Iowa Acad. Sci. 35: 184-197.

Pannier, P. 1959. El efecto de distintas concentrationes sotenas sobre el desarrolo de *Rhizophora mangle* L. Acta cient. Venez. 10: 68-78.

Pannier, P. 1962. Estudio fisiologico sobre la viviparia de *Rhizophora mangle* L. Acta cient. Venez. 13: 184-197.

Panshin, A. J. 1932. An anatomical study of the woods of the Philippine mangrove swamps. Philipp. J. Sci. 48: 143-208.

Panzer, W. 1935. Sandkrabben-Spuren an der Kuste von Neu-Guinea. Nat. Volk 65: 36-46.

Parham, J. W. 1964. Plants of the Fiju Islands. Govt. Press, Suva, Fiji.

Parkes, K. C. and R. W. Dickerman. 1967. A new subspecies of mangrove warbler (*Dendroica petechia*) from Mexico. Ann. Carnegie Mus. 39: 85-90.

Passler, J. 1912. Die untersuchgensergebnisse der Deutsch-Ostafrika eingesandtern Mangroverinden. Pflanzer Jg. 8: 65.

Patil, R. P. 1961. A note on the vegetation at Sazina Khali in the Sunderbans. Indian For. 87: 481-483.

Patil, R. P. 1964. Cultivation of mangrove seedlings in pots at Allahabad U.P. Sci. Cult. 30: 43-44.

Patil, R. P. and S. S. Sidhu. 1961. Seedling anatomy of *Bruguiera parviflora* W. and A. Proc. 48th Indian Sci. Congr. 3: 280.

Patton, R. T. 1942. Ecological studies in Victoria. VI. Salt marsh. Proc. R. Soc. Vict. 54: 131-144.

Paulsen, O. 1918. Chenopdiaceae from western Australia. Dan. Bot. Ark. 2: 56-66.

Payet, M., P. Pene, M. Sankale, G. Delorme, and H. Delatoure. 1962. Le micro-climat de la presque ile du Cap-Bert: son determinisme

physiologique. Bull. Soc. med. Afr. noire Lang. fr. 13: 40-58.
Paynter, R. A., Jr. 1955. The ornithogeography of the Yucatan Peninsula. Peabody Mus. Nat. Hist., Yale Univ., Bull. No. 9, 374 pp.
Pearman, R. W. 1955. Tanning materials of the British Commonwealth. Colonial Plant and Animal Products 5: 96.
Pearman, R. W. 1957. Mangrove bark – its value as a tanning material. Leather Trades Rev. 125: 315-316.
Pearse, A. S. 1932. Animals in brackish water pools at Dry Tortugas. Pap. Tortugas Lab. 28: 125-142.
Pellegrin, F. 1952. Les Rhizophoracees de l'Afrique Equatiriale Francaise. Mus. Nat. Hist. Nat. Notulae Syst. 14: 292-300.
Penfound, W. T. 1952. Southern swamps and marshes. Bot. Rev. 18: 413-446.
Penfound, W. T. and E. S. Hathaway. 1938. Plant communities in the marshlands of southeastern Louisiana. Ecol. Monogr. 8: 1-56.
Perrier de la Bathie, H. 1953. La mangrove (paletuviers) de Madagascar: biologie et utilite. Rev. Int. Bot. Appl. Agric. Trop. 33: 373-374 and 581-582.
Petit-Thouars, A. du. 1813. Notice sur le manglier. J. Bot. 2: 27-41.
Phillips, J. 1959. Agriculture and ecology in Africa. Flora 131: 1-40.
Phillips, O. P. 1903. How the mangrove tree adds new land to Florida. J. Geogr. 2: 10-21.
Pidgeon, I. 1940. The ecology of the central coastal area of New South Wales. III. Types of primary succession. Proc. Linn. Soc. N.S.W. 65: 221-249.
Pickening, C. 1879. Chronological History of Plants. Little, Brown and Co., Boston, 1222 pp.
Piexoto, A. R. 1959. Tropical-almound *Terminalia;* Fruit, oil, and tanning. Bol. Agric. Minas Gerais Dept. Prod. Veg. 8: 69-71.
Pijl, L. van der. 1969. Evolutionary action of tropical animals on the reproduction of plants. Biol. J. Linn. Soc. London 1: 85-96.
Pitot, A. 1958. Rhizophores et racines chez *Rhizophora* sp. Bull. Inst. Franc. Afr. Noire 20: 1103-1138.
Pittier, H. 1898. Primitiae florae costaricensis. Anal. Inst. fis.-geogr. Nacl. 8: 1-26.
Pittier, H. 1908. Ensayo Sobre las Plantas Usuales de Costa Rica. H. L. and J. B. McQueen, Washington, D. C., p. 112.
Pittier, H. 1926. Manual de las Plantas Usuales de Venezuela. Pub. by the author, Caracas, p. 286.
Pollard, C. L. 1902. Plant agencies in the formation of the Florida keys. Pl. World 5: 8-10.
Pope, W. T. 1929. Manual of Wayside Plants of Hawaii. Advertiser Pub. Co., Honolulu, pp. 157-159.
Porsch, O. 1924. Vogel blumenstudien. I. Jb. wiss. Bot. 63: 553-706.
Post, E. 1963a. Systematische und pflanzen-Geographische Notizen zur *Bostrichia-Caloglassa* Assoziation. Rev. Algel. 9: 1-84.

Post, E. 1963b. Zur Verbreitung und Okologie der *Bostrychia-Caloglossa* Assoziation. Int. Rev. ges. Hydrobiol. Hydrogr. 48: 47-152.

Prain, D. 1903. Flora of the Sunderbans. Rec. Bot. Surv. India 2: 231.

Prakash, U. 1960. A Survey of the Deccan Intertrappean beds flora of India. J. Palaentol. 34: 1027-1040.

Price, J. L. 1955. A survey of the freshwater fishes of the island of Trinidad. J. Agric. Soc. Trinidad and Tobago, Pap. No. 863: 1-28.

Price, W. A. 1954. Shorelines and coasts of the Gulf of Mexico. U. S. Fish. Bull. 89: 39-65.

Pustelnik, W. 1953. Analisis de la Corteza de Mangle Colorado (*Rhizophora mangle* L.) del oriente de Venezuela y su importancia en la elaboracion de extractos curientes solidos. Agron. Trop. 3: 107-116 (also in Rev. Soc. Venez. 5: 19-31).

Putnam, R. C. and A. V. Bowles. 1955. The chemistry of vegetable tannins. XI. Mangrove (Borneo cutch). J. Am. Leather Chemists Assoc. 50: 42-46.

Pynaert, L. 1933. La mangrove congolaise. Bull. Agric. Congo Bel. 23: 184-207.

Quesnel, V. C. 1964. Suitability of sulphurous acid for hydrolysis of condensed tannins. Tetrahedron Letters 48: 3699-3702.

Quinones, L. R. and J. F. Puncochar. 1943. Informe preliminar sobre la utilizacion practica de la corteza del mangrove. Caribb. For. 5: 44-47.

Rai, J. N., J. P. Tewari, and K. G. Mukerji. 1969. Mycoflora of mangrove mud. Mycopathol. Mycol. Appl. 38: 17-31.

Rains, D. W. and E. epstein. 1967. Prederential absorption of potassium by leaf tissue of the mangrove, *Avicennia marina:* an aspect of halophytic competence in coping with salt. Aust. J. Biol. Sci. 20: 847-857.

Rajagopalan, V. R. 1949. Cytological studies in Combretaceae I. Proc. Indian Sci. Congr. 36: 137-138.

Rao, A. N. and L. C. Wan. 1969. A new record of folian sclerids in *Scyphiphora hydrophyllacea.* Curr. Sci. 38: 594-595.

Rao, R. S. 1957. Observations on the mangrove vegetation of the Godavri Estuary. Proc. Mangrove Swamp Symp., Ministry of Food and Agriculture, Calcutta, India, pp. 36-44.

Raunkaiaer, C. 1934. The Life Forms of Plants and Statistical Plant geography, Being the Collected Papers of C. Raunkiaer. Oxford Univ. Press, Oxford, 632 pp.

Rawitscher, F. K. 1944. Algumas nocoes sobre a vegetacao do litoral brasileiro. Bol Assoc. dos Geogr. Bras. 5: 13-28.

Reinders-Gouwentak, C. A. 1953. Sonneratiaceae and other mangrove-swamp families, anatomical structure and water relations. Fl. Males. 4: 513-515.

Reintjes, J. W. 1963. The importance of the occurrence of menhaden in the coastal waters and estuaries of peninsular Florida. Proc. Gulf Caribb. Fish. Inst. 16: 108-113.

Rendle, A. B. and S. LeM. Moore. 1921. A systematic account of the plants

collected in New Caldonia and the Isle of Pines by Prof. R. H. Compton, 1914. I. Flowering plants (angiosperms). J. Linn. Soc. London 15: 245-418.

Revelle, R. and R. W. Fairbridge. 1957. Carbonates and carbon dioxide. Mem. geol. Soc. Am. 67: 239-295.

Reyes, L. J. 1938. Philippine woods. Dept. Agric. Commerce, Manila, Tech. Bull. No. 7.

Richards, P. W. 1952. The Tropical Rain Forest: an Ecological Study. Cambridge Univ. Press. Cambridge, 299-312.

Ricklefs, R. E. 1971. Foraging behavior of mangrove swallows at Barro Colorado Island. Auk 88: 635-651.

Ridgway, R. 1873. On some new forms of American birds. Am. Nat. 7: 602-619.

Ridgway, R. 1902. The Birds of North and Middle America, Part 2. U. S. Natl. Mus. Bull. No. 50, 834 pp.

Ridley, H. N. 1920. New and rare plants from the Malay Peninsula. J. Fed. Malay. St. Mus. 10: 128-156.

Ridley, H. N. 1922. The flora of the Malay Peninsula, Vol. 1, pp. 692-703, L. Reeve and Co., London.

Ridley, H. N. 1930. The Dispersal of Plants Throughout the World. L. Reeve and Co., London, 744 pp.

Rimbach, A. 1932. The forests of Equador. Trop. Woods. 31: 1-9.

Robas, A. K. 1970. South Florida's mangrove-bordered estuaries: their role in sport and commercial fish production. Univ. Miami Sea Grant Inf. Bull. No. 4, 28 pp.

Robertson, R. 1959. The mollusk fauna of Bahamian mangroves. Am. Macacol. Union Ann. Rep. Bull. 1959: 22-23.

Robertson, W. B. 1962. Fire and vegetation in the Everglades. Proc. 1st Ann. Tall Timbers Fire Ecol. Conf., pp. 67-80.

Robyns, W. 1971. Over viviparie en biotecnose bij planten. Meded. Kon. Vlaamse. Acad. Wetensch. Lett. Schone Kusten Belgie 33: 3-14.

Rodriguez, G. 1959. The marine communities of Margarita Island, Venezuela. Bull. Mar. Sci. Gulf Caribb. 9: 237-280.

Rodriguez, G. 1963. The intertidal estuarine communities of Lake Maracaibo, Venezuela. Bull. Mar. Sci. Gulf Caribb. 13: 197-218.

Rodriguez, G. 1964. Physical parameters of the Maracaibo estuary and their implications. Proc. Gulf. Caribb. Fish. Inst., 17th Ann. Sess., pp. 42-50.

Rogers, J. S. 1951. Native sources of tanning materials. U.S.D.A. Yearb., Wash., D. C., pp. 709-715.

Roig y Mesa, J. T. 1945. Plantas Medicinales, Aromáticas o Venenosas de Cuba. Cultural, S. A., Habana, pp. 447-449.

Roonwall, M. L. 1954. The marine borer, *Bactronophorus thoracites* (Gould) Mollusca, Eulamellibranchiata, Teredinidae) as a pest of living trees in the mangrove forests of the Sundurhaus, Bengal, India. Proc. Zool. Soc. Bengal 7: 91-105.

Roos, P. J. 1964. The distribution of reef corals in Curacao. Natuurwetensch. Studiekring Suriname Ned. Antillen Uitganen, 1-51.
Rosevear, D. R. 1947. Mangrove swamps. Farm For. 8: 23-30.
Russell, A. 1943. *Conocarpus erecta* (buttonwood, Zaragoza-mangrove), a new domestic source of tannin. Chemurg. Dig. 2: 27-29.
Roth, I. 1965. Histogenese der Lentizellenam Hypocotyl von *Rhizophora mangle* L. Ostero. Bot. Z. 112: 640-653.
Ruhland, W. 1915. Untersuchungen uber die Hautdrusender Plumbaginaceen. J. Wiss. Bot. 55: 409-498.
Russell, A. 1942. Natural tanning materials of the southeastern United States. J. Am. Leather Chemists Assoc. 37: 340-356.
Putzler, K. 1969. The mangrove community, aspects of its structure, faunistics and ecology. *in* Lagunas Costeros, Un Simposio, Mem. Simp. Intern. Lagunas Costeras, UNAM-UNESCO, pp. 515-536.
Rutzler, K. 1970. Oil pollution: damage observed in tropical communities along the Atlantic seabord of Panama. Bioscience 20: 222-224.
Rzedowski, J. and R. McVaugh. 1966. La vegetacion de Nuera Galicia. Con. Univ. Mich. Herb. 9: 1-133.
Safford, W. E. 1905. The useful plants of the island of Guam. Contrib. U. S. Natl. Herb., Vol. II.
Sagrario, C. D. del. 1957. Recovery of tannin from mangrove bark and its conversion into a colorless tannin extract. Chem. Abstr. 53: 4792D.
Salvosa, F. M. 1936. *Rhixophora.* Nat. Appl. Sci. Bull., Univ. Philippines 5: 179-237.
Sawman-Wanakit, L. 1936. Forests in the south. Vanasarn For. J., Thailand, Sp. Ed., No. 3, December.
Sanchez, R. and M. Elana. 1963. Datos relativos a los mangalares de Mexico. Ann. Esc. Nac. Cienc. Biol. Mex. 12: 61-72.
Sandison, E. E. 1966. The effect of salinity fluctuations on the life cycle of *Balanus pallidus stutsburi* Darwin in Lagos Harbour, Nigeria. J. Anim. Ecol. 35: 363-378.
Sandison, E. E. and M. B. Hill. 1966. The distribution of *Balanus pallidus stutsburi* Darwin, *Gryphaea gasar* (Adanson) Dautzenberg, *Mercierella enigmatica* Fauvel and *Hydroides uncinata* Philippi in relation to salinity in Lagos Harbour and adjacent creeks. J. Anim. Ecol. 35: 235-250.
Santos, T. A. and I. B. Siapno. 1968. Cutting operations of mangrove forest in Zamboanga peninsula (Mindanao). Philipp. Lumberman 14: 14-20.
Sargent, C. S. 1893. The mangrove tree. Gard. For. 6: 97-98.
Sargent, C. S. 1903. Combretaceae. Silvic. N. Am. 5: 19-29.
Sargent, C. S. 1922. Manual of Trees of North America (Exclusive of Mexico). 2nd ed., Houghton Mifflin Co., Boston, pp. 763-764.
Sargent, C. S. 1962. Effects of recent tropical cyclones on the coastal vegetation of Mauritius. J. Ecol. 50: 275-290.
Sasaki, S. 1912. Plants of the Kojurin Forest, Formosa. Trans. Nat. Hist. Soc. Formosa 2: 45-50.

Sasaki, S. 1923. *Kandelia rheedii* is disappearing from the Taiwanian flora. Trans. Nat. Hist. Soc. Formosa 13: 37-39.
Satayanaraynan, Y. 1959. Ecological studies of the Elephante Island. Inst. Franc. de Pondicherry, Trav. Sec. Scien. Tech. 1: 99-114.
Sauer, J. D. 1961. Coastal plant geography of Mauritius. Coast. Stud. Ser., Louisiana St. Univ. 5: 1-153.
Sauer, J. D. 1967. Geographic reconnaissance of seashore vegetation along the mexican gulf coast. Coastal St. Res. Inst. La. St. Univ., Tech. Rep. No. 56.
Saunders, J. B. 1958. Recent foraminifera of mangrove swamps and river estuaries and their fossil counterparts in Trinidad. Micropaleontology 4: 79-92.
Saver, J. D. 1965. Geographic reconnaissance of western Australian seashore vegetation. Aust. J. Bot. 13: 39-69.
Savory, H. J. 1953. A note on the ecology of *Rhizophora* in Nigeria. Kew Bull. 1: 127-128.
Scheffen, W. 1937. Strandbeobachtungen im Malayischen Archipel. Geol. Meere Binnengew. 1.
Scheffer, T. C. and C. G. Duncan. 1947. The decay resistance of certain Central American and Ecuadorian woods. Trop. Woods. 92: 1-24.
Schenck, H. 1889. Ueber die Luftwurzeln von *Avicennia tomentosa* und *Laguncularia racemosa*. Flora 72: 83-88.
Schimper, A. F. W. 1891. Die Indo-Malayische Strandflora. Bot. Mit. Trop. 3: 1-204.
Schimper, A. F. W. 1892-1893. Rhizophoraceae. Nat. Pflanzenfam. 7: 42-56.
Schimper, A. F. W. 1903. Plant Geography on a Physiological Basis. Oxford Univ. Press. Oxford, 839 pp.
Schimper, A. F. W. and F. C. von Faber. 1935. Pflanzengeographie auf Physiologischer Grundlage. 2 vols., 3rd Ed., Jena.
Schmidt, J. 1902. Rhizophoraceae. Bot. Tidsskr. 24: 249-554.
Schmidt, J. 1903. Skuddene has den gamle verdens mangrovetraer. Nordiske Forlag Econst. Bojeuen, 113 pp.
Schmidt, J. 1904. Zur Frage der Salzausscheidung der Mangrovepflanzen. Flora 93: 260-261.
Schmidt, J. 1905. Bidrag til Kundskab om Skuddene hos den Gamle Verdens Mangrove-traer. Bot. Tidsskr. 26: 1-113.
Schmidt, J. 1906. Die Kustenvegetation von Koh Chang. Vegetationsbilder 3: 78-91.
Schneider, E. E. 1916. Commercial woods of the Philippines: their preparation and uses. Philipp. Bur. Forest., Bull. No. 14, pp. 179-182.
Schneider, J. 1921. Uber Mangrove auf den Philippinen. Gerger 47: 53-54.
Schnepper, W. C. R. 1933. Vloedbosch culturen. Tectona 26: 907-919.
Schnetter, M. 1969. Observaciones ecologicas en la isla de Salamanca (Departmento del Magdalene) Colombia. Caldasia 10: 299-315.
Scholander, P. F. 1965. From the frozen forest to the tropical mangroves. *in* Science in Alaska: Proc. 15th Alaskan Sci. Conf., College, Alaska, 31 Aug. -

4 Sept. 1964, AAAS, Washington, D. C.
Scholander, P. F. 1968. How mangroves desalinate seawater. Physiol. Plantarum 21: 258-268.
Scholander, P. F., E. D. Bradstreet, H. T. Hammel, and E. A. Hemmingsen. 1966. Sap concentrations in halophytes and some other plants. Plant Physiol. 41: 529-532.
Scholander, P. F., L. van Dam, and S. I. Scholander. 1955. Gas exchange in the roots of mangroves. Am. J. Bot. 42: 92-98.
Scholander, P. F., H. T. Hammel, E. A. Hemmingsen, and E. D. Bradstreet. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. U. S. Natl. Acad. Sci. 52: 119-125.
Scholander, P. F., H. T. Hammel, E. Hemmingsen, and W. Carey. 1962. Salt balance in mangroves. Plant Physiol. 37: 722-729.
Scholander, P. F., H. T. Hammel, E. D. Bradstreet, and E. A. Hemmingsen. 1965a. Sap pressure in vascular plants. Science 148: 339-346.
Scholander, P. F., H. T. Hammel, E. D. Bradstreet, and E. A. Hemmingsen. 1965b. Sap pressure in vascular plants (Addendum). Science 148: 1488.
Scholander, P. F. and M. de Oliveira Perez. 1968. Sap tension in flooded trees and bushes of the Amazon. Plant Physiol. 42: 1870-1873.
Scholl, D. W. 1963. Sedimentation in modern coastal swamps, southwestern Florida. Bull. Am. Assoc. Pet. Geol. 47: 1581-1603.
Scholl, D. W. 1964. Recent sedimentary record in mangrove swamps and rise in sea level over the southwestern coast of Florida. I. Mar. Geol. 1: 344-366.
Scholl, D. W. 1965a. High interstitial water cholorinity in estuarine Mangrove swamps. Florida. Nature 207: 284-285.
Scholl, D. W. 1965b. Recent sedimentary record in mangrove swamps and rise in sea level over the southwestern part of Florida. II. Mar. Geol. 2: 343-364.
Scholl, D. W. Modern coastal swamp statigraphy and the ideal cyclotherm. *in* Environments of Coal Deposition, E. C. Dapples and M. B. Hopkins (eds.), Geol. Soc. Amer. Spec. Pap., *in press.*
Scholl, D. W. and M. Stuijer. 1967. Recent submergence of southern Florida: a comparison with adjacent coasts and other eustatic data. Geol. Soc. Am. Bull. 78: 437-454.
Schone, H. 1963. Menotaktische Orientierung nach polarisiertem und unpolarisierten Light bei der Mangrovekrabbe *Goniopsis.* Z. Vgl. Physiol. 46: 496-514.
Schone, H. and H. Schone. 1963. Balz und Verhaltensweisen der Mangrovekrabbe *Goniopsis cruentata* Latr. und das Winkverhalten der eulitoralen Brachyuren. Z. Tierpsychol. 20: 641-656.
Schratz, E. 1934. Beitrage zur Biologie der Halophyten. I. Zur Keimungphysiologie. Jahrb. f. wiss. Bot. 80: 112-142.
Schuster, W. H. 1962. Das marine Littoral als Lebensraum Terrestrischer Kleinarthropoden. Int. ges. rev. Ges. Hydrobiol. 47: 359-412.

Schuster, W. H. 1952. Fish culture in brackish water ponds of Java. Indo-Pacific Fish. Counc. Spec. Publ., No. 1, pp. 1-143.

Seifriz, W. 1943. The plant life of Cuba. Ecol. Monogr. 13: 375.426.

Sen Gupta, J. C. 1935. Die Osmotischen Verhaltnisse bei einigen Pflanzen in Bengal (Indian). Ber. Dtsch. bot. Ges. 53: 783-795.

Sen Gupta, J. C. 1938a. Ueber die osmotische Werte und den Chloranteil in Pflanzen einiger Salzgebietes Bengal. Ber. Dtsch. bot. Ges. 56: 474-485.

Sen Gupta, J. C. 1938b. Der histochemische Nachweis vor Natrium bei einigen Pflanzen von Bengal. Ber. Dtsch. bot. Ges. 56: 486-494.

Sen Gupta, J. C. 1942. Investigation of the annual variation of the water content, osmotic value and chloride fractions of some plants in Port Canning, near calcutta. 50th Ann. Vol R. Bot. Gard. Calcutta, 1942.

Seshadri, T. R. and B. Venkataramani. 1959. Leucocyanin from mangroves. Indian J. Sci. Res. 18B: 261-262.

Setchell, W. L. 1926. Phytogeographical notes on Tahiti. Univ. Cal. Publ. Bot. 12: 241-290.

Setten, G. G. K. 1947. A note on the growth and yield of Beruus (*Bruguiera cylindrica* (L.) (Bl.). Malay. For. 16: 74.

Setten, G. G. K. 1955. A note on the growth and yield of Berus (*B. cylindrica* (L.) Bl. syn. *B. caryophylloides* Bl.) in Selangor. Res. Pamphl. For. Res. Instit. Fed. Malaya, No. 16, 12 pp.

Shantz, H. L. and C. F. Marbut. 1923. The vegetation and soils of Africa. Am. Geogr. Soc. Res. Ser. 13: 1-242.

Shah, J. J. and K. P. Sundarraj. 1965. Stipular sclerids in *Rhizophora mucronata* L. Curr. Sci. 34: 155-156.

Shelford, V. E. 1963. The Ecology of North America. Univ. Ill. Press, Urbana, 610 pp.

Shier, D. E. 1969. Vermetid reefs and coastal development in the Ten Thousand Islands, southwest Florida. Geol. Soc. Bull. 80: 485-508.

Shieve, F. 1937. The vegetation of the cape region of Baja California. Madrona 4: 105-113.

Shimada, Y. 1924. Investigations on *Kandelia rheedii* in Komoko, Taiwan. Trans. Nat. Hist. Soc. Formosa 14: 25-29 (in Japanese).

Sidhu, S. S. 1960. Studies on mangroves. Natl. Acad. Sci. India, No. 1960, pp. 111-112.

Sidhu, S. S. 1961. Vegetation of East Godavari. Proc. 48th Indian Sci. Congr. 3: 358-359.

Sidhu, S. S. 1961. Chromosomal studies on some mangrove species. Proc. 48th Indian Sci. Congr. 3: 302-304.

Sidhu, S. S. 1962. Chromosomal studies of some mangrove species. Indian For. 88: 585-592.

Sidhu, S. S. 1963a. Studies on the mangroves of India. I. East Godavari region. Indian For. 89: 337-351.

Sidhu, S. S. 1963b. Studies on mangroves. *in* Symposium on Ecological Problems in the Tropics. Proc. Natl. Acad. Sci. India, Sect. B. 33: 129-136.

Sidhu, S. S. 1968. Further studies on the cytology of mangrove species in India. Caryologia 21: 353-357.
Simpson, C. T. 1932. Florida Wild Life. Macmillan, New York, pp. 9-11, 118.
Simpson, J. G. and R. C. Griffiths. 1967. The fisheries resources of Venezuela and their exploitation. Serie Recursos y Expl. Presqueros 1: 171-206.
Silveira, F. 1937. Mangrove. Rodriguesia 3: 131-154.
Simberloff, D. S. and E. O. Wilson. 1969. Experimental zoogeography of islands: the colonization of empty islands. Ecology 50: 278-296.
Singh. T. C. N. and A. T. Natarajan. 1953-1954. Morphology of the pollen grains of the constituents of the mangrove vegetation (abstr.) Proc. Indian Sci. Congr. 40: 99-100.
Slooten, D. V. van. 1937. Die Verbreitung von *Lumnitzera* und anderen Mangrovegewachsen. Blumea Suppl. 1: 162-175.
Slooten, H. J. van der. 1960. Resina de Fenol-Formaldehido para contrachapeado obtenida del tanio de *Rhizophora mangle*. Inst. For. Latino Amer. de Invest. Capacit., Merida, Venezuela, Bol. 6: 34-39.
Slud, P. 1964. The birds of Costa Rica, distribution and ecology. Bull. Am. Mus. Nat. Hist. 128.
Small, J. K. 1914. Exploration in the Everglades and on the Florida keys. J. N. Y. Bot. Gard. 15: 69-79.
Small, J. K. 1923. Mangroves, J. N. Y. Bot. Gard. 24: 211.
Small, J. K. 1924. Mangroves. J. N. Y. Bot. Gard. 25: 73-74.
Small, J. K. 1933. Manual of the Southeastern Flora. Univ. North Carolina Press, Chapel Hill, pp. 938-939.
Smith, A. C. and I. M. Johnson. 1945. A phytogeographic sketch of Latin America. *in* Plants and Plant Science in Latin America, F. Verdoorn, (ed.), Chronica Botanica Co. Waltham, Massachusetts.
Smith, F. G. W., R. H. Williams, and C. C. Davis. 1950. An ecological survey of the sub-tropical inshore waters adjacent to Miami. Ecology 31: 119-146.
Smith, J. 1882. A Dictionary of Popular Names of the Plants Which Furnish the Natural and Acquired Wants of Man in All Matters of Domestic and General Economy. Macmillan, London, pp. 263-264.
Sodiro, R. P. L. 1901. El mangle rojo. Estudios de Medicina, Quito ¾: 3-18.
Sokoloff, B., J. B. Redd, and R. Dutscher. 1950. Nutritive value of mangrove leaves (*Rhizophora mangle* L.). Q. J. Fla. Acad. Sci. 12: 191-194.
Solereder, H. 1885. Zur Anatomie und Systematik der Combretaceen. Bot. Centralbl. 23: 161-166.
Solereder, H. 1908. Systematic Anatomy of the Dicotyledons. Clarendon Press, Oxford, 423 pp.
Southwell, C. R. and J. D. Bultman. 1971. Marine borer resistances of untreated woods over long periods of immersion in tropical waters. Biotropica 3: 81-107.
Spackman, W., C. P. Dolsen, and W. Riegal. 1966. Phytogenic organic sediments and sedimentary environments in the Everglades-mangrove complex. Part I. Evidence of a transgressing sea and its effect on

environments of the Shark River area of southwest Florida. Paleontrophica 117: 135-152.

Spackman, W., D. W. Scholl, and W. H. Taft. 1964. Field Guide Book to Environments of Coal Formation in Southern Florida. Geol. Soc. Am., 67 pp.

Specht, R. L. and C. P. Mountford. 1958. Records of the American-Australian Scientific Expedition to Arnhem Land. 3. Botany and Plant Ecology. Melbourne Uni. Press, Melbourne.

Speck, N. H. 1960. Vegetation of the North Kimberly area. CSIRO Aust. Land Res. Ser. 4: 41-63.

Speck, N. H. and M. Lazarides. 1964. Vegetation and pastures of the West Kimberly area. CSIRO Aust. Land Res. Ser. 9: 140-174.

Spooner, G. M. and H. B. Moore. 1940. the ecology of the Tamar estuary. 6. An account of the macroflora of the intertidal muds. J. mar. biol. Assoc. U.K. 24: 283-291.

Sprague, T. A. and L. A. Boodle. 1909. Kokoti (*Anopyxis ealaensis* Sprague). Bull. Misc. Inf., R. Bot. Gard., Kew, London, pp. 309-312.

Stace, C. A. 1961. Cuticular characters as an aid to the taxonomy of the South-West African species of *Combretum.* Mitt. Bot. Staatssam. Munchen 4: 9-17.

Stace, C. A. 1965. The significance of the leaf epidermis in the taxonomy of the Combretaceae. J. Linn Soc. London Bot. 59: 229-252.

Stace, C. A. 1966. The use of epidermal characters in phylogenetic considerations. New Phytol. 65: 304-318.

Stamp, L. D. 1925. The aerial survey of the Irrawaddy delta forests. J. Ecol. 13: 262-276.

Standley, P. C. 1924. Trees and shrubs of Mexico. Cont. No. 23., U. S. Natl. Herb., Washington, D. C.

Standley, P. C. 1928. Flora of the Panama Canal Zone. Contrib. No. 27, U. S. Natl. Herb., Washington, D. C. p. 283.

Standley, P. C. 1930. Flora of Yucatan. Field Mus. Nat. Hist. Bot. Ser. III, No. 3, p. 371.

Standley, P. C. 1937. Flora of Costa Rica, Part II. Field Mus. Nat. Hist., Chicago, pp. 766-767.

Standley, P. C. and S. J. Record. 1936. The forests and flora of British Honduras. Field Mus. Nat. Hist. Pub. No. 350, Bot. Ser. XII, pp. 275-276.

Stearn, W. T. 1958. A key to the West Indian mangroves. Kew Bull. 13: 33-37.

Steenis, C. G. G. J. van. 1935. Maleische vegettieschetsen. Tijdschr. K. ned. aardrijsk. Genoot. 52: 363-398.

Steenis, C. G. G. J. van. 1941. Kustaanwas en mangrove. Natuurwet. Tijdschr. Ned. Ind. 101: 82-85.

Steenis, C. G. G. J. van. 1954a. Vegetatie en Flora. New Guinea 2: 218-275.

Steenis, C. G. G. J. van. 1954b. Plumbaginaceae. Fl. Males. 1: 107-112.

Steenis, C. G. G. J. van. 1958a. Rhizophoraceae. Flora Malesiana 5: 431-493.

Steenis, C. G. G. J. van. 1958b. Tropical shore formations. Proc. Symp. Humid Trop. Veg., Tijiawi (Indonesia), pp. 215-217.
Steenis, C. G. G. J. van. 1962a. The distribution of mangrove plant genera and its significance foe paleogeography. Kon. Neder. Akad. van Wetensch. 65: 164-169.
Steenis, C. G. G. J. van. 1962b. The land bridge theory in botany. Blumea 2: 235-372.
Steenis, C. G. G. J. van. 1963. Pacific Plant Areas. 1. Monogr. Natl. Inst. Sci. Technol. Philipp., Manila, Monogr. No. 8, 279 pp.
Steenis, C. G. G. J. van. 1965. Concise plant geography of java. *in* Flora Java, C. G. G. J. van Steenis (ed.), Vol. 2, pp. 1-72.
Steenis, C. G. G. J. van. 1968. Do *Sonneratia caseolaris* and *S. ovata* occur in Queensland or the Northern Territory? N. Queensland Nat. 35: 3-6.
Steenis, C. G. G. J. van and M. M. J. van Balgoog. 1966. Pacific Plant Areas. 2. Blumea, Suppl. 5, 312 pp.
Steers, J. A. 1929. The Queensland coast and the Great Barrier Reef. Geogr. J. 74: 232-257.
Steers, J. A. 1937. The coral islands and associated features of the Great Barrier Reef. Geogr. J. 89: 1-28 and 119-146.
Steers, J. A. 1940. Sand cays and mangroves in Jamaica. Geogr. J. 96: 305-323.
Steers, J. A., V. J. Chapman, J. Colman, and J. A. Lofthouse. 1940. Sand cays and mangroves in Jamaica. Geogr. J. 96: 305-328.
Stehle, H. 1945. Forest types of the Caribbean Islands, Part 1. Caribb. For. Suppl. to Vol. 6, pp. 1-16.
Stephens, W. M. 1962. Trees that make land. Sea Front. 8: 219-230.
Stephens, W. M. 1963. Mangroves: trees that make land. Smithson. Inst. Rep. 1962, pp. 491-496.
Setphens, W. M. 1969. The mangrove. Oceans 2: 51-55.
Stephenson, T. A. 1939. The constitution of the intertidal fauna and flora of South Africa. Part 1. J. Linn. Soc. London 40: 487-536.
Stephenson, T. A. and A. Stephenson. 1950. Life between tide marks in North America. I. The Florida Keys. J. Ecol. 38: 354-402.
Stephenson, T. A., A. Stephenson, G. Tandy, and M. A. Spender. 1931. The structure and ecology of Low Isles and other reefs. Br. Mus. Nat. Hist. Sci. Rep., Great Barrier Reef Exped., 1928-1929. 3: 17-112.
Stephenson, W., R. Endean, and I. Bennett. 1958. An ecological survey of the marine fauna of Low Isles, Queensland. Aust. J Mar. Freshwater Res. 9: 261-318.
Stern, W. L. and G. K. Voight. 1959. Effect of salt concentration on growth of red mangrove in culture. Bot. Gaz. 121: 36-39.
Stewart, A. 1916. Some observations concerning the botanical conditions of the Galapagos Islands. Wis. Acad. Sci. Trans. 18: 272-340.
Steup, F. K. M. 1946. Boschbeheer in de vloedbosschen van riouw. Tectona 36: 289-298.

Stocker, O. 1924. Beitrage zum Halophytenproblem. Okologische untersuchungen der Strandund Dunenpflanzen des Dars (Vorpommern). Z. Bot. 16: 289-330.

Stocker, O. 1925. Beitrage zum Halophytenproblem. Okologische untersuchungen der Strandund Dunenpflanzen des Dars (Vorpommern). Z. Bot. 17: 1-24.

Stocker, O. 1935. Transpiration und Wasserhaushalt in verschieden Klima Zonen. III. Jb. wiss. Bot. 81: 464-471.

Stocking, C. R. 1956. Osmotic pressure and osmotic value. Handb. Pflphysiol. 2: 63-64.

Stoddart, D. R. 1962. Three Caribbean atolls: Turneffe Islands, Lighthouse Reef, and Glover's, British Honduras. Tech. Rep. Coastal Stud. Inst. La. State Univ. 11, 151 pp.

Stokes, A. P. D. and R. C. Roberts. 1934. Saline peat profiles of Puerto Rico. J. Wash. Acad. Sci. 24: 175-182.

Story, R. 1969. Vegetation of the Adelaide-Alligator area. CSIRO Aust. Land REs. Ser. 25: 114-130.

Straatmans, W. 1954. Reclamation of tidal mud flats in Tonga. Tech. Pap. South Pac. Comm., No. 53, 18 pp.

Strenzke, K. 1958. *Axelsonia tubifera* n. sp., ein neuer arthropleoner Collembole mit Geschlechtsdimorphismus aus der brasilianischen Mangrove. Acta. Zool. Cracov. 2: 607-619.

Strunk, L. 1906. Chemische untersuchungen von mangrovenrinden. Tropenpflanzer 10: 116.

Studholme, W. P. and W. R. Philipson. 1966. A comparison of the cambium in two woods with included phloem: *Heimerliodendron brunonianum* and *Avicennia vesinifera.* N. Z. J. Bot. 4: 332-341.

Sundararaj, D. D. 1954. Mangrove (*Avicennia officinalis* L.) leaves as cattle feed. I. Sci. Cult. 19: 339.

Sukhamananda, M. 1951. Sutan mangrove Forest. Vanasarn For. J. 3: 1-16.

Suter, H. H. 1960. A General and Economic Geology of Trinidad and Tobago. H.M.S.O., London, 145 pp.

Svenson, H. K. 1945. A brief review of the Galapagos flora. *in* Plants and plant Science in Latin America. F. Verdoorn (ed.), Cronica Botanica Co., Waltham, Massachusetts.

Svenson, H. K. 1946. Vegetation of the coast of Equador and Peru and its relation to the Galapagos Islands. Am. J. Bot. 33: 394-426.

Swain, T. 1965. The tannins. *in* Plant Biochemistry, J. Bonner and J. E. Varner (eds.), Academic Press, New York, 1054 pp.

Swart, H. J. 1958. An investigation of the mycoflora in the soil of some

mangrove swamps. Acta Bot. Neerl. 7: 741-768.
Swart, H. J. 1963. Further investigations of the mycoflora in the soil of some mangrove swamps. Acta Bot. Neerl. 12: 98-111.
Symington, C. F. 1940. Three malayan species of *Bruguiera.* Malay. For. 9: 131-139.
Tabb, D. C. and R. B. Manning. 1961. A checklist of the flora and fauna of northern Florida Bay and adjacent brackish waters of the Florida mainland collected during the period July 1957 through September 1960. Bull. Mar. Sci. Gulf Caribb. 11: 552-649.
Tabb, D. C., D. L. Bubrow, and R. B. Manning. 1962. The ecology of northern Florida Bay and adjacent estuaries. Fla. State Board Conserv. Tech. Ser. Publ. No. 39.
Tadeo, C. B. 1962. Plants with edible fruits in the Philippines. For. Leaves 14: 57.
Tanner, W. F. 1960. Florida coastal classification. Trans. Gulf Coast Assoc. Geol. Soc. 10: 259-266.
Tansley, A. G. and F. E. Fritsch. 1905. The flora of the Ceylon littoral. New phytol. 4: 27-36.
Taylor, B. W. 1959. The classification of lowland swamp communities in northeastern Papua. Ecology 40: 703-711.
Taylor, J. S. 1931. A note on the flora of mangrove. Entomol. Rec. J. Var., N. S. 43: 41-42.
Taylor, W. R. 1925. The marine flora of the Dry Tortugas. Rev. Algol. 2: 113-135.
Taylor, W. R. 1928. The marine algae of Florida, with special reference to the Dry Tortugas. Carnegie Inst. Wash., Publ. No. 379; Pap. from the Tortugas Lab. No. 25, 219 pp.
Taylor, W. R. 1954. Sketch of the character of marine algae vegetation of the shores of the Gulf of Mexico. *in* The Gulf of Mexico. It's Origin, waters, and marine life. P.S. Galtsoff, Coordinator, U.S.D.I., Fish. Bull. 55: 177-192.
Teas, H. J. and F. Montgomery. 1968. Ecology of red mangrove seedling establishment. Bull. Assoc. Southeast. Biol. 15: 56-57.
Teijsmann, J. E. 1957. Dagverhaal eener Botanische Reis over de Westkust van Sumatra. Nat. Tijd. Ned. Ind. 14: 249- 376.
Teixerira, C. and M. B. Kutner. 1961. Contribuicao para o conhecimento das diatomaceas da regiao de Cananeia. Bol. Inst. Oceanogr. Sao Paulo 11: 41-74.
Teixeira, C. and M. B. Kutner. 1963. Plankton studies in a mangrove environment. I. First assessment of standing stock and principal ecological factors. Bol. Inst. Oceanogr., Sao Paulo 12: 101-124.
Teixeira, C., M. B. Kutner, and F. M. S. Torgo. 1965. O efeito da respiracao bacteriana no estudo da producao primaria. Rev. bras. Biol. 25: 287-294.
Teixeira, C., J. Tundisi, and M. B. Kutner. 1965. Plankton studies in a mangrove environment. II. The standing stock and some ecological factors. Biol. Inst. Oceanogr., Sao Paulo, Publ. 14: 13-41.

Teixeira, C., J. Tundisi and J. Santoro Ycaza. 1967. Plankton studies in a mangrove environment. IV. Size fractionation of the phytoplankton. Biol. Inst. Oceanogr. Sao Paulo 16: 39-42.

Teixeira, C., J. Tundisi, and J. Santoro Ycaza. 1969. Plankton studies in a mangrove environment. VI. Primary production, zooplankton standing-stock and some environmental factors. Int. rev. Ges. Hydrobiol. 54: 289-301.

Teng, K. Forests in the sea at Hainan Island. Chin. For. 2: 30-31.

Thom, B. G. 1967. Mangrove ecology and deltaic geomorphology: Tobasco, Mexico. J. Ecol. 301-343.

Thome, S. and A. F. Moller. 1901. Mangrove. Tropenpflanzen 5: 339.

Thompson, J. M. 1954. The genera of oysters and the Australian species. Aust. J. Mar. Freshwater Res. 5: 132-168.

Thorne, R. F. 1954. Flowering plants of the waters and shores of the Gulf of Mexico. U. S. Fish Wildl. Serv., Fish. Bull. 89: 193-202.

Thornton, I. and M. E. C. Giglioli. 1965. The mangrove swamps of Keneba, Lower Gambia River Basin. II. Sulphur and pH in the profiles of swamp soils. J. appl. Ecol. 2: 257-269.

Tieghem, Ph. van. 1898. Avicenniacees et Symporemacees. Place de ces deux noubelles familles dans la classification. J. Bot., Paris 12: 345-358.

Tinley, K. L. 1958. A preliminary report on the ecology of the Kosi lake system. Unpublished report of the Natal Parks, Fish and Game Preservation Board, Natal, 90 pp.

Tischler, G. 1910. Untersuchungen an Mangrove-und Orchideen-Wurzeln mit specieller Bezichung auf die Statolithen des Geotropismus. Ann. Jard. Bot. Buit., Suppl. 3, pp. 131-186.

Tobler, F. 1914. Die Mangrove der Insel Ulenge (Deutsch-Ostafrika). Bot. Jahrb. 50: 398-411.

Todd, R. and P. Bronnimann. 1957. Recent foraminifera and Thecamoebina from the eastern Gulf of Paria. Cushman Found. Foram. Res. Spec. Publ. No. 3.

Tokioka, T. 1942. Ascidians found on the mangrove trees in Iwayama Bay, Palao. Palao Trop. Biol. Stn. Stud. (Tokyo) 2: 497-506.

Talken, H. R. 1967. The species of *Arthrocnemum* and *Salicornia* (Chenopodiaceae) in southern Africa. Bothalia 9: 255-307.

Tomlinson, T. E. 1957. Relationship between mangrove vegetation, soil texture and reaction of surface soil after empoldering saline swamps in Sierra Leone. Trop. Agric. Trinidad 34: 41-50.

Tomlinson, T. E. 1957. Changes in a sulphide-containing mangrove soil on drying and their effect upon the suitability of the soil for the growth of rice. Empire J. Exp. Agric. 25: 108-118.

Tralau, H. 1964. The genus *Nypa* van Wurmb. K. Svensk, Vetensk. Akad. Handl., Ser. 5, 10: 5-29.

Treub, M. 1883. Notes sur l'embryon, le sac embryonaire et l'ovule de *Avicennia officinalis.* Ann. Jard. Bot. Buit. 3: 79-87.

Triana, I. and I. E. Planchon. 1862. Prodromus Florae Novo- Grandensis ou enumeration des plantes de la Nouvelle-Grenade, avec descriptions des especes nouvelles. Ann. Soc. Nat. Quart. Ser., Bot. 17: 1-382.

Trimble, H. 1892. Mangrove tannin. Contr. Bot. Lab. Univ. Penn. 1: 50-55.

Troll, W. 1930. Ueber die sogenannten Atemwurzeln der Mangroven. Ber. Stsch. bot. Ges. 48: 81-99.

Troll, W. 1931. Botanische Mitteillungen aus den Tropen. III. *Dictyotposis paopagalifera* W. Troll, eine neue Brackwasseralge ostindischer Mangrovegebiete. Flora, N. F. 25: 474-502.

Troll, W. 1932. Uber die sogenannten Atemwurzelin der Mangroven. S. Br. Ges. Morph. Physiol. 40: 46-48.

Troll, W. 1933a. Uber *Acrostichum aurem* und *Acrostichum* speciosum und nestene Formen des letzeren. Flora, N. F. 28' 301-328.

Troll, W. 1933b. *Camptostemon schultzii* Mart. und *C. philippensis* Becc. als neue Vertreter der australasiatischen Mangrove-vegetation. Flora, N. F. 28: 348-352.

Troll, W. 1942. Die Mangrovegewache. *in* "vergleichende Morphologie der Hoheran Pflanzen" Erster Band, Dritter Teil, Berlin-Zehlendorf.

Troll, W. and O. Dragendorff. 1931. Uber die Luftwurzeln von *Sonneratia* Linn. und ihre biologische Bedeutung. Planta 13: 311-473.

Truman, R. 1961. The eradication of mangroves. Aust. J. Sci. 24: 198-199.

Trumbull, S. 1954. Mangrove wood, leaves, may bar expansion of Everglades Park. Miami Herald, Apr. 14.

Tschirley, F. H. 1969. Defoliation in Vietnam. Science 163: 779-786.

Tsopa, E. 1939. La vegetation des halophytes du Nord de la Roumanie en connexion avec celle du reste du pays S.I.G.M.A. 70: 1-22.

Tundisi, J. 1969. Plankton studies in a mangrove environment – its biology and primary production. *in* Lagunas Costeras, un Simposio. Mem. Simp. Intern. Lagunas Costeras. UNAM – UNESCO, Nov. 28-30, 1967, Mexico, D. F., pp. 485-494.

Tundisi, J. and C. Teixeira. 1965. Plankton studies in a mangrove environment. *in* Simposio sobre a Oceanografic do Atlantico Sul Occidental, Rio de Janiero, Brazil, 14-18 Setembro. 1964. Ann. Acad. Brasil, Cienc. 37 (Suppl.): 192-193.

Tundisi, J. and C. Teixeira. 1968. Plankton studies in a mangrove environment. VII. Size fractionation of the phytoplankton: some studies on methods. Bol. Inst. Oceanogr. 17; 89-94.

Tundisi, J. and T. M. Tundisi. 1968. Plankton studies in a mangrove environment. V. Salinity tolerances of some planktonic crustaceans. Bol. Inst. Oceanogr., Sao Paulo 17: 57-65.

Turrill, W. B. and E. Milne-Redhead. 1956. Flora of tropical East Africa: Rhizophoraceae. Crown Agents, London, 311 pp.

Uhvits, R. 1946. Effect of osmotic pressure on water absorption and germination of alfalfa seed. J. Bot. 33: 278-285.

Ule, E. 1901. Die vegetation von Cabo Frio an der Kuste von Brasilien. Bot. Jb. 28: 511-528.
Ulken, A. 1970. Phycomyceten aus der Mangrove bei Cananeia (Sao Paulo, Brazilien). Veroff. Inst. Meeresforsch. Bremerhaven 12: 313-319.
Unwin, A. H. 1920. West african forests and forestry. E. P. Dutton and Co., New York, 416 pp.
Uphof, J. C. T. 1935. Die holzigen Halophyten-vereinen des Kustengebietes Floridas. Mitt. Dut. Dendrol. Ges. 47: 39-53.
Uphof, J. C. T. 1937. Die nordliche Verbreitung der Mangroven im atlantischen Gebiet. Arch. Hydrobiol. 31: 141-144.
Vahrmeijer, J. 1966. Notes on the vegetation of north Zululand. Afr. Wildl. 20: 151-161.
Vann, J. H. 1959a. The physical geography of the lower coastal plain of the Guiana coast. Geogr. Branch, O.N.R., Washington, D.C., Project NR. 388-028, Tech. Rep. 1, 91 pp.
Vann, J. H. 1959b. Landform-vegetation relationships in the Atrato delta. Ann. Assoc. Am. Geogr. 49: 345-360.
Vanwijk, C. L. 1951. Soil survey of the tidal swamps of South Borneo in connection with agricultural practices. Tectona 41: 75-110.
Vaughan, R. E. and P. O. Wiehe. 1937. Studies on the vegetation of Mauritius. I. J. Ecol. 25: 301-311.
Vaughan, T. W. 1909. The geologic work of mangroves in southern Florida. Smithson. Misc. Collect. 52: 461-464.
Vazquez Bota, J. 1963. Classification de las melsas forestales de campeche. Bol. Tech. Inst. Macl. For. Mex., Vol 10.
Venkateswarlu, J. 1935. A contribution to the embryology of Sonneratiaceae. Proc. Indian Acad. Sci. 5: 23-29.
Venkateswarlu. J. 1944. The estuarial flora of the Godavari. J. Bombay Nat. Hist. Soc. 44: 431-435.
Benkateswarlu J. and R. S. P. Rao. 1964. The wood anatomy and taxonomic position of Sonneratiaceae. Curr. Sci. 33: 6-9.
Versteegh, F. 1952. Problems of silviculture and management of mangrove forests in Indonesia. Doc. FAO Asia-Pac. For. Comm. 77, 5 pp.
Verwey, J. 1930. Einiges uber die Biologie Ost-Indisher Mangrovekrabben. Terubia 12: 167-261.
Vibul Vanakij, L. 1937. Mangrove forest. Vanasarn For. J., No. 6.
Vieillard, E. 1862. Plantes utiles de la Navelle-Caledonie. Ann. Sci. Nat. Paris, ser. IV, vol. 16.
Visser, S. A. 1963. The occurrence of microorganisms in tropical swamps. Biochem. J. 89: 83-84.
Vu Van Cuong Humbert. 1964a. Flore et vegetation de la mangrove de la region de Saigon-Cap Saint Jacques, Sud Viet-Nam. These Doct., 3rd Cycle, spec. Sci. Biol., Paris, 199 pp.
Vu Van Cuong Humbert 1964b. Nouveautes pour la Flore du Camboge, du Laos, et de Vietnam (Rhizophoraceae, Sonneratiaceae, Myrtacae).

Addisonia 4: 343-347.
Wadsworth, F. H. 1959. Growth and regeneration of white mangrove in Puerto Rico. Caribb. For. 20: 59-71.
Wadsworth, F. H. and G. H. Englerth. 1959. Effects of the 1956 hurricane on forests in Puerto Rico. Caribb. For. 20: 38-57.
Waisel, Y. 1958. Germination behavior of some halophytes. Bull. Res. Coun. Israel (Botany) 60: 187-189.
Walker, F. S. 1937. The management and exploitation of the Klang mangrove forests. Malay. For. 6: 71-78.
Walker, F. S. 1938. Regeneration of Klang mangroves. Malay. For. 7: 71-76.
Walker-Arnott, G. A. 1938. On the Rhizophoraceae. Ann. Mag. Nat. Hist. 1: 359-374.
Walsh, G. E. 1967. An ecological study of a Hawaiian mangrove swamp. *in* Estuaries, G. H. Lauff (ed.), AAAS Publ. 83, Washington, pp. 420-431.
Walter, H. 1936. Die Okologischen Verhaltnisse in der Nabel Wuste, Namib (Deutsch Sudwestafrika). Ber. Dtsch. bot. Ges. 54: 17-32.
Walter, H. 1936a. Der wasser-und Salzgehalt der Ost-Afrikanischen Mangroven. Ber. Dtsch. bot. Ges. 54: 33-49.
Walter, H. 1936b. Uber den Wasserhaushalt der Mangrovpflanzen. Ber. Schweiz. bot. Ges. 46: 217-228.
Walter, H. and M. Steiner. 1936. Die Okologie der Ost-Afrikanischen Mangroven. Z. Bot. 30: 65-193.
Warburg, O. 1893. *Rhizophora mangle*, tropische Fragmente. Bot. Jb. 40: 517-548.
Ward, J. M. 1967. Studies in ecology on a shell barrier beach. I. Vegetatio 14: 241.
Waring, G. A. 1926. Geology of the Islands of Trinidad and Tobago, B.W.I. John Hopkins Press, Baltimore, 180 pp.
Warming, E. 1877. Om *Rhizophora mangle* L. Bot. Notes 1877: 14-21.
Warming, E. 1883. Tropische Fragmente. II. *Rhizophora mangle* L. Bot. Jb. 4: 519-548.
Warming, E. and M. Vahl. 1925. Oecology of Plants. Oxford Univ. Press, London, 422 pp.
Warner, G. F. 1967. The life history of the mangrove tree crab, *Aratus pisoni* L. Zool. 153: 321-335.
Warner, G. F. 1969. The occurrence and distribution of crabs in a Jamaican mangrove swamp. J. Anim. Ecol. 38: 379-389.
Watanabe, K. and M. Kutner. 1965. Bacteriological analysis of mangrove waters at Cananeia. *in* Simposio Sobre a Oceanografia do Atlantico Sul Occidental, Rio de Janeiro, Brazil, 14-18 Septembro, 1964. Ann. Acad. Brazil Cienc. 37 (Suppl.): 3.
Watanabe, K. and M. Kutner. 1965. Plankton studies in a mangrove environment. III. Bacterial analysis of waters in Cananeia. Bol. Inst. Oceanogr. 14: 43-51.
Waterman, A. M. 1946. The effect of water soluble extracts from the

heartwood of tropical American woods on the growth of two wood-decay fungi. Trop. Woods. 88:3

Watson, J. G. 1928. Mangrove forests of the Malay Peninsula. Malay. For. Rec. 6: 1-275.

Watson, J. G. 1934. Stick thinnings. Malay. For. 3: 12-17.

Webb, K. L. 1966. NaCl effects on growth and transpiration in *Salicornia bigelovii* salt marsh halophyte. Plant and Soil 24: 261-268.

Webb, L. J. 1966. The identification and conservation of habitat types in the wet tropical lowlands of North Queensland. Proc. R. Soc. Queensland. 78: 59-86.

Webber, J. H. 1898. Strandflora of Florida. Science (N.S.) 8: 658-671.

Weberling, F. 1960. Weitere Untersuchungen uber das Vorkommen rudimentarer Stipeln bei den Myrtales (Combretaceae, Melastomataceae). Flora 149: 189-205.

Webster, L. 1967. Distribution of mangroves in South Australia. A.A. (hon.) thesis, Geogr. Dept., Univ. Adelaide, Adelaide, Australia.

Wei, S. 1947. The relation of typhoons to plant distribution in Taiwan. Bull. Taiwan For. Res. Inst. 6: 1-14.

Weiss, M. H. 1966a. Apercu preliminaire sur les rapports entre le milieu et la distribution des paletuviers le long d'une mangrove littorale proche de Tulear. Rec. Trav. Stn. Mar. Endoume, Marseille, Suppl. 5: 165-173.

Weiss, M. H. 1966b. Apercu preliminaire sur une mangrove naturelle a l'interieur des terres au Sud de Tulear. Rec. Trav. Stn. Mar. Endoume, Marseille, Suppl. 5: 175-178.

Weiss, M. H. and A. Kiener. 1971. Observations relatives a la nature chimique des eaux de la region de Tulear. Diversite, variations, relations avec les zonations biocenotiques. Tethys (Suppl.) 1: 215-236.

Welch, B. L. 1963. From coral reef to tropical island via *Thalassia* and mangrove. Va. J. Sci. 14: 213-214.

Wenzel, H. 1925. Die Mangroverinden als Gerbmaterialen. Anatomische Untersuchungen der gerbstoffreichsten Mangroverinden. Bot. Arch. 59-96.

West, E. and L. E. Arnold. 1956. Native Trees of Florida. 2nd ed., Univ. Florida Press, Gainesville, p. 162.

West, O. 1945. Distribution of mangroves in the eastern Cape Provence. S. Afr. J. Sci. 41: 238-242.

West, R. C. 1954. The mangrove swamp of the Pacific littoral of Colombia. Coast. Geogr. Conf., Feb. 1954, OWR-NRC, Washington, D. C., pp. 45-50.

West, R. C. 1956. Mangrove swamps of the Pacific coast of Colombia. Ann. Assoc. Am. Geogr. 46: 98-121.

West, R. C. 1957. The Pacific lowlands of Colombia, a negroid area of the American tropics. La. St. Univ. Press, Baton Rouge, 278 pp.

Westermaier, M. 1900. Zur Kenntnis der Pneumatophoren. Bot. Unters. im Anaschluss an eine Tropenreise, vol. 1.

Westing, A. H. 1971a. Ecological effects of military defoliation on the forests of South Vietnam. Bioscience 21: 893-898.

Westing, A. H. 1971b. Forestry and the war in South Vietnam. J. For. 69: 777-784.
Westing, A. H. 1971c. Herbicides as weapons in South Vietnam, a bibliography. Bioscience 21: 1225-1227.
Wetmore, A. 1965. The birds of the Republic of Panama. I. Tinamidae (Tinamous) to the Rhynchopidae (Skimmers). Smithson. Misc. Coll., No. 150.
Weyl, R. 1953a. Lithogenetische Studien in den Mangroven der Pazifik-Kuste. Neues Jb. Beol. Palaontol., Mh, No. 5, pp. 202-218.
Weyl, R. 1953b. In den Mangroven El Salvadors. Nat. Volk 83: 26-30.
Wharton, W. J. L. 1883. Mangrove as a destructive agent. Nature 29: 76-77.
Wherry, E. T. 1920. Plant distribution around salt marshes. Ecology 1: 42-48.
White, C. T. 1926. A variety of *Ceriops tagal* C. B. Rob. (=C. *candolleana* W. and A.). J. Bot. London 64: 220-221.
Whiteleather, R. T. and H. H. Brown. 1945. An Experimental Fishery Survey in Trinidad, Tobago and British Guiana. U. S. Govt. Printing Office, Washington, D. C. 130 pp.
Whitford, H. 1911. The forests of the Philippines. Bur. For. Bull. 10 (1): 1-94.
Whitford, H. 1911. The forests of the Philippines. Bur. For. Bull. 10 (2): 1-113.
Whitlo, C. K. 1947. Animal life in mangroves. W. Aust. Nat. 1: 53-56.
Whittaker, R. H. 1953. A consideration of climax theory: the climax as a population and pattern. Ecol. Monogr. 23: 41-78.
Wiehe, P. O. 1935. A quantitative study on the influence of tide upon populations of *Salicornia europaea*. J. Ecol. 23: 323-333.
Wiele, N. 1882. Om Stammens og Bladets Byning hos *Avicennia nitida* L. Bot. Tidsskr. 13: 156.
Wieler, A. 1898. Die function der Pneumathoden und das Aerenchym. Jb. wiss. Bot. 32: 503-524.
Wiens, H. J. 1956. The geography of Kapingamarangi Atoll in the eastern Carolines. Atoll Res. Bull. 48, June 1956.
Wiens, H. J. 1962. Atoll Environment and Ecology. Yale Univ. Press, New Haven, 532 pp.
Wijk, C. L. van. 1953. Soil survey of the tidal swamps of South Borneo in connection with the agricultural possibilities. Contrib. Cent. Agr. Res. Stat., Bogor, Indonesia, No. 123.
Williams, R. R. 1911. The economic possibilities of the mangrove swamps of the Philippines. Philipp. J. Sci. A. 6: 45-61.
Willis, J. H. 1944. Excursion to Seaholm salt marsh flora and mangroves. Viet. Nat. 61: 40-41.
Wilson, E. O. and D. S. Simberloff. 1969. Experimental zoo-geography of islands: defaunation and monitoring techniques. Ecology 50: 267-278.
Wilson, O. 1924. Latin America as a source of tanning materials. Chem. Metall. engin. 30: 303-305, 344-346, 398-399.

Wilson, W. P. 1889. The production of aerating organs on the roots of swamp and other plants. Proc. Acad. Nat. Sci. Philadelphia, pp. 67-69.

Winkler, H. 1931. Einige Bemerkungen uber Mangrove-Pflanzen und den *Amorphophallus titanum* im Hamburger Botanischer Garten. Ber. Dtsch. bot. Ges. 49: 87-102.

Womersley, H. B. S. and S. J. Edmonds. 1952. Marine coastal zonation in southern Australia in relation to a general scheme of classification. J. Ecol. 40: 89-90.

Womersley, H. B. S. and S. J. Edmonds. 1958. A general account of the intertidal ecology of south Australian coasts. Aust. J. Mar. Freshwater Res. 9: 217-260.

Wyatt-Smith, J. 1953a. The malayan species of *Bruguiera*. Malay. For. 16: 156-161.

Wyatt-Smith, J. 1953b. The malayan species of *Sonneratia*. Malay. For. 16: 213-216.

Wyatt-Smith, J. 1954a. The malayan species of *Avicennia*. Malay. For. 17: 21-25.

Wyatt-Smith, J. 1954b. Mangrove flora replaced by fresh water forest. Malay. For. 17: 25-26.

Wyatt-Smith, J. 1960. Field key to the trees of mangrove forests in Malaya. Malay. For. 23: 126-132.

Wyatt-Smith, J. and A. J. Vincent. 1962. Progressive development in the management of tropical lowland evergreen rain forest and mangrove forest in Malaya. Malay. For. 25: 199-223.

Yamamoto, Y. 1940. Enumeration of the mangrove plants, including herbs, climers, epiphytes, and the lower forms of plant life from the Dutch East Indies. Trop. Agric. 12: 157.

Yamanaka, T. 1957. The forest vegetation of the Amani islands with special reference to the *Shiia sieboldii* forest. Rep. U.S.A. Biol. Stat. Kochi Univ. 4, 14 pp.

Yamashiro, M. 1961. Ecological study on *Kandelia candel* (L.) Druce, with special reference to the structure and falling of seedlings. Hikobia 2: 209-214.

Young, C. M. 1930. A Year on the Great Barrier Reef. Putnam, New York, 246 pp.

Yonge, C. M. 1953. Aspects of life in muddy shores. *in* Essays in Marine Biology, S. M. Marshall and A. P. Orr (eds.), Oliver and Boyd, Edinburgh, pp. 29-49.

Zieman, J. C. Origin of circular beds of *Thallasia* (Spermatophyta: Hydrocharitaceae) in south Biscayne Bay, Florida and their relationship to mangrove hammocks. Bul. Inst. Mar. Sci. *in press.*

Zilch, A. 1954. Moluscos de los manglares de El Salvador. Com. Inst. Trop. Invest. Cient. (El Salvador) 3: 77-87.

Zinderen Bakker, E. M. van. 1965. On swamp vegetation and structure of

swamps in south and east Africa. Bot. Jb. Syst. Pflanzengeogr. 84: 215-231.

Zullo, V. A. 1969. Additional records of *Tetrabalanus polygenus* Cornwall, 1941 (Cirripedia, Thoracica). Occas. Pap. Calif. Acad. Sci. 74: 1-8.

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Reprinted by
U.S. Environmental Protection Agency
from
Pesticides Monitoring Journal, Volume 6, Number 4, March 1973

Organochlorine Residues in Estuarine Mollusks, 1965-72—National Pesticide Monitoring Program[1]

Philip A. Butler[2]

Part I. General Summary and Conclusions

Part II. Residue Data—Individual States

ABSTRACT

This paper describes the development of the national program for monitoring estuarine mollusks in 15 coastal States and reports the findings for the period 1965-72. The report is presented in two parts: Part I. General Summary and Conclusions, and Part II. Residue Data—Individual States.

Analyses of the 8,095 samples for 15 persistent organochlorine compounds showed that DDT residues were ubiquitous; the maximum DDT residue detected was 5.39 ppm. Dieldrin was the second most commonly detected compound with a maximum residue of 0.23 ppm. Endrin, mirex, toxaphene, and polychlorinated biphenyls were found only occasionally. Results indicate a clearly defined trend towards decreased levels of DDT residues, beginning in 1969-70. At no time were residues observed of such a magnitude as to imply damage to mollusks; however, residues were large enough to pose a threat to other elements of the biota through the processes of recycling and magnification.

[1] Contribution No. 155 from the Gulf Breeze Environmental Research Laboratory, U.S. Environmental Protection Agency, Gulf Breeze, Fla. 32561, an Associate Laboratory of the National Environmental Research Center, Corvallis, Oreg.

[2] Ecological Monitoring Branch, Technical Services Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Gulf Breeze, Fla. 32561.

Part I. General Summary and Conclusions

Introduction

Initial investigations of the effects of pesticides on estuarine fauna were undertaken at the Gulf Breeze Laboratory in 1958 to determine if the pesticide lindane might be safely used directly in estuarine waters to control crabs preying on shellfish populations. The unexpected acute toxicity of this chemical, not only to crabs but also to nontarget organisms, revealed by these early experiments prompted a broad investigation of both the direct and indirect effects of persistent synthetic pesticides. The extent of the problem was not known, and the investigators were concerned about the potential harm to estuarine fauna exposed to drainage waters from large river basins where significant quantities of pesticides were used. Marine commercial fisheries were recognized as being especially vulnerable since a major portion of their catch, both in tonnage and dollar value, is made up of estuarine-dependent species.

The acute toxicity of a broad spectrum of pesticides was determined under laboratory conditions (*14-17*). These data, however, could be most useful only if there were information on the actual levels of organochlorines reaching the estuarine environment. Accordingly, a search was undertaken for meaningful tools to measure this type of pollution (*6*).

The decision to use mollusks as bioassay tools was based on the findings of laboratory experiments designed to measure the uptake and flushing rates of an array of organochlorine pesticides under controlled conditions. Various species of mollusks, but primarily the eastern oyster, *Crassostrea virginica,* were exposed to appropriate concentrations of pesticides added continuously to a flowing seawater system. Results indicated that oysters detect DDT in the ambient water supply at levels as low as 10 parts per trillion (10^{-11}). By the process of biomagnification, residues of DDT as high as 25 ppm accumulate in oyster tissues within 96 hours at a level of environmental contamination of only 1.0 ppb (*1*). Oysters tolerate tissue residues of DDT at least as high as 150 ppm without apparent ill effect *provided* residues are accumulated slowly. However, as little as 0.1 ppm of DDT in the oyster's water supply terminates feeding activities and at summer water temperatures (31°C) will cause death.

Organochlorine residues are flushed rapidly from molluscan tissues when the water supply is no longer contaminated. In one experimental series, for example, DDT residues of about 25 ppb in oysters and soft clams, *Mya arenaria,* diminished by 50-90% after a week of flushing in clean water. Consequently, it is possible to learn much about the periodicity of organochlorine pollution in estuaries from samples of sedentary species collected at appropriately brief intervals.

As a result of these studies, it was possible for the Bureau of Commercial Fisheries to undertake a program for monitoring pesticide residues in estuarine mollusks to determine the extent of organochlorine pollution. The collection of samples was not begun immediately in some areas, while in others, sample collection was terminated at an early date. The program was continuously operative, however, from July 1965 through June 1972. In 1971, the Gulf Breeze Laboratory and the monitoring program became a part of the U.S. Environmental Protection Agency.

The following report describes the 7-year (1965-72) data collection and discusses, specifically, the well-defined trends in the magnitude of DDT residues in estuarine mollusks. Except where noted, the term DDT includes the metabolites TDE and DDE. All residue analyses are presented, by State, in Part II of this report. A report summarizing the first 3 years of this program was published in 1969 (*3*).

Data Interpretation

Although the eastern oyster has a wide distribution, it was obvious that some other species might be more available for monitoring in different geographical areas or salinity regimes; thus, different species of mollusks were tested in the laboratory to determine their relative capabilities in the uptake and retention of organochlorine pollutants (*2*). Such information is all important for the understanding of these monitoring data.

In the tests, all species were exposed to the same hydrographic conditions with low turbidity and a salinity level about 80% that of seawater. It is probable that species accustomed to different ecological conditions would react more efficiently in nature than in the Laboratory. Caution must be exercised in the extrapolation of laboratory results to field conditions, and, at best, such data serve only as guidelines for the interpretation of residue levels in monitored samples.

In general, any of three species of oysters, four species of mussels and two species of clams were found to be reliable indicators of the magnitude of organochlorine pollution (Table 1). In some areas it was necessary to use the hard clam, *M. mercenaria,* although it is the least satisfactory of the species evaluated. Under similar

laboratory conditions, for example, hard clams accumulated pesticide residues less than half as large as those in oysters. Moreover, the residues were flushed from the clam much more quickly than from the oyster when clean water was restored.

Sample Collection and Preparation

The management of estuarine molluscan resources is the responsibility of the individual States; therefore, in each coastal area there is a cadre of specialists who are not only interested in estuarine pollution but who also have the knowledge and equipment necessary to collect shellfish samples. Without the continuing cooperation of these agencies (see Acknowledgment), this program could not have achieved its objectives.

TABLE 1.—*Pelecypod mollusks used to monitor organochlorine residues in 15 States—1965-72*

Scientific and Common Names of Mollusks	
Crassostrea gigas	Pacific oyster
Crassostrea virginica	eastern oyster
Ostrea lurida	Olympia oyster
Modiolus demissus	ribbed mussel
Modiolus modiolus	northern horse mussel
Mytilus californianus	Californian mussel
Mytilus edulis	blue mussel
Mercenaria mercenaria	hard clam
Mya arenaria	soft clam
Corbicula fluminea	Asiatic clam, fresh water

State	Species Collected
Alabama	*C. virginica*
California	*C. gigas* *O. lurida* *M. demissus* *M. californianus* *M. edulis* *C. fluminea*
Delaware	*C. virginica* *M. demissus* *M. mercenaria*
Florida	*C. virginica*
Georgia	*C. virginica*
Maine	*M. modiolus* *M. edulis* *M. arenaria*
Maryland	*C. virginica*
Mississippi	*C. virginica*
New Jersey	*C. virginica*
New York	*C. virginica* *M. demissus* *M. edulis* *M. mercenaria* *M. arenaria*
North Carolina	*C. virginica*
South Carolina	*C. virginica*
Texas	*C. virginica*
Virginia	*C. virginica*
Washington	*C. gigas*

Estuaries with well defined drainage basins and bays that could be considered "nursery areas" for estuarine fauna were selected for monitoring.

Some sites were monitored because of suspected pollution problems. To insure continuity of data, permitting detection of not only seasonal but yearly trends in pesticide pollution levels, it was essential, too, that the stations selected have shellfish populations large enough for monthly collections over a number of years. Duplicate samples of 15 or more mature mollusks were collected and prepared for shipment at about 30-day intervals. About 10% of all samples were analyzed in replicate; the remaining duplicates were discarded after satisfactory analysis of the sample. Sample collections were interrupted by the loss of shellfish populations to vandals, floods, and hurricanes, but the overall continuity of the data was good.

Coverage of coastal estuaries was incomplete in this program because other agencies were monitoring shellfish in some states, notably Alabama, Louisiana, and Massachusetts. The number of sample collections by State and year is tabulated in Table 2. The original plan was to monitor each area for 5 years so that trends in pesticide residue levels could be detected. The general absence of residues in Washington estuaries, however, prompted an earlier termination of monitoring in that State. In addition to the samples tabulated, about 2,000 miscellaneous samples of other species of vertebrates and invertebrates were collected and analyzed. These frequently had more varied pesticide residues and at higher levels than mollusks but are omitted from this report because of difficulty in determining their source.

The analysis of all samples by a single laboratory to insure uniformity seemed important in planning the program. Various potential preservatives were examined to find a method for shipping samples without resorting to refrigeration. Eventually, it was discovered that by dehydrating the homogenized tissues of mollusks or other marine animals with a desiccant mixture, the dry samples could be wrapped in aluminum foil and held without refrigeration for 15 or more days without degradation or loss of organochlorine residues (*2*). This made it possible to ship the samples by regular mail.

In practice, samples of 15 or more mature oysters or other mollusks were collected and taken to the cooperating agency's laboratory. Samples not to be processed immediately could be refrigerated for 2 or 3 days in the shell. If longer storage was necessary, animals were shucked and the undrained meats frozen in mason jars. The shucked meats were homogenized in an electric blender, and approximately 25-g aliquots were blended

with precisely three times their weight of desiccant to yield a total sample weight of about 100 g. Alternate blending and chilling (not freezing) of sample is required to achieve a dry, free-flowing product. The amount of desiccant used depends on the moisture content of the sample. Less desiccant is required for fish (two times body weight), while up to nine times as much desiccant may be used with small samples, plankton for example, to achieve a 100-g final weight of the sample to be processed. The desiccant is made up of about 90% sodium sulfate and 10% Quso (Quso G30, manufactured by Philadelphia Quartz Co., Philadelphia, Pa.), a micro fine precipitated silica.

Analytical Procedures

Throughout the monitoring program samples were routinely screened for the following substances: aldrin, chlordane, *p,p'*-DDT, *p,p'*-TDE (DDD), *p,p'*-DDE, dieldrin, endrin, heptachlor, heptachlor epoxide, lindane, methoxychlor, mirex, and toxaphene. On the few occasions when the *o,p'* isomers of DDT were detected in quantifiable amounts, they were included with the *p,p'* residues.

On receipt in the laboratory, samples were extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and transferred to 250-ml separatory funnels. The extracts were diluted to 25 ml with petroleum ether and partitioned with two, 50-ml portions of acetonitrile previously saturated with petroleum ether. The acetonitrile was evaporated just to dryness, and the residue eluted from a Florisil column (*12*). The sample was then identified and quantitated by electron capture gas chromatography. Three columns of different polarity (DC-200, QF-1, and mixed DC-200/QF-1) were used to confirm identification Operating parameters on Varian Aerograph gas chromatographs were as follows:

Columns:	Pyrex glass 6' x ⅛" (o.d.) packed with 3% DC-200, 5% QF-1, and a 1:1 ratio of 3% DC-200 and 5% QF-1, all on 80/100 mesh Gas Chrom Q
Temperatures:	Detector—210° C Injector—210° C Oven—190° C
Carrier gas:	Prepurified nitrogen at a flow rate of 40 ml/min

A few samples were analyzed by thin layer chromatography. All residues are reported on a wet-weight basis. The lower limit of quantification was 10 ppb. Laboratory tests conducted during the sampling period gave the following recovery rates: *p,p'*-DDE, 80-85%; *p,p'*-TDE, 92-95%; *p,p'*-DDT, 91-95%. Data in this report do not include a correction factor for percent recovery.

Toxaphene sometimes interfered with the quantification of DDT residues which, in these cases, are reported as approximate values. The lower limit of quantification of toxaphene was 250 ppb. The presence of polychlorinated biphenyls (PCB's) also interfered with the quantification of DDT residues. In most instances, DDT was calculated as though PCB's were not present. Acquisition

TABLE 2.—*Summary of sample collections by State and year—1965-72*

State	Principal Species Monitored	Number of Sample Collections								
		1965	1966	1967	1968	1969	1970	1971	1972	Totals
Alabama	*C. virginica*				13	20				33
California	*C. gigas*		136	180	167	139	45	75	30	772
Delaware	*M. mercenaria*		16	101	99	71				287
Florida	*C. virginica*	6	80	102	82	44	35	19	6	374
Georgia	*C. virginica*			112	127	124	121	120	60	664
Maine	*M. arenaria*	6	95	89	79	83	44			396
Maryland	*C. virginica*		18	20	26	9	15			88
Mississippi	*C. virginica*	30	71	72	72	63	66	60	36	470
New Jersey	*C. virginica*		23	44	45	39	33	27	8	219
New York	*M. mercenaria*		148	183	175	174	148	143	88	1,059
North Carolina	*C. virginica*		96	201	204	204	124	136	66	1,031
South Carolina	*C. virginica*	72	142	143	145	108				610
Texas	*C. virginica*	53	133	125	93	97	103	95	29	728
Virginia	*C. virginica*	56	117	123	120	112	105	27	9	669
Washington	*C. gigas*	40	218	223	214					695
Total		263	1,293	1,718	1,661	1,287	839	702	332	8,095

of the appropriate standards permitted the identification of Aroclor 1254® in samples from California, Florida, Georgia, Texas, and Virginia, and Aroclor 1242® in samples from Virginia. Since 1970, PCB residues have been approximately quantified in samples from Florida and, more recently, from Virginia.

There is some question as to how much interference by PCB's exists in the sample analyses reported in the early years of the monitoring program. At this time there is no way of knowing with certainty. It is considered significant that in the period 1965-70 there was a 3-8% annual increase in the domestic sale of these chemicals, and total domestic sales in 1970 were more than double sales in 1960; however, PCB residues were identified in samples fron relatively few estuaries in 1971.

During the course of the program, several States extended the monitoring of their estuaries and collected more samples than the Gulf Breeze Laboratory was equipped to process. Analytical equipment similar to that used at Gulf Breeze was provided to these agencies as well as a manual of operations (Prepared by A. J. Wilson, Jr., Research Chemist, Gulf Breeze Laboratory), to insure similar methodology in analytical techniques. For the first few collections under the new arrangement, samples were split and analyses made by both the State and Federal laboratories. Excellent comparability in data was obtained (*13*) and thereafter, the State agency submitted only the monthly data reports to the Gulf Breeze Laboratory. Such arrangements were in effect during portions of the monitoring program in California, Georgia, Maine, New York, and Virginia.

Data Summaries and Discussion

DDT with its analogs was the most commonly identified pesticide and occurred in 63% of all samples analyzed (Table 3). Dieldrin was the second most commonly detected residue with an incidence of 15%. DDT and dieldrin were detected in some samples from all States monitored (Tables 4 and 5). Other organochlorine residues were encountered infrequently and generally at low levels, with the exception of toxaphene. The large number of Georgia samples containing toxaphene reflects the direct contamination of the marine environment by the effluent from a single manufacturing plant.

The incidence of DDT residues varied markedly from one drainage basin to another and was not correlated with the magnitude of the residues. Only in New Jersey and Alabama, for example, did all samples contain detectable residues of DDT, but the size of DDT residues was greater in several other States (Table 4). It is true that in both Alabama and New Jersey, monitored oyster populations were exposed primarily to the runoff from a single, although complex, drainage basin. In other States, samples were collected from several distinct drainage basins.

TABLE 3 —*Summary of organochlorine residues detected in estuarine mollusks by State—1965-72*

State	Total Number of Samples	Number of Samples with Residues >5 PPB (µG/KG) and Maximum Residue ( ) Detected in PPB (µG/KG)					
		DDT	Dieldrin	Endrin	Mirex	Toxaphene	PCB's
Alabama	33	33 (616)	6 (21)				
California	772	712 (3,970)	194 (57)	14 (19)		4 (11,000)	[1] 21
Delaware	287	216 (205)	37 (25)				
Florida	374	230 (5,390)	27 (28)				25 (390)
Georgia	664	96 (96)	141 (230)			128 (54,000)	[1] 16
Maine	396	72 (359)	14 (38)				
Maryland	88	71 (70)	11 (22)				
Mississippi	470	285 (135)	19 (20)				
New Jersey	219	219 (278)	52 (26)				[1] 6
New York	1,059	858 (596)	456 (132)				
North Carolina	1,031	768 (566)	12 (19)				
South Carolina	610	332 (154)	24 (154)		12 (540)		
Texas	728	530 (1,249)	134 (87)	22 (32)		[1] 1	[1] 5
Virginia	669	585 (678)	112 (40)				19 (2,800)
Washington	695	78 (176)	1 (120)				

[1] Present but not quantified.

TABLE 4.—*Listing of States in order of frequency and maximum value of DDT residues in mollusks*

State	Frequency of Residues (%)	State	Maximum Value in PPB
Alabama	100	Florida	5,390
New Jersey	100	California	3,970
California	92	Texas	1,249
Virginia	87	Virginia	678
New York	81	Alabama	616
Maryland	81	New York	596
Delaware	75	North Carolina	566
North Carolina	75	Maine	359
Texas	73	New Jersey	278
Florida	62	Delaware	205
Mississippi	61	Washington	176
South Carolina	54	South Carolina	154
Maine	18	Mississippi	135
Georgia	15	Georgia	96
Washington	11	Maryland	70

NOTE: These comparisons are limited in that the number of samples, number of sampling stations, periods (years) of sampling, and species of mollusks differ for each State.

TABLE 5.—*Listing of States in order of frequency and maximum value of dieldrin residues in mollusks*

State	Frequency of Residues (%)	State	Maximum Value in PPB
New York	43	Georgia	230
California	25	South Carolina	154
New Jersey	24	New York	132
Georgia	21	Washington	120
Alabama	18	Texas	87
Texas	18	California	57
Virginia	17	Virginia	40
Delaware	13	Maine	38
Maryland	13	Florida	28
Florida	7	New Jersey	26
Mississippi	4	Delaware	25
South Carolina	4	Maryland	22
Maine	4	Alabama	21
North Carolina	1	Mississippi	20
Washington	<1	North Carolina	19

NOTE: These comparisons are limited in that the number of samples, number of sampling stations, periods (years) of sampling, and species of mollusks differ for each State.

The magnitude of all DDT residues was low compared to residues reported in carnivores such as fish-eating birds. By extrapolation from laboratory experiments, the monitoring data indicate that, in most cases, estuarine pollution with DDT was intermittent and at levels in the low parts-per-trillion range. In only 38 samples (0.5%) did the residue exceed 1.0 ppm. These samples were collected in California, Florida, and Texas in drainage basins having intensive agricultural development. The single highest residue of 5.39 ppm (DDT-3.70 ppm, TDE-0.76 ppm, DDE-0.93 ppm) was observed in oysters from the Caloosahatchee River drainage basin in Florida where the seasonal pattern of residue fluctuations indicated an agricultural or at least a scheduled use of the pesticide (Fig. 1). It is significant that extensive acreage in this drainage basin was devoted to sugarcane and sweetcorn that would be maturing and receiving fairly heavy applications of pesticides during the peak residue periods indicated in Fig. 1 (R. G. Curtis, 1972, Florida Cooperative Extension Service, *personal communication*). In controlled feeding experiments in the laboratory, from 50 to 100% mortality was observed in small populations of commercial species of shrimp, crabs, and fish fed exclusively diets containing less than 3.0 ppm of *p,p'*-DDT (*4*).

In a survey of 7,000 analyses of mollusk samples completed in the period 1965-71, the mean residue composition was 24% DDT, 39% TDE, and 37% DDE. Exceptions to this average picture were Station 2 in New Jersey where DDT comprised only 4% (mean of 47 samples in 5 years) and Station 18 in Washington where DDT made up 75% of the residues (mean of 36 samples in 3 years). Biotic recycling of persistent residues is usually associated with the high percentages of DDT metabolites found in dominant carnivores. It is of interest that the metabolites were the only residues detected in many of these analyses of filter-feeding mollusks. Results of a study by Johnson *et al. (10)* indicated that there are some animals, however, such as aquatic insects, in which direct exposures to DDT result in tissue residues that are more than 80% DDE. The large percentage of the parent compound DDT in residues from Washington mollusks does imply a direct contamination of the estuarine environment, perhaps, for insect control. But in general, the percentage distribution of DDT metabolites in these samples revealed little about the kinetics of DDT in the estuary.

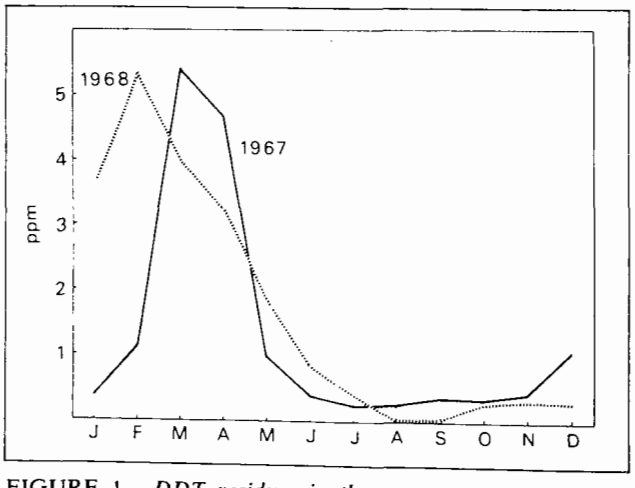


FIGURE 1.—*DDT residues in the eastern oyster from the Caloosahatchee River Basin, Lee County, Fla., by month of collection—1967 and 1968*

Trends in DDT Residues

Many of the estuaries were monitored over a sufficient period of time to permit detection of clearly defined trends in DDT residue patterns. Average DDT residue levels detected in the first 2 to 5 years and average levels in the final year of monitoring in each State are presented in Table 6. The overall picture is that of a pronounced decline, about 55%, in the number of samples containing DDT residues in excess of 100 ppb. There was a 20% decrease in the 10-100 ppb range, and a concomitant 44% increase in the number of samples having negligible or undetectable DDT residues.

There are important exceptions to this average picture. In California, New York, and Virginia, for example, more samples had residues in excess of 10 ppb in 1971 than in earlier years. On the other hand, the percent of samples having residues higher than 100 ppb declined in these States. It would appear that in some areas, DDT pollution has become more widespread, but has resulted in residues of lower magnitude in the estuarine food web.

Since organochlorine residues in mollusks showed a continuing decline in most areas during the years that domestic sales and presumably usage of PCB compounds were increasing, PCB's were not considered to be a significant factor in the early monitoring data.

Too few samples from Alabama were analyzed in this program to indicate any trend in residue magnitude. The mean value of 88 ppb of DDT in 33 samples collected in 1969-70 may be compared, however, with a mean residue level of 330 ppb in a series of 82 samples of oysters collected in 1965-66 (*7*).

Exact comparisons by States of the data in Table 6 are not valid since in succeeding years there were different numbers of samples and occasionally different species of mollusks collected at the same station. A more critical review of data on DDT residues is possible for 10 stations in North Carolina. These stations were selected for the continuity of sampling of the eastern oyster at monthly intervals for more than 5 years. The number of samples containing less than 11 ppb of DDT increased steadily until, in 1971, 76% of all residues were in this category as compared to only 8% in 1966 and 1967. The corresponding decrease in the number of samples containing larger residues is shown in Fig. 2 and Table 7.

TABLE 6.—*Percent distribution of DDT residues of different magnitude in estuarine mollusks by State—1965-71 (7,000 samples)*

State	Percent Distribution of Samples							
	<11 PPB		11-100 PPB		101-1,000 PPB		>1,000 PPB	
	First 2 to 5 Years	1971	First 2 to 5 Years	1971	First 2 to 5 Years	1971	First 2 to 5 Years	1971
Alabama			69		31			
California	14	7	30	64	51	28	5	1
Delaware	23	30	62	67	15	3		
Florida (1 station)	43	100	57					
Georgia	85	96	15	4				
Maine	82	98	17	2	1			
Maryland	19	50	81	50				
Mississippi	42	72	56	27	2	1		
New Jersey		7	69	74	31	19		
New York	26	22	60	74	14	4		
North Carolina	22	76	68	24	10			
South Carolina	52	82	47	18	1			
Texas	34	52	53	45	13	3	<1	
Virginia	18		67	95	15	5		
Washington (1 station)	92	94	8	6				
Mean	39	56	49	39	11	5	<0.5	

TABLE 7.—*Trends in magnitude of DDT residues in oysters, 10 stations, North Carolina*

Year	Total Number of Samples	<11 PPB		11-100 PPB		101-1,000 PPB	
		Number of Samples	Percent Distribution	Number of Samples	Percent Distribution	Number of Samples	Percent Distribution
1966	60	5	8	45	75	10	17
1967	119	9	8	90	76	20	16
1968	120	26	22	70	58	24	20
1969	120	29	24	77	64	14	12
1970	109	61	56	46	42	2	2
Subtotal	528	130	25	328	62	70	13
1971	115	87	76	26	22	2	2
Percent change in 1971 from average for 1966-70		+204%		−65%		−85%	

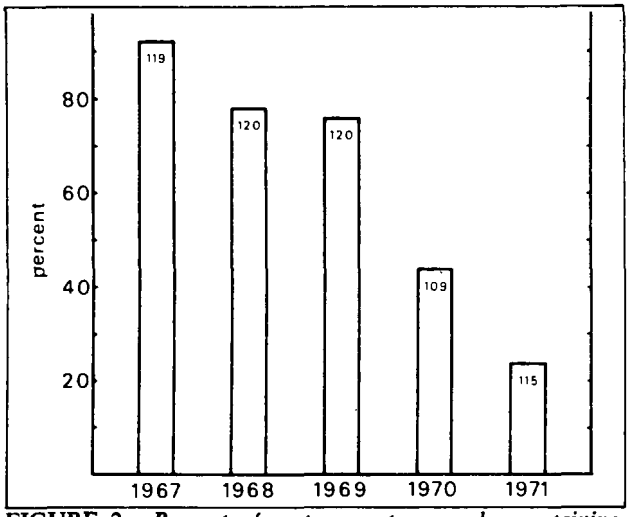


FIGURE 2.—*Percent of eastern oyster samples containing more than 10 ppb of DDT, average of monthly samples collected at 10 stations in North Carolina (Numbers in bars indicate total number of samples)*

Conclusions

The data demonstrate that in most estuaries monitored, detectable DDT residues have declined in both number and magnitude in several species of estuarine mollusks in recent years. DDT pollution in many estuaries, as judged by the magnitude of the residues in mollusks, peaked in 1968 and has been declining markedly since 1970.

The sensitivity of mollusks to organochlorine pollutants plus the fact that they are filter-feeders warrant the assumption that the contribution of particulate DDT to estuaries from one or more primary sources such as drainage basin runoff waters, atmospheric fallout, and persistent reservoirs in bottom sediments, has declined significantly.

In view of the efficiency of mollusks in detecting and storing residues of the persistent organochlorines, it is clear that relatively low levels of this type of pollution were present in the monitored areas during the period 1965 to 1972.

Appropriate correlations of the residue data reported here with available records of drainage-basin discharge rates, precipitation, and hydrographic factors in the various types of estuaries should provide a useful model for predicting the effects of future introductions of unspecified synthetic substances chemically similar to DDT.

See Appendix for chemical names of compounds discussed in this paper.

LITERATURE CITED

(1) *Butler, P. A. 1966.* Pesticides in the marine environment. J. Appl. Ecol. 3(Suppl.): 253-259.

(2) ———. *1968.* Pesticide residues in estuarine mollusks. Proc. Natl. Symp. Estuarine Pollut. Stanford University, Stanford, Calif., 1967. p. 107-121.

(3) ———. *1969.* Monitoring pesticide pollution. BioScience 19(10): 889-891.

(4) ———. *1969.* Significance of DDT residues in estuarine fauna. p. 205-220. *In* Chemical Fallout. Charles C. Thomas, Springfield, Ill.

(5) *Butler, P. A., A. J. Wilson, Jr., and R. Childress. 1972.* The association of DDT residues with losses in marine productivity. p. 262-266. *In* Marine pollution and sea life. Fishing News (Books) Ltd., London, Eng.

(6) *Butler, P. A., A. J. Wilson, Jr., and A. J. Rick. 1960.* Effect of pesticides on oysters. Proc. Natl. Shellfish. Assoc. 51:23-32.

(7) *Casper, V. L., R. J. Hammerstrom, E. A. Robertson, Jr., J. C. Bugg, Jr., and J. L. Gaines. 1969.* Study of chlorinated pesticides in oysters and estuarine environment of the Mobile Bay area. USDHEW, Consum. Prot. Environ. Health Serv. 47 p.

(8) *Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. 1970.* A polychlorinated biphenyl (Aroclor 1254®) in the water, sediment, and biota of Escambia Bay, Florida. Bull. Environ. Contam. Toxicol. 5(2): 171-180.

(9) *Foehrenbach, J., G. Mahmood, and D. Sullivan. 1971.* Chlorinated hydrocarbon residues in shellfish (Pelecypoda) from estuaries of Long Island, New York. Pestic. Monit. J. 5(3): 242-247.

(10) *Johnson, B. T., C. R. Saunders, H. O. Sanders, and R. S. Campbell. 1971.* Biological magnification and degradation of DDT and aldrin by freshwater invertebrates. J. Fish. Res. Board Can. 28(5): 705-709.

(11) *May, E. B. 1971.* A survey of the oyster and oyster shell resources of Alabama. Ala. Mar. Resourc. Bull. 4:1-53.

(12) *Mills, P. A., J. H. Onley, and R. A. Gaither. 1963.* Rapid method for chlorinated pesticide residues in nonfatty foods. J. Assoc. Off. Agric. Chem. 46(2): 186-191.

(13) *Modin, J. C. 1969.* Chlorinated hydrocarbon pesticides in California bays and estuaries. Pestic. Monit. J. 3(1): 1-7.

(14) *U.S. Department of the Interior. 1962.* Effects of pesticides on fish and wildlife in 1960. Fish Wildl. Serv. Circ. 143. Washington, D.C. 52p.

(15) _____. 1963. Pesticide-wildlife studies: a review of Fish and Wildlife Service investigations during 1961 and 1962. Fish Wildl. Serv. Circ. 167. Washington, D.C. 109p.

(16) _____. *1964.* Pesticide-wildlife studies 1963: a review of Fish and Wildlife Service investigations during the calendar year. Fish Wildl. Serv. Circ. 199. Washington, D.C. 129p.

(17) _____. *1965.* The effects of pesticides on fish and wildlife. Fish Wildl. Serv. Circ. 226. Washington, D.C. 77p.

Acknowledgments

I should like to but cannot acknowledge individually the many people in administrative and technical positions whose interest in this program made its efficient conduct possible. It is my pleasure to thank especially Louis D. Stringer, Thomas C. Carver, Chester E. Danes, Anne Gibson, now of the National Marine Fisheries Service, and my secretary, Madeleine Brown, for their continuing cooperation and assistance.

We are greatly indebted to the graduate students and technicians whose diligence in the collection and processing of samples made the program a reality. I trust that the results will make them pleased with their participation.

The program could not have been developed without the interest and skills of Alfred J. Wilson, Jr., Research Chemist at the Gulf Breeze Laboratory.

Lastly, I thank the administrators and professional staffs of the cooperating agencies who kindly let me think that the monitoring program had the number one priority on their busy schedules.

In view of the volume of data in this report, it is inevitable that there are sins of both omission and commission. The writer would be most grateful to have these called to his attention so that the record can be appropriately emended.

COOPERATING AGENCIES—This alphabetical listing by States includes the names of investigators and, where appropriate, chemists and their titles at the time they were participating in the program. Where chemists are not listed, the samples were analyzed at the Gulf Breeze Laboratory under the supervision of Alfred J. Wilson, Jr., with the assistance of Jerrold Forrester and Johnny Knight. The listing of more than one principal investigator or agency in any one State reflects changes taking place during the monitoring period 1965-72. Operational funds were provided by the U.S.F.W.S., Bureau of Commercial Fisheries (BCF) for the collection of samples and for analytical equipment where contracts are indicated. In States participating by agreement, the BCF provided equipment and chemicals. In 1971-72, the program was jointly funded by the National Marine Fisheries Service (NMFS) and the Environmental Protection Agency.

ALABAMA	Alabama Marine Resources Laboratory Johnie H. Crance, Director; E. B. May, Principal Investigator. Agreement.
CALIFORNIA	California Dept. of Fish and Game, Marine Resources Operations Dr. H. C. Orcutt, Laboratory Supervisor; John Modin, Chemist. Contracts, BCF: 14-17-0007-332; 14-17-0002-211; -265; -337; -532. California Department of Fish and Game, Resources Agency W. H. Griffith, Principal Investigator. Contract, NMFS: N-042-10-72(N).
DELAWARE	University of Delaware Dr. F. C. Daiber, Principal Investigator. Contracts, BCF: 14-17-0002-117; -261; -326.
FLORIDA	State Board of Conservation Marine Laboratory R. M. Ingle, Director of Research. Agreement. Bureau of Commercial Fisheries—Environmental Protection Agency, Gulf Breeze Laboratory. Dr. T. W. Duke, Director. Agreement.
GEORGIA	The University of Georgia Dr. T. L. Linton, Principal Investigator. Contracts, BFC: 14-17-0002-220; -267. C. J. Durant, Principal Investigator and Chemist. Contracts, BFC: 14-17-0002-344; -454. Dr. R. J. Reimold, Principal Investigator. Contract, NMFS: N-042-12-71(N).
MAINE	Department of Sea and Shore Fisheries L. Varney, Principal Investigator; John Hurst, Laboratory Director and Chemist. Contracts, BCF: 14-17-0007-333; 14-17-0002-206; -263; -332; -434.
MARYLAND	BCF Biological Laboratory Dr. A. Rosenfield, Principal Investigator. Agreement.
MISSISSIPPI	Gulf Coast Research Laboratory Dr. W. P. Abbott, Principal Investigator. Contracts, BCF: 14-17-0002-133; -172; -235; -341. Dr. G. Gunter, Laboratory Director. Contract, NMFS: N-042-11-71(N).
NEW JERSEY	Rutgers—The State University, Oyster Research Laboratory Dr. H. H. Haskin and D. E. Kunkle, Principal Investigators. Agreement.
NEW YORK	New York State Department of Environmental Conservation D. H. Wallace, Director of Marine Fisheries; J. Foehrenbach, Chemist. Contracts, BCF: 14-17-0002-163; -219; -268; -345; -455; NMFS: N-042-14-71(N).
NORTH CAROLINA	University of North Carolina, Institute for Marine Sciences Dr. A. F. Chestnut, Principal Investigator. Contracts, BCF: 14-17-0002-182; -239; -343; NMFS: N-042-15-71(N).
SOUTH CAROLINA	Bears Bluff Laboratories, Inc. Dr. G. R. Lunz, Director (deceased). Contracts, BCF: 14-17-0002-130; -171; -234; -340; -426.
TEXAS	State of Texas, Parks and Wildlife Department T. R. Leary, Coastal Fisheries Coordinator; R. Childress, Principal Investigator. Agreement.
VIRGINIA	Virginia Institute of Marine Science Dr. M. L. Brehmer, Principal Investigator; Dr. R. J. Huggett, Principal Investigator and Chemist. Contracts, BCF: 14-17-0002-138; -174; -237; -342; -452; NMFS: N-042-13-71(N).
WASHINGTON	State of Washington, Department of Fisheries C. Lindsay, R. E. Westley, Principal Investigators. Contracts, BCF: 14-17-0002-134; -173; -236.

Part II. Residue Data—Individual States

The following sections present residue data for the 15 coastal States where estuarine mollusks were monitored for organochlorine residues. A map showing sampling sites in the respective States together with a discussion of the findings are included in each section.

SECTION A.—ALABAMA

Samples of the eastern oyster, *Crassostrea virginica*, were collected in Alabama at 3-month intervals during 1968-69 from four commercial reefs in or near Mobile Bay. Samples were processed at the Alabama Marine Resources Laboratory and mailed to the Gulf Breeze Laboratory for chemical analysis.

Approximate station locations are shown in Fig. A-1. Stations 1 and 2 on the eastern shore of Mobile Bay are influenced more by the presumably cleaner Gulf of Mexico waters than Stations 3 and 4 which are more exposed to drainage waters from the Alabama-Tombigbee River Basin. Both Stations 1 and 4 are influenced to an unknown extent by small drainage basins in the coastal areas of Alabama. A summary of data on organochlorine residues in the monitored species, *C. virginica*, is presented in Table A-1, and the distribution of residues in this species for each sampling station by date of collection in Table A-2. Many of these data have already been published by the cooperating agency (*10*).

All 33 samples contained detectable amounts of DDT, but the sampling series was conducted in Alabama for too few years to indicate annual trends in pollution levels. An earlier study of pesticide residues in Mobile Bay oysters (*7*) also reported a 100% incidence of DDT in 82 samples analyzed; however, maximum DDT residues at Shell Bank and Cedar Point reefs were 13 and 25 times higher in 1965 than those observed in this study in 1969. Because of differences in sample preparation in the two studies, 1965 residues could be expected to be only about 10% higher than the 1969 data had there been no change in DDT pollution levels in the bay. Alabama and New Jersey were the only States of the 15 monitored in which 100% of the samples contained detectable residues of DDT. The maximum level of DDT in Alabama oysters (616 ppb) was lower than residues found in four other States.

Dieldrin residues were small, but the 18% incidence was significantly higher than the average incidence for all States of 15%. The incidence and magnitude of dieldrin residues in the 1965 study (*7*) were significantly higher.

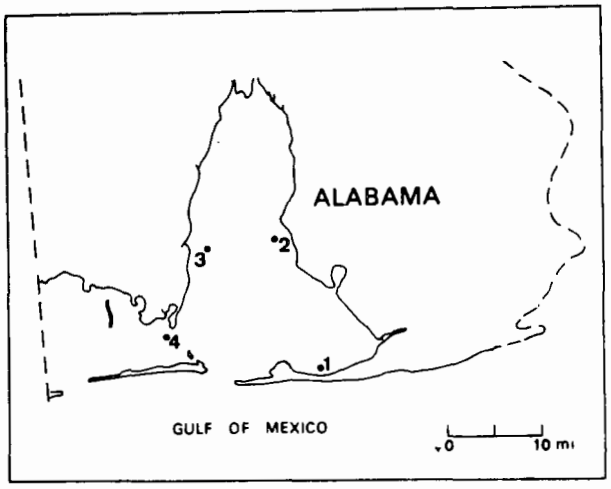


FIGURE A-1.—*Diagram of coastal Alabama showing approximate location of monitoring stations*

1. Shellbank—Bon Secour Bay
2. Klondike—Mobile Bay
3. Whitehouse—Mobile Bay
4. Cedar Point Reef—Mississippi Sound

TABLE A-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1968-69—Alabama*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)	
				DDT	Dieldrin
1	Shellbank	1968-69	8	8 (214)	1 (14)
2	Klondike	1968-69	8	8 (445)	1 (14)
3	White House	1968-69	7	7 (616)	2 (21)
4	Cedar Point	1968-69	8	8 (372)	2 (13)
	Occasional stations (2)	1968-69	2	2 (237)	
Total number of samples			33		
Percent of samples positive for indicated compound				100	18

[1] Each sample represents 15 or more mature mollusks.

TABLE A-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Alabama*

[Blank = no sample collected; — = no residue detected above 5 ppb; T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—SHELL BANK—8 SAMPLES[1]													
1968	DDE				110			110			33		
	TDE				88			52			15		
	DDT				16			12			—		
1969	DDE	48			94			48		26	T		
	TDE	31			70			25		17	T		
	DDT	—			—			—		13	—		
	Dieldrin	—			14			—		—	—		
STATION 2.—KLONDIKE—8 SAMPLES[1]													
1968	DDE				230			210			45		
	TDE				180			130			37		
	DDT				35			34			12		
1969	DDE	120			18			170		73	22		
	TDE	100			94			110		53	18		
	DDT	—			44			23		62	—		
	Dieldrin	—			14			—		—	—		
STATION 3.—WHITE HOUSE—7 SAMPLES[1]													
1968	DDE				320			120			32		
	TDE				240			57			20		
	DDT				56			11			—		
	Dieldrin				20			—			—		
1969	DDE	110			15			56		46			
	TDE	83			98			37		40			
	DDT	—			36			—		36			
	Dieldrin	—			21			—		—			
STATION 4.—CEDAR POINT—8 SAMPLES[1]													
1968	DDE				180			86			41		
	TDE				160			51			23		
	DDT				32			17			23		
1969	DDE	84			77			110		26	30		
	TDE	55			71			78		22	23		
	DDT	—			30			—		26	T		
	Dieldrin	—			13			—		—	T		

[1] Each sample represents 15 or more mature mollusks.

SECTION B.—CALIFORNIA

The monthly collection of mollusks to monitor pesticide pollution in 12 estuaries in California was initiated in January 1966. Some of these stations were terminated and other estuaries were added during the course of the program. Samples were analyzed at the Gulf Breeze Laboratory until May 1968; from then until May 1970 they were analyzed at the Marine Resources Operations Laboratory of the Department of Fish and Game, Menlo Park, Calif. During the period July 1970 June 1972, samples were collected and analyzed at approximately 3-month intervals by the Department of Fish and Game, Pesticides Investigations at Sacramento, Calif.

Six different mollusks (*Crassostrea gigas, Corbicula fluminea, Modiolus denissus, Mytilus californianus, Mytilus edulis, and Ostrea lurida*) were utilized for monitoring; for the most part, a single species was collected at each station. The relative ability of these different mollusks to store organochlorine residues appears to be reasonably similar and, thus, comparisons of the magnitude of residues in different estuaries can be made with some confidence. In general, residue levels at different stations followed patterns of suspected pollution loading in the associated drainage basin, regardless of the species monitored.

The approximate station locations are shown in Fig. B-1. A summary of data on organochlorine residues in the monitored species is presented in Table B-1, and the distribution of residues in these species for each sampling station by date of collection in Table B-2. Results of some of the analyses conducted by the Gulf Breeze Laboratory during the period January 1966 December 1967 have been published by the cooperating agency (*13*).

DDT residues in mollusks were consistently larger in California than in any other area monitored with the exception of a single station in south Florida. There is a clear pattern of maximum pesticide residues being correlated with proximity of the monitoring station to runoff from agricultural lands. In southern California, where most samples contained typically large residues, residues were consistently higher at Hedionda and Mugu Lagoons, the recipients of agricultural runoff waters, than at Anaheim Slough which receives intermittent runoff from the urban and industrialized sections of Los Angeles. Residues in samples from estuaries draining the intensely cultivated central and southern parts of the State were larger, by one order of magnitude usually, than those in samples collected from watersheds north of San Francisco Bay where dairy land predominates.

The incidence of dieldrin residues (25%) was second only to New York samples although residues were lower in magnitude than in five other States. California and Texas were the only States where endrin and toxaphene

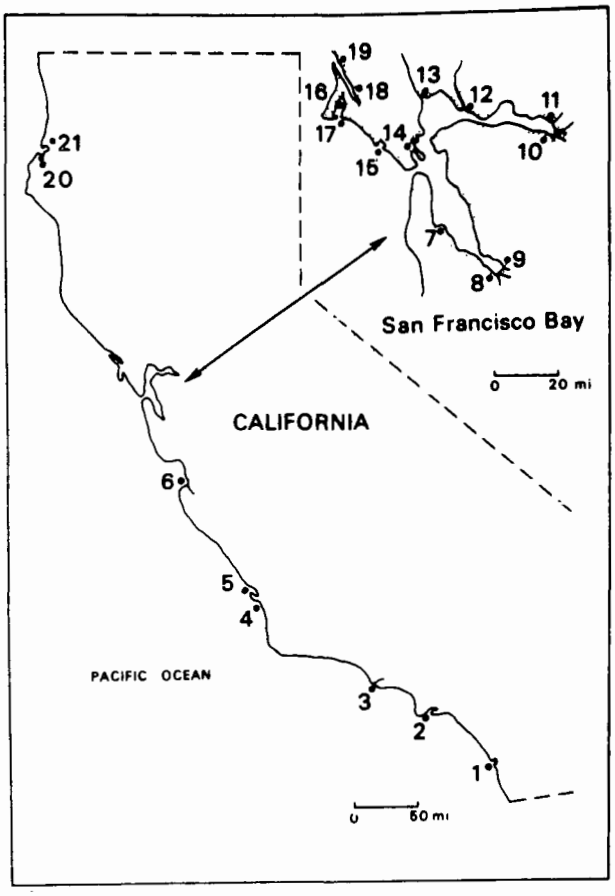


FIGURE B-1.—*Diagram of coastal California and the San Francisco Bay area showing approximate location of monitoring stations*

1. Hedionda Lagoon
2. Anaheim Slough
3. Point Mugu
4. Baywood Park—Morro Bay
5. Los Osos Creek—Morro Bay
6. Elkhorn Slough
7. Coyote Point—San Francisco Bay, South
8. Guadalupe Slough—San Francisco Bay, South
9. Alviso Slough—San Francisco Bay, South
10. West Island—Sacramento-San Joaquin River Basin
11. False River—Sacramento-San Joaquin River Basin
12. Napa River—San Pablo Bay
13. Petaluma River—San Pablo Bay
14. Point San Quentin—San Francisco Bay, North
15. Bolinas Lagoon
16. Schooner Bay—Drakes Estero
17. Berries Bay—Drakes Estero
18. Tomales Bay—Tomales Bay
19. Nicks Cove—Tomales Bay
20. Gunther Island—Humboldt Bay
21. Bird Island—Humboldt Bay

from presumably agricultural sources were detected. Polychlorinated biphenyl compounds were detected in samples beginning in 1971, but were not quantified. They occurred in a few samples from nearly all drainage basins monitored.

Late in 1970 or early 1971, there was a sharp decline in DDT residues in samples collected in estuaries draining predominantly agricultural areas, i.e., San Francisco Bay and the southern parts of the State. Decreased frequency

of sample collection in 1970-71 makes it impossible to pinpoint when this decline in DDT pollution occurred. The typically small DDT residues in samples from drainage basins north of San Francisco Bay remained about the same throughout the monitoring period.

TABLE B-1.—*Summary of data on organochlorine residues in the monitored species, 1966-72—California*

Station Number	Location	Monitoring Period	Principal Monitored Species	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)				
					DDT	Dieldrin	Endrin	Toxaphene	PCB's [2]
1	Hedionda Lagoon	1967-72	*M. edulis*	31	31 (3,970)	4 (T)		2 (11,000)	2
2	Anaheim Slough	1967-72	*M. edulis*	33	33 (833)	10 (31)	1 (T)		2
3	Point Mugu	1967-72	*M. edulis*	29	29 (1,758)	9 (16)	1 (T)		2
4	Baywood Park	1966-72	*C. gigas*	52	52 (601)	3 (24)			
5	Los Osos Creek	1966-72	*C. gigas*	52	52 (412)	4 (27)			1
6	Elkhorn Slough	1966-72	*C. gigas*	57	57 (2,305)	24 (57)	2 (19)		2
7	Coyote Point	1966-72	*O. lurida*	55	54 (362)	26 (43)	2 (19)		1
8	Guadalupe Slough	1968-72	*M. demissus*	27	25 (407)	9 (37)			1
9	Alviso Slough	1968-72	*M. demissus*	28	28 (328)	6 (25)			1
10	West Island	1967-72	*C. fluminea*	28	28 (2,280)	23 (22)	3 (T)		1
11	False River	1967-71	*C. fluminea*	26	26 (1,850)	12 (24)	1 (18)		
12	Napa River	1968-72	*M. demissus*	28	26 (210)	5 (T)	2 (T)		1
13	Petaluma River	1968-72	*M. demissus*	28	25 (268)	4 (10)			1
14	Point San Quentin	1966-70	*C. gigas*	50	49 (440)	22 (23)			
15	Bolinas Lagoon	1966-68	*C. gigas*	17	14 (45)				
16	Schooner Bay	1966-72	*C. gigas*	33	25 (43)	2 (T)			1
17	Berries Bar	1966-68	*C. gigas*	27	25 (44)		1 (19)		
18	Tomales Bay	1966-72	*C. gigas*	34	28 (45)	2 (T)	1 (T)		
19	Nicks Cove	1966-68	*C. gigas*	25	20 (37)				
20	Gunther Island	1966-72	*C. gigas*	33	31 (78)	5 (T)			1
21	Bird Island	1966-68	*C. gigas*	25	3 (T)				
	Occasional stations (15)	1966-72	Mixed	54	51 (1,144)	25 (26)		2 (1,000)	4
Total number of samples				772					
Percent of samples positive for indicated compound					92	25	2	<1	3

NOTE: T = >5 but <10 ppb.
[1] Each sample represents 15 or more mature mollusks.
[2] Present but not quantified.

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*

[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—HEDIONDA LAGOON—*M. EDULIS* UNLESS OTHERWISE INDICATED—31 SAMPLES [1]													
1967	DDE										100	[3] 130	[3] 90
	TDE										72	240	84
	DDT										130	3,600	740
	Toxaphene										—	11,000	970

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—HEDIONDA LAGOON—*M. EDULIS*, UNLESS OTHERWISE INDICATED—31 SAMPLES [1]—Continued													
1968	DDE	130	52	200	210	91	130	120	168	[3] 105	120	136	
	TDE	73	31	88	220	103	154	80	171	74	73	58	
	DDT	920	200	440	300	42	86	59	129	63	120	164	
1969	DDE	52	211	242	118	227	95		139	347	466	76	
	TDE	—	207	101	172	124	53		35	99	115	64	
	DDT	123	291	486	214	99	91		34	61	68	108	
1970	DDE											114	
	TDE											102	
	DDT											54	
	Dieldrin											T	
1971	DDE		19				36		54			18	
	TDE		11				58		56			13	
	DDT		16				T		285			10	
	Dieldrin		T				—		—			—	
	PCB's		—				—		—			[4]	
1972	DDE	14				31							
	TDE	T				31							
	DDT	10				10							
	Dieldrin	T				T							
	PCB's	—				[4]							
STATION 2.—ANAHEIM SLOUGH—*M. EDULIS*, UNLESS OTHERWISE INDICATED—33 SAMPLES [1]													
1967	DDE										360	330	200
	TDE										100	150	87
	DDT										85	120	120
1968	DDE	270	[6] 110	[3] 170	310	464	203	265	432	[3] 464	440	354	
	TDE	91	45	62	110	186	102	68	109	127	170	118	
	DDT	160	43	110	77	108	52	33	51	65	110	70	
	Dieldrin	—	31	—	—	—	T	T	—	—	12	—	
	Endrin	—	—	—	—	—	—	T	—	—	—	—	
1969	DDE	157	273	127	51	388	547	323	466	451	168	494	
	TDE	37	55	—	136	172	189	107	115	107	129	130	
	DDT	123	217	94	222	131	97	37	60	64	282	88	
1970	DDE		157									305	
	TDE		49									126	
	DDT		38									10	

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 2.—ANAHEIM SLOUGH—*M. EDULIS*, UNLESS OTHERWISE INDICATED—33 SAMPLES [1]—Continued													
1971	DDE		75			103			185			92	
	TDE		53			164			101			41	
	DDT		23			T			22			10	
	Dieldrin		T			T			T			T	
	PCB's		—			—			—			[4]	
1972	DDE	64				80							
	TDE	24				53							
	DDT	18				10							
	Dieldrin	T				T							
	PCB's	—				[4]							
STATION 3.—POINT MUGU—*M. EDULIS*, UNLESS OTHERWISE INDICATED—29 SAMPLES [1]													
1967	DDE										130	160	220
	TDE										150	230	280
	DDT										270	440	650
	Dieldrin										—	—	T
1968	DDE	250	200	[8] 370	170	366	207	255	465	[3] 269		360	
	TDE	230	210	350	180	494	168	65	388	278		443	
	DDT	460	340	790	430	749	363	32	432	566		955	
	Dieldrin	—	—	16	—	—	—	T	—	—		T	
	Endrin	—	—	—	—	—	—	—	—	—		T	
1969	DDE	226		560	334	365	[6] 273	[6] 298		[6] 918	[6] 349	112	
	TDE	121		—	301	63	31	116		117	34	146	
	DDT	161		391	248	120	92	40		580	176	185	
1970	DDE											[6] 238	
	TDE											141	
	DDT											56	
1971	DDE		[6] 49				[6] 65		[6] 22			[6] 112	
	TDE		24				73		T			50	
	DDT		45				11		—			20	
	Dieldrin		T				T		—			T	
	PCB's		—				—		—			[4]	
1972	DDE	[6] 37				[6] 24							
	TDE	12				10							
	DDT	T				10							
	Dieldrin	T				T							
	PCB's	—				[4]							

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 4.—BAYWOOD PARK—*C. GIGAS*—52 SAMPLES[1]													
1966	DDE	54	74	82	75	76	52	55	62	59	69	69	100
	TDE	26	34	35	25	32	19	22	25	35	34	33	37
	DDT	25	25	26	20	24	14	—	18	26	24	25	46
	Dieldrin	—	—	—	24	16	—	—	—	—	—	—	—
1967	DDE	110	110	62	130	80	120	51	82	55	48	35	46
	TDE	29	42	50	47	34	49	29	40	23	21	10	13
	DDT	58	73	96	130	67	70	37	49	46	26	15	10
1968	DDE	96	43	40	160	48	49	48	48	44		74	
	TDE	24	13	T	40	17	19	—	13	—		—	
	DDT	25	13	30	61	—	T	T	T	—		—	
1969	DDE	123	111	139	180	148	119	110		97	165	184	162
	TDE	23	38	—	70	57	40	57		31	53	70	75
	DDT	24	164	131	351	189	150	43		31	58	64	69
1970	DDE		220	226	215	56							
	TDE		74	87	58	21							
	DDT		69	64	46	—							
1971	DDE								22			21	
	TDE								11			16	
	DDT								—			10	
1972	DDE	33											
	TDE	12											
	DDT	T											
	Dieldrin	T											
STATION 5.—LOS OSOS CREEK—*C. GIGAS*, UNLESS OTHERWISE INDICATED—52 SAMPLES[1]													
1966	DDE	83	58	43	88	65	40	43	53	73	10	71	72
	TDE	33	27	17	39	25	16	16	22	34	27	33	31
	DDT	23	21	14	30	20	—	—	14	23	23	25	37
	Dieldrin	—	—	—	27	10	—	—	—	—	—	—	—
1967	DDE	62	120	63	110	93	130	64	81	56	43	37	29
	TDE	29	47	43	42	57	56	46	44	33	20	14	10
	DDT	41	96	130	120	92	80	52	49	72	25	20	12
1968	DDE	100	61	42	70	[7] 65	42	31	25	55		69	
	TDE	32	21	13	24	T	T	—	11	T		—	
	DDT	37	21	T	36	T	—	T	—	—		—	
1969	DDE	66	70	126	104	155	144	201		115	223		137
	TDE	T	37	—	56	61	80	83		43	72		51
	DDT	T	72	131	239	183	188	128		34	93		35

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 5.—LOS OSOS CREEK—*C. GIGAS*, UNLESS OTHERWISE INDICATED—52 SAMPLES [1]—Continued													
1970	DDE		186	182	221	63							
	TDE		60	54	70	13							
	DDT		53	50	41	—							
1971	DDE								29			25	
	TDE								15			21	
	DDT								T			10	
1972	DDE	30				12							
	TDE	12				T							
	DDT	T				10							
	Dieldrin	T				T							
	PCB's	—				[4]							
STATION 6.—ELKHORN SLOUGH—*C. GIGAS*, UNLESS OTHERWISE INDICATED—57 SAMPLES [1]													
1966	DDE	160	220	96	96	89	88	86	72	79	84	130	190
	TDE	160	220	120	110	95	82	79	77	66	65	77	160
	DDT	250	290	110	96	85	65	64	55	41	56	76	210
	Dieldrin	—	19	11	20	18	—	—	—	—	10	—	30
1967	DDE	200	220	200	230	210	300	160	200	190	62	190	250
	TDE	160	230	200	260	340	390	200	260	210	55	150	230
	DDT	260	440	390	690	860	920	390	500	390	110	340	370
	Dieldrin	26	25	29	30	39	33	10	10	15	—	14	17
1968	DDE	260	130	120	170	214		173	122	168		95	65
	TDE	160	85	92	160	212		129	70	100		71	63
	DDT	250	97	61	230	411		237	159	200		113	110
	Dieldrin	13	—	—	—	—		27	—	—		—	—
	Endrin	—	—	—	—	—		19	—	—		—	—
1969	DDE	178	191	126	280	215	324	424			1,373	237	191
	TDE	102	338	156	393	223	253	358			502	171	117
	DDT	120	441	346	808	304	96	704			630	284	189
1970	DDE	208	230	445	270	353	325					[7] 31	
	TDE	173	300	582	285	276	236					19	
	DDT	204	444	808	491	411	375					26	
	Dieldrin	—	—	—	—	—	—					T	
	Endrin	—	—	—	—	—	—					T	
1971	DDE		[7] 67			[7] 29			[7] 43			[7] 28	
	TDE		37			24			71			37	
	DDT		72			10			—			17	
	Dieldrin		—			T			—			11	
	PCB's		—			—			—			[4]	

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 6.—ELKHORN SLOUGH—*C. GIGAS*, UNLESS OTHERWISE INDICATED—57 SAMPLES [1]—Continued													
1972	DDE		[7] 42			[7] T							
	TDE		25			T							
	DDT		36			10							
	Dieldrin		T			57							
	PCB's		—			(4)							
STATION 7.—COYOTE POINT—*O. LURIDA*—55 SAMPLES [1]													
1966	DDE	54	93	42	71	71	47	30	35	50	42	33	63
	TDE	74	120	54	88	91	66	38	46	65	71	51	79
	DDT	63	100	43	70	74	46	39	42	55	62	40	75
	Dieldrin	—	27	20	29	23	—	—	—	—	21	15	21
1967	DDE	41	49	51	61	65	52	51	47	39	33	46	46
	TDE	60	68	74	82	76	78	84	58	58	37	86	84
	DDT	58	72	79	89	69	70	80	50	50	43	110	51
	Dieldrin	26	26	28	23	21	25	17	43	13	—	16	16
	Endrin	—	—	—	—	—	19	—	—	—	—	—	—
1968	DDE	44	59	47	47	46	27	42	—	30		33	
	TDE	57	95	69	71	103	41	57	—	57		69	
	DDT	53	100	84	89	103	45	60	—	56		58	
	Dieldrin	13	—	18	20	—	—	10	—	—		—	
	Endrin	—	—	—	10	—	—	—	—	—		—	
1969	DDE	81	52	172	25	—	65	34	163	36		52	24
	TDE	46	—	—	—	89	87	38	99	T		78	30
	DDT	48	158	171	—	176	88	33	100	39		59	26
1970	DDE	18	33	102	93	37	33						
	TDE	33	55	102	100	—	50						
	DDT	33	42	87	76	42	24						
1971	DDE		17									14	
	TDE		67									24	
	DDT		47									24	
	Dieldrin		T									T	
1972	DDE		15			T							
	TDE		32			T							
	DDT		53			T							
	Dieldrin		T			T							
	PCB's		—			(4)							

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	STATION 8.—GUADALUPE SLOUGH—*M. DEMISSUS*—27 SAMPLES [1]												
1968	DDE		36	77	48	74	67	34	24	19	24	34	T
	TDE		90	180	100	185	140	68	53	57	58[2]	T	24
	DDT		110	150	60	91	130	34	T	40	26	—	T
	Dieldrin		18	23	14	—	14	—	—	—	—	—	—
1969	DDE	—	—	34	70	26	11	10	24	—	29		
	TDE	42	—	—	—	27	108	22	34	48	50		
	DDT	—	—	—	—	—	204	T	T	T	T		
1970		No Samples Collected											
1971	DDE		T			T			—			T	
	TDE		40			26			—			T	
	DDT		28			10			—			10	
	Dieldrin		T			T			—			37	
1972	DDE		11			T							
	TDE		12			T							
	DDT		10			10							
	Dieldrin		T			T							
	PCB's		—			[4]							
	STATION 9.—ALVISO SLOUGH—*M. DEMISSUS*—28 SAMPLES [1]												
1968	DDE		43	46	69	74	47	26	28	18	11	—	13
	TDE		140	59	170	169	95	72	80	45	79	T	30
	DDT		93	78	77	85	66	35	34	35	11	T	27
	Dieldrin		18	12	25	—	—	—	—	—	—	—	—
1969	DDE	38	—	55	98	52	12	17	30	—	39		
	TDE	59	61	55	—	73	161	42	45	56	27		
	DDT	—	—	88	—	108	111	T	20	T	T		
1970	DDE		42										
	TDE		76										
	DDT		33										
1971	DDE		13			T			—			T	
	TDE		38			15			T			T	
	DDT		17			T			—			10	
	Dieldrin		—			T			—			—	
1972	DDE	T				T							
	TDE	T				T							
	DDT	10				10							
	Dieldrin	T				T							
	PCB's	—				[4]							

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 10.—WEST ISLAND—*C. FLUMINEA*—28 SAMPLES [1]													
1967	DDE	280	330	320	270	320	230	170	140	170	180	690	390
	TDE	250	370	350	250	250	210	130	93	150	150	490	310
	DDT	210	300	310	250	260	270	150	130	230	270	1,100	770
	Dieldrin	20	20	22	17	12	18	T	15	20	10	20	18
	Endrin	T	T	T	—	—	—	—	—	—	—	—	—
1968	DDE	390	500	280	370	251	196	134	104	41		71	
	TDE	290	400	200	220	224	183	160	182	97		138	
	DDT	240	290	190	210	320	223	150	235	150		184	
	Dieldrin	16	22	15	16	—	21	19	13	—		—	
1969	DDE			177									
	TDE			—									
	DDT			168									
1970		No Samples Collected											
1971	DDE					198			91			15	
	TDE					126			71			T	
	DDT					173			111			—	
	Dieldrin					T			T			T	
1972	DDE		11			T							
	TDE		10			T							
	DDT		10			10							
	Dieldrin		—			T							
	PCB's		—			(4)							
STATION 11.—FALSE RIVER—*C. FLUMINEA*—26 SAMPLES [1]													
1967	DDE				470	460	320	420	270				400
	TDE				410	320	200	260	180				350
	DDT				970	910	640	780	500				210
	Dieldrin				24	19	20	16	16				17
1968	DDE	470	500	340	330	315	199	250	122	53		96	
	TDE	400	590	230	230	281	167	190	212	109		144	
	DDT	220	420	200	190	312	225	290	296	92		103	
	Dieldrin	17	22	19	23	—	—	—	16	—		—	
	Endrin	18	—	—	—	—	—	—	—	—		—	
1969	DDE		151	54		93	41	88	139	57	42	—	
	TDE		152	66		75	47	88	165	91	76	91	
	DDT		378	167		214	46	135	136	61	43	44	
1970		No Samples Collected											

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 11.—FALSE RIVER—*C. FLUMINEA*—26 SAMPLES [1]—Continued													
1971	DDE		21										
	TDE		41										
	DDT		20										
	Dieldrin		T										
STATION 12.—NAPA RIVER—*M. DEMISSUS*—28 SAMPLES [1]													
1968	DDE		T	25	21	24	23	15	21	18		T	
	TDE		22	46	72	100	83	38	42	62		24	
	DDT		T	26	39	45	47	—	23	18		T	
1969	DDE	10	—	100	62	—	10	13	T	—	T	12	
	TDE	30	—	48	—	—	24	45	30	58	24	37	
	DDT	T	—	—	—	—	T	T	T	—	T	T	
1970	DDE		11									16	
	TDE		33									93	
	DDT		T									46	
	Dieldrin		—									T	
	Endrin		—									T	
1971	DDE		27			13			T				T
	TDE		143			68			T				T
	DDT		41			10			T				T
	Dieldrin		T			T			—				—
	Endrin		T			—			—				—
1972	DDE		T			T							
	TDE		21			T							
	DDT		23			10							
	Dieldrin		T			T							
	PCB's		—			(4)							
STATION 13.—PETALUMA RIVER—*M. DEMISSUS*—28 SAMPLES [1]													
1968	DDE		—	27	27	26	47	19	—	—		92	
	TDE		—	58	63	72	104	35	T	13		68	
	DDT		—	15	19	31	41	T	—	—		108	
1969	DDE	T	—	124	49	12	T	17	—	—	22	10	
	TDE	T	—	37	—	28	T	38	—	57	37	22	
	DDT	—	—	—	—	—	T	T	—	—	T	T	
1970	DDE		T									28	
	TDE		T									71	
	DDT		T									26	
	Dieldrin		—									10	

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 13—PETALUMA RIVER—*M. DEMISSUS*—28 SAMPLES [1]—Continued													
1971	DDE		T			T			—				T
	TDE		18			24			T				T
	DDT		10			T			—				—
	Dieldrin		T			—			—				—
1972	DDE		T			T							
	TDE		T			T							
	DDT		10			10							
	Dieldrin		T			T							
	PCB's		—			(4)							
STATION 14.—POINT SAN QUENTIN—*C. GIGAS*—50 SAMPLES [1]													
1966	DDE	12	30	47	52	69	59	37	52	57	51	55	55
	TDE	20	37	60	83	120	92	47	82	90	88	84	110
	DDT	14	12	19	23	45	38	24	43	49	33	40	98
	Dieldrin	—	—	—	14	20	—	—	—	—	11	15	20
1967	DDE	52	34	30	42	23	39	45	53	30	31	100	45
	TDE	130	65	59	75	55	85	120	130	74	68	50	84
	DDT	88	49	49	70	34	64	89	63	38	36	85	45
	Dieldrin	23	11	15	19	13	21	19	17	11	—	10	11
1968	DDE	43	44	43	43	36	59	59	25	38			40
	TDE	79	96	78	97	95	110	110	60	86			120
	DDT	44	89	67	63	69	100	100	T	52			82
	Dieldrin	12	17	17	12	—	—	12	—	—			18
1969	DDE	74	53	62	47	143	25	13	30		—	—	31
	TDE	80	149	134	—	143	54	23	41		—	55	51
	DDT	130	193	76	182	154	24	T	T		—	32	26
1970	DDE	18	19	54	66	51							
	TDE	18	T	31	64	39							
	DDT	T	T	37	45	23							
STATION 15.—BOLINAS LAGOON—*C. GIGAS*—17 SAMPLES [1]													
1966	DDE									10	T	T	—
	TDE									—	T	10	—
	DDT									—	—	—	—
1967	DDE	T	T	10	10	11	T	11	13	—	T	T	T
	TDE	11	13	16	17	16	14	21	20	—	15	11	T
	DDT	T	T	T	11	14	12	13	11	—	11	—	—

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 15.—BOLINAS LAGOON—*C. GIGAS*—17 SAMPLES [1]—Continued													
1968	DDE	—											
	TDE	—											
	DDT	—											
STATION 16.—SCHOONER BAY—*C. GIGAS*—33 SAMPLES [1]													
1966	DDE	—	T	T	—	T	T	T	T	—	T	T	—
	TDE	—	—	T	—	T	—	—	—	—	T	T	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	T	T	10	11	T	11	T	15	—	—	T	—
	TDE	—	—	T	13	13	13	T	18	—	—	—	—
	DDT	—	—	—	10	—	T	—	10	—	—	—	—
1968	DDE	—											
	TDE	—											
	DDT	—											
1969		No Samples Collected											
1970	DDE		11									10	
	TDE		T									11	
	DDT		T									10	
1971	DDE		14			T			T				T
	TDE		16			T			T				T
	DDT		10			T			T				10
	Dieldrin		T			—			—				—
1972	DDE		T			T							
	TDE		T			T							
	DDT		10			10							
	Dieldrin		—			T							
	PCB's		—			[4]							
STATION 17.—BERRIES BAR—*C. GIGAS*—27 SAMPLES [1]													
1966	DDE	—	13	T	10	13	10	17	11	12	T	T	11
	TDE	—	15	T	17	16	10	13	11	T	T	T	10
	DDT	—	T	—	—	T	—	—	—	—	—	—	—
1967	DDE	T	11	17	14	16	12	13	T	13	15	14	T
	TDE	T	14	20	18	17	14	15	12	16	18	17	T
	DDT	—	—	T	10	11	10	T	—	10	T	T	—

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 17—BERRIES BAR—*C. GIGAS*—27 SAMPLES [1]—Continued													
1968	DDE	—			12				17				
	TDE	—			13				14				
	DDT	—			T				—				
	Endrin	19			—				—				
STATION 18.—TOMALES BAY—*C. GIGAS*—34 SAMPLES [1]													
1966	DDE	T	T	—	—	T	—	14	11	14	14	T	T
	TDE	—	T	—	—	11	—	T	T	11	12	—	—
	DDT	—	—	—	—	T	—	—	—	—	—	—	—
1967	DDE	T	12	11	11	T	11	T	T	T	—	11	—
	TDE	—	T	T	12	T	14	T	—	—	—	T	—
	DDT	T	10	T	11	T	13	T	—	—	—	T	—
1968	DDE	—			11				T				
	TDE	—			—				T				
	DDT	—			10				—				
1969	DDE									22		T	
	TDE									T		T	
	DDT									18		T	
1970	DDE											T	
	TDE											T	
	DDT											10	
	Dieldrin											T	
	Endrin											T	
1971	DDE		12			T							T
	TDE		T			T							T
	DDT		10			10							10
1972	DDE		T										
	TDE		T										
	DDT		10										
	Dieldrin		T										
STATION 19.—NICKS COVE—*C. GIGAS*—25 SAMPLES [1]													
1966	DDE	—	12	11	—	T	T	11	11	T	T	T	T
	TDE	—	T	—	—	—	—	—	T	—	—	T	—
	DDT	—	T	—	—	—	—	—	—	—	—	—	—
1967	DDE	12	13	13	14	10	T	11	T	T	—	14	—
	TDE	T	10	12	12	T	T	T	—	T	—	—	—
	DDT	T	11	T	11	T	T	T	—	—	—	—	—

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 19—NICKS COVE—*C. GIGAS*—25 SAMPLES [1]—Continued													
1968	DDE	—											
	TDE	—											
	DDT	—											
STATION 20.—GUNTHER ISLAND—*C. GIGAS*—33 SAMPLES [1]													
1966	DDE	—	—	—	—	—	T	11	T	T	T	T	T
	TDE	—	—	—	—	—	T	17	—	21	T	T	T
	DDT	T	10	—	47	11	14	—	11	—	18	14	20
1967	DDE	10	T	T	T	T	T	T	T	—	T	T	T
	TDE	T	—	—	T	—	T	T	—	T	T	T	T
	DDT	30	28	12	19	19	19	24	12	16	15	21	22
1968	DDE	—			13								
	TDE	—			11								
	DDT	—			54								
1969		No Samples Collected											
1970	DDE											11	
	TDE											11	
	DDT											10	
	Dieldrin											T	
1971	DDE		T			T			T				T
	TDE		13			T			—				T
	DDT		17			10			—				10
	Dieldrin		T			T			—				T
1972	DDE			T			T						
	TDE			T			T						
	DDT			10			10						
	Dieldrin			—			T						
	PCB's			—			(6)						
STATION 21.—BIRD ISLAND—*C. GIGAS*—25 SAMPLES [1]													
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	T	—	—	T	—	T

TABLE B-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—California*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 21—BIRD ISLAND—*C. GIGAS*—25 SAMPLES [1]—Continued													
1968	DDE	—											
	TDE	—											
	DDT	—											

[1] Each sample represents 15 or more mature mollusks.
[2] DDE, TDE, and DDT values approximated because of presence of toxaphene.
[3] *C. gigas*.
[4] Present but not quantified.
[5] *M. demissus*.
[6] *M. californianus*.
[7] *M. edulis*.

SECTION C.—DELAWARE

Samples were collected at nine stations at monthly intervals during the period October 1966 August 1969. The eastern oyster (*Crassostrea virginica*) ribbed mussel (*Modiolus demissus*), and hard clam (*Mercenaria mercenaria*) were each collected at three stations. All samples were analyzed at the Gulf Breeze Laboratory. The approximate locations of the stations are shown in Fig. C-1. The Cape Henlopen station was in Delaware Bay; the other stations were adjacent to the Bay but exposed primarily to the runoff from large agricultural areas in separate drainage basins. A summary of data on organochlorine residues in the monitored species is presented in Table C-1, and the distribution of residues in these species for each sampling station by date of collection in Table C-2.

The use of three different species for monitoring obscured pollution patterns in Delaware estuaries to some extent. The relative inefficiency of hard clams in storing organochlorine residues makes Rehoboth Bay (Stations 7 and 8) appear to be generally free from this type of pollution. The first samples of clams collected in adjacent Indian River Bay (Station 9) also were free of detectable residues; however, subsequent monitoring using the ribbed mussel, showed Indian River Bay to be moderately but continuously polluted. It is probable that Rehoboth Bay was similarly polluted during the monitoring period. This same reasoning suggests that the waters at Cape Henlopen were continually more polluted with DDT than the small residues in the hard clams would imply.

The magnitude of DDT residues in clams and oysters showed no trend towards increased or decreased levels during the 3-year monitoring period. In ribbed mussels, however, there was a marked decline in the average level of residues in the final year at Stations 1 and 2 as well as Station 9. Delaware monitoring samples ranked 6th in frequency and 10th in magnitude of DDT residues in comparison with the other 14 States. The 13% incidence of dieldrin residues was about the average for all States.

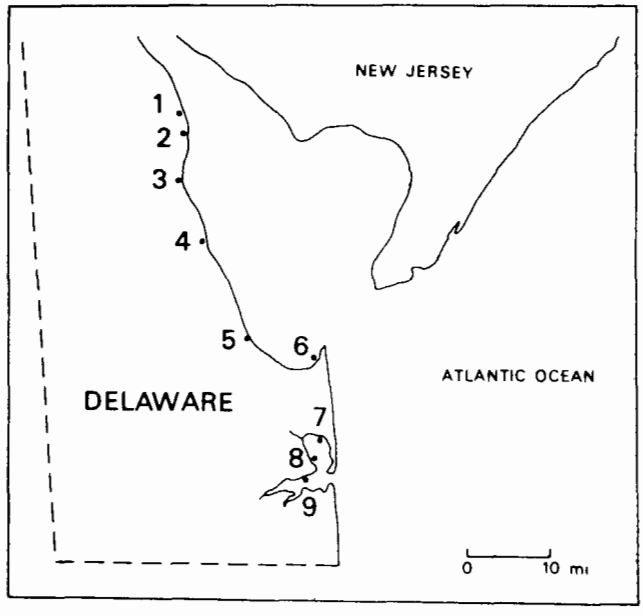


FIGURE C-1.—*Diagram of coastal Delaware showing approximate location of monitoring stations*

1. Leipsic River
2. Simons River
3. Bowers Beach—Murderkill River
4. Mispillion River
5. Broadkill River
6. Cape Henlopen—Delaware Bay
7. Thompson Island—Rehoboth Bay
8. Arrowhead Point—Rehoboth Bay
9. West Gables—Indian River Bay

TABLE C-1.—*Summary of data on organochlorine residues in the monitored species, 1966-69—Delaware*

Station Number	Location	Monitoring Period	Principal Monitored Species	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)	
					DDT	Dieldrin
1	Leipsic River	1967-69	M. demissus	27	23 (156)	4 (13)
2	Simons River	1967-69	M. demissus	25	23 (205)	6 (19)
3	Bowers Beach	1966-69	C. virginica	34	34 (172)	25 (25)
4	Mispillion River	1966-69	C. virginica	35	33 (90)	2 (10)
5	Broadkill River	1966-69	C. virginica	34	34 (90)	
6	Cape Henlopen	1966-69	M. mercenaria	32	30 (65)	
7	Thompson Island	1966-69	M. mercenaria	33	5 (16)	
8	Arrowhead Point	1966-69	M. mercenaria	34	4 (35)	
9	West Gables	1966-69	M. demissus	33	30 (96)	
Total number of samples				287		
Percent positive for indicated compound					75	13

[1] Each sample represents 15 or more mature mollusks.

TABLE C-2—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—Delaware*

[Blank = no sample collected; — = no residue detected above 5 ppb; T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—LEIPSIC RIVER—*M. DEMISSUS*—27 SAMPLES [1]													
1967	DDE			12	25	26	47	33	21	17	22	18	32
	TDE			11	53	46	91	77	51	47	29	47	77
	DDT			—	T	12	18	23	51	47	—	T	17
	Dieldrin			—	10	13	—	10	—	10	—	—	—
1968	DDE			27	30	32	26	33	T	12	23	19	
	TDE			68	69	91	41	45	20	18	29	37	
	DDT			17	17	14	—	22	—	--	T	18	
1969	DDE	—	--	T	15	T	—	17	--				
	TDE	—	--	14	18	T	—	33	—				
	DDT			—	—	—	—	19	—				
STATION 2.—SIMONS RIVER—*M. DEMISSUS*—25 SAMPLES [1]													
1967	DDE	13		17	31	31	43	37	25		18	29	29
	TDE	43		37	65	150	89	79	47		88	75	66
	DDT	--		—	15	24	19	28	38		29	18	16
	Dieldrin	--		—	15	19	12	T	—		12	—	—
1968	DDE			28	23	39			23	—	20	11	
	TDE			65	78	100			30	—	26	16	
	DDT			18	39	16			—	—	22	—	
	Dieldrin			—	—	10			—	—	—	—	

TABLE C-2—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—Delaware*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 2.—SIMONS RIVER—*M. DEMISSUS*—25 SAMPLES [1]—Continued													
1969	DDE	—	13	T	21	14	21	13	21				
	TDE	—	22	T	23	19	29	26	35				
	DDT	—	—	—	—	—	24	26	31				
STATION 3.—BOWERS BEACH—*C. VIRGINICA*—34 SAMPLES [1]													
1966	DDE											25	19
	TDE											29	17
	DDT											—	—
	Dieldrin											16	—
1967	DDE	27	25	(2)	(2)	42	43	41	40	40	42	41	32
	TDE	35	26	(2)	(2)	65	70	66	64	56	98	51	38
	DDT	—	—	(2)	(2)	T	10	24	68	17	25	14	10
	Dieldrin	18	14	20	25	24	18	11	13	16	16	13	15
1968	DDE	50	46	48	52	66	75	82	49	41	52	57	41
	TDE	56	42	47	48	73	78	53	34	32	44	57	35
	DDT	T	—	T	—	10	T	25	T	T	13	18	T
	Dieldrin	12	13	14	15	12	16	—	—	—	—	16	15
1969	DDE	52	48	42	49	41	79	60	39				
	TDE	37	47	36	39	39	70	57	29				
	DDT	—	—	—	—	—	20	29	11				
	Dieldrin	14	15	11	—	11	—	—	—				
STATION 4.—MISPILLION RIVER—*C. VIRGINICA*—35 SAMPLES [1]													
1966	DDE										31	21	22
	TDE										27	24	24
	DDT										15	—	—
1967	DDE	17	24	(2)	25	35	23	22	16	20	T	32	27
	TDE	18	22	(2)	31	41	26	30	20	23	44	36	32
	DDT		—	(2)	—	—	—	T	11	T	T	—	T
	Dieldrin	—	—	10	—	10	—	—	—	—	—	—	—
1968	DDE	23	28	Lost	41	39	39	47	34	32	25	39	38
	TDE	40	31		40	36	34	33	23	25	18	32	27
	DDT	T	—		—	15	—	—	—	—	—	—	—
1969	DDE	40	50	36	29	36	26	35	28				
	TDE	29	31	28	25	32	26	22	20				
	DDT	—	—	—	—	—	—	—	—				

TABLE C-2—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—Delaware*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 5.—BROADKILL RIVER—*C. VIRGINICA*—34 SAMPLES[1]													
1966	DDE										18	22	
	TDE										20	17	
	DDT										T	—	
1967	DDE	28	18	23	17	23	24	16	30	27	35	35	37
	TDE	23	11	17	13	18	21	19	30	27	27	31	33
	DDT	—	—	—	—	—	T	T	16	11	T	T	T
1968	DDE	25	29	23	32	39	43	36	48	37	31	44	51
	TDE	21	22	16	21	45	32	20	32	25	22	33	39
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	40	38	43	41	39	42	43	35				
	TDE	24	28	26	20	28	28	24	15				
	DDT	—	—	—	—	—	—	—	—				
STATION 6.—CAPE HENLOPEN—*M. MERCENARIA*—32 SAMPLES[1]													
1966	DDE											12	
	TDE											11	
	DDT											—	
1967	DDE	12	13	—	[2]	14	12	20	12	T	14	16	16
	TDE	16	12	—	[2]	14	14	24	14	T	13	15	15
	DDT	—	—	—	[2]	—	—	—	—	—	—	—	—
1968	DDE	13	15	18	18	28	39	25	25	19	13	T	21
	TDE	12	14	14	15	24	26	16	15	12	T	T	11
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	12	14	22	Lost	16	19	22	35				
	TDE	—	—	T		11	T	10	20				
	DDT	—	—	—		—	—	—	—				
STATION 7.—THOMPSON ISLAND—*M. MERCENARIA*—33 SAMPLES[1]													
1966	DDE										—		T
	TDE										T		11
	DDT										T		—
1967	DDE	T		—	T	T	—	—	—	—	—	—	—
	TDE	T		—	—	T	—	—	—	—	—	—	—
	DDT	—		—	—	T	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—

TABLE C-2—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—Delaware*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 7.—THOMPSON ISLAND—*M. MERCENARIA*—33 SAMPLES [1]—Continued													
1969	DDE	—	—	—	—	—	—	—	—				
	TDE	—	—	—	—	—	—	—	—				
	DDT	—	—	—	—	—	—	—	—				
STATION 8.—ARROWHEAD POINT—*M. MERCENARIA*—34 SAMPLES [1]													
1966	DDE										—	—	T
	TDE										24	—	T
	DDT										11	—	—
1967	DDE	—		[2]	T	—	—	—	—	—	—	—	—
	TDE	—		[2]	T	—	—	—	—	—	—	—	—
	DDT	—		[2]	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	—	—	—	—	—	—				
	TDE	—	—	—	—	—	—	—	—				
	DDT	—	—	—	—	—	—	—	—				
STATION 9.—WEST GABLES—*M. DEMISSUS* UNLESS OTHERWISE INDICATED—33 SAMPLES [1]													
1966	DDE										[3]	[3]	
	TDE										—	—	
	DDT										—	—	
1967	DDE	11		18	17	22	19	15	18	13	19	19	21
	TDE	25		37	32	33	24	21	29	13	29	30	33
	DDT	32		19	14	24	13	14	41	21	21	22	26
1968	DDE	19	24	18	20	18	23	T	T	—	T	12	13
	TDE	29	35	28	32	30	37	11	T	—	T	16	18
	DDT	21	30	22	26	23	36	T	—	—	—	T	—
1969	DDE	10	16	13	16	17	21	13	[3] 11				
	TDE	14	16	17	20	22	27	18	T				
	DDT	—	—	—	T	10	T	11	—				

[1] Each sample represents 15 or more mature mollusks.
[2] Present but not quantified.
[3] *M. mercenaria.*

SECTION D.—FLORIDA

Investigation of the effects of pesticide pollution on estuarine fauna in Florida was initiated at the Gulf Breeze Laboratory, Gulf Breeze, Fla., in 1959. During the next 5 years, sufficient headway in the understanding of uptake and flushing rates of persistent synthetic compounds as well as the technology for handling samples made a continuing monitoring program feasible. Local oysters (Station 9, East Bay) were analyzed monthly during 1964, and the concept of a national monitoring program was developed and implemented in 1965. The eastern oyster, *C. virginica,* was the only species monitored in Florida; all samples were analyzed at the Gulf Breeze Laboratory. The approximate locations of monitoring stations are shown in Fig. D-1. A summary of data on organochlorine residues in the monitored species, *C. virginica,* is presented in Table D-1, and the distribution of residues in this species for each sampling station by date of collection in Table D-2.

Oyster samples from Florida contained the highest levels of DDT residues and the most persistent contamination with PCB's observed in the entire monitoring program.

The polychlorinated biphenyl, Aroclor 1254®, was identified in studies of estuarine fauna following a 1969 fish kill in Escambia Bay, Fla., (*8*). Station 9 is about 25 miles from the presumed source of this PCB pollution and is in a contiguous but distinct drainage basin. Monitoring samples from this station contained PCB residues about one-third the magnitude of residues in Escambia Bay oysters and continued to have residues of similar magnitude for at least 3 years after the presumed primary source of PCB's had been eliminated.

The trend in DDT residues is most clearly shown in the Station 9 data. Some DDT had been used in this geographic area for agricultural purposes. However, its primary use had been for the control of stable-fly larvae, *Stomoxys calcitrans,* that develop in seaweed windrows on estuarine beaches. In 1969, methoxychlor was substituted for this purpose, and DDT residues virtually disappeared from all succeeding monitoring samples. Methoxychlor residues were not detected in the monitored samples. There are not enough recent data to determine DDT pollution trends in other estuaries along the Florida Gulf coast.

The incidence of DDT in Florida samples (62%) is about the average for all States monitored. The incidence of dieldrin (7%) may be compared with the average incidence of 15% for all States.

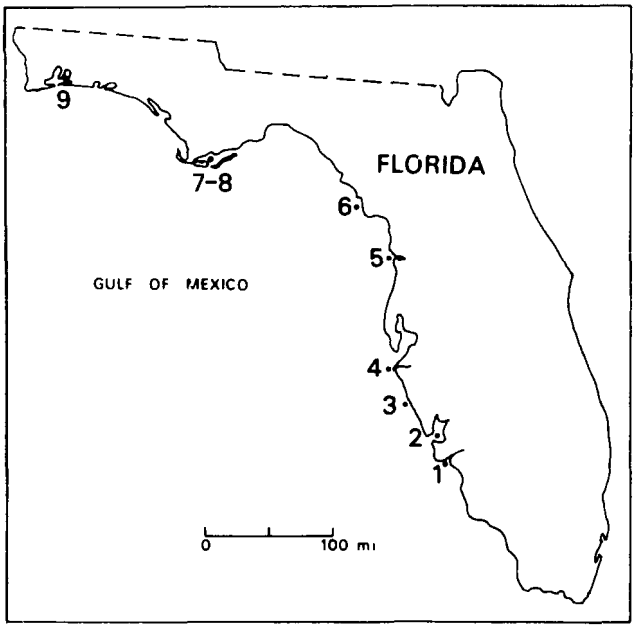


FIGURE D-1.—*Diagram of coastal Florida showing approximate location of monitoring stations*

1. Iona Point—Caloosahatchee River
2. Charlotte Harbor—Peace River
3. Coral Cove—Little Sarasota Bay
4. Manatee River
5. Crystal River
6. Suwanee River
7. St. Vincents Bar (North)—Apalachicola Bay
8. St. Vincents Bar (South)—Apalachicola Bay
9. East Bay—Blackwater River

TABLE D-1.—*Summary of data on organochlorine residues in the monitored species* (C. Virginica), *1965-72—Florida*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)		
				DDT	Dieldrin	PCB's [2]
1	Iona Point	1967-69	31	31 (5,390)	1 (11)	
2	Charlotte Harbor	1966-69	31	28 (338)	13 (27)	
3	Coral Cove	1966-69	32	32 (129)		
4	Manatee River	1966-69	32	32 (159)		
5	Crystal River	1966-71	43	7 (27)		
6	Suwanee River	1966-69	32	6 (22)		

TABLE D-1.—*Summary of data on organochlorine residues in the monitored species* (C. Virginica), *1965-72—Florida*—Continued

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)		
				DDT	Dieldrin	PCB's [2]
7	St. Vincents Bar (North)	1966-67	17	12 (50)	3 (28)	
8	St. Vincents Bar (South)	1966-67	16	10 (70)	3 (22)	
9	East Bay	1965-72	84	46 (65)		25 (390)
	Occasional stations (21)	1966-71	56	26 (101)	7 (12)	
Total number of samples			374			
Percent of samples positive for indicated compound				62	7	7

[1] Each sample represents 15 or more mature mollusks.
[2] Calculated as Aroclor 1254®

TABLE D-2—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Florida*
[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—IONA POINT—31 SAMPLES [1]													
1966	DDE								30	13	24	35	T
	TDE								39	20	48	79	T
	DDT								—	—	—	28	—
1967	DDE	91	320	930	1,450	290	110	53	60	87	72	140	240
	TDE	94	170	760	705	310	200	110	160	160	150	220	310
	DDT	190	630	3,700	2,550	350	68	57	32	97	110	68	520
1968	DDE	760	1,200	1,100	1,500	780	340	180	—	T	77	84	82
	TDE	44	560	580	560	390	310	190	T	16	160	120	120
	DDT	2,800	3,600	2,300	1,200	650	220	33	—	—	69	140	60
	Dieldrin	—	—	—	—	—	—	—	—	11	—	—	—
1969	DDE		710	940									
	TDE		1,400	400									
	DDT		1,700	1,100									
STATION 2.—CHARLOTTE HARBOR—31 SAMPLES [1]													
1966	DDE								T	T	17	—	52
	TDE								10	16	23	—	91
	DDT								—	—	15	—	41
1967	DDE	83	14	15	39	18	30	13	T	T	—	T	18
	TDE	85	20	24	33	27	43	20	13	T	—	11	28
	DDT	170	T	13	—	T	13	T	—	—	—	—	21
	Dieldrin	—	—	—	—	—	—	14	11	19	—	—	15

TABLE D-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Florida*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 2.—CHARLOTTE HARBOR—31 SAMPLES [1]—Continued													
1968	DDE	19	23	18	34	27	22	20	—	T	—	T	T
	TDE	22	26	18	36	29	18	26	T	17	—	11	T
	DDT	14	13	—	20	16	10	T	—	—	—	—	—
	Dieldrin	—	11	—	18	11	—	27	—	16	—	13	19
1969	DDE		14	17									
	TDE		19	22									
	DDT		—	12									
STATION 3.—CORAL COVE—32 SAMPLES [1]													
1966	DDE								24	T	10	25	17
	TDE								21	—	T	33	16
	DDT								—	—	—	T	T
1967	DDE	34	26	24	25	20	24	25	23	12	16	13	10
	TDE	28	23	22	26	21	21	24	20	16	16	10	10
	DDT	12	12	T	13	T	10	17	T	T	T	T	T
1968	DDE	29	27	21	35	49	39	31	19	20	28	21	23
	TDE	30	30	14	36	43	40	26	16	23	28	23	29
	DDT	10	14	—	13	37	49	32	22	14	28	11	T
1969	DDE	30	41	36									
	TDE	38	40	40									
	DDT	T	20	15									
STATION 4.—MANATEE RIVER—32 SAMPLES [1]													
1966	DDE								23	37	25	T	30
	TDE								39	47	33	T	33
	DDT								—	13	11	—	12
1967	DDE	37	39	22	31	18	T	19	21	23	33	34	13
	TDE	30	45	24	41	23	T	20	42	46	59	45	14
	DDT	19	19	10	13	T	—	T	20	17	13	14	14
1968	DDE	26	24	18	42	16	18	31	16	18	18	17	24
	TDE	24	29	30	65	19	61	88	37	38	16	19	27
	DDT	26	13	10	22	T	25	40	T	13	—	—	14
1969	DDE	22	32	24									
	TDE	33	46	26									
	DDT	—	17	T									

TABLE D-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Florida*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 5.—CRYSTAL RIVER—43 SAMPLES[1]													
1966	DDE							—	12	—	—	T	—
	TDE							—	—	—	—	T	—
	DDT							—	—	—	—	—	—
1967	DDE	—	T	—	—	—	—	T	—	—		—	—
	TDE	—	—	—	—	—	—	T	—	—		—	—
	DDT	—	—	—	—	13	—	T	—	—		—	—
1968	DDE	—	—	—	T	—	11	—		—	—	—	—
	TDE	—	—	—	T	—	—	—		—	—	—	—
	DDT	—	—	—	—	—	16	—		—	—	—	—
1969	DDE	—	—	—			—		—		—		—
	TDE	—	—	—			—		—		—		—
	DDT	—	—	—			—		—		—		—
1970	DDE		—		—		—		—		—		—
	TDE		—		—		—		—		—		—
	DDT		—		—		—		—		—		—
1971	DDE	—			—								
	TDE	—			—								
	DDT	—			—								
STATION 6.—SUWANEE RIVER—32 SAMPLES[1]													
1966	DDE							—	T	—	—	T	—
	TDE							—	—	—	—	T	—
	DDT							—	—	—	—	—	—
1967	DDE	—	12	T	—	—	—	—	—	—	—	—	—
	TDE	—	T	T	—	—	—	—	—	—	—	—	—
	DDT	—	T	T	—	11	11	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—		—	—	—	—
	TDE	—	—	—	—	—	—	—		—	—	—	—
	DDT	—	—	—	—	—	—	—		—	—	—	—
1969	DDE	—	—	—									
	TDE	—	—	—									
	DDT	—	—	—									
STATION 7.—ST. VINCENTS BAR (NORTH)—17 SAMPLES[1]													
1966	DDE			T	16	T			14	—	—	11	T
	TDE			T	19	T			15	—	—	10	T
	DDT			—	T	—			—	—	—	—	—
	Dieldrin			—	10	—			—	—	—	—	—

TABLE D-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Florida*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 7.—ST. VINCENTS BAR (NORTH)—17 SAMPLES [1]—Continued													
1967	DDE	T	—	T		22	T	13	—	—		T	
	TDE	—	—	T		23	T	10	—	—		—	
	DDT	—	—	—		T	—	T	—	—		—	
	Dieldrin	11	—	—		28	—	—	—	—		—	
STATION 8.—ST. VINCENTS BAR (SOUTH)—16 SAMPLES [1]													
1966	DDE			18	25				17	—	—	—	—
	TDE			21	30				T	—	—	—	—
	DDT			—	15				—	—	—	—	—
	Dieldrin			—	13				—	—	—	—	—
1967	DDE	T	—	14		21	T	13	T	T		—	
	TDE	—	—	13		22	T	12	T	—		—	
	DDT	—	—	—		T	38	T	—	—		—	
	Dieldrin	—	—	15		22	—	—	—	—		—	
STATION 9.—EAST BAY—84 SAMPLES [1]													
1965	DDE							19	T	T	—	T	T
	TDE							18	T	T	—	T	—
	DDT							13	T	T	—	T	—
1966	DDE	12	13	13	17	26	24	15	14	T	—	T	T
	TDE	—	14	—	15	24	19	11	—	—	—	—	T
	DDT	—	—	—	15	15	14	—	—	—	—	—	—
1967	DDE	T	18	21	18	18	12	20	T	T	T	T	T
	TDE	—	18	17	22	24	13	23	—	—	—	—	—
	DDT	—	14	16	15	15	—	18	—	10	—	—	—
1968	DDE	16	12	17	22	15	15	—	T	20	—	T	—
	TDE	15	20	—	—	—	—	—	T	—	—	—	—
	DDT	10	12	—	—	—	—	—	—	—	—	—	—
1969	DDE	T	16	11	—	13	14	—	—	10	T	—	—
	TDE	T	14	10	—	13	—	—	—	T	—	—	—
	DDT	—	T	—	—	—	—	—	—	14	—	—	—
1970	DDE	—	T	—	T	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	PCB's [2]	—	—	—	—	380	180	170	73	92	50	55	140
1971	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	PCB's [2]	160	160	200	220	230	390	190	230	100	55	120	—

TABLE D-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Florida*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 9.—EAST BAY—84 SAMPLES [1]—Continued													
1972	DDE	—	—	—	—	—	—						
	TDE	—	—	—	—	—	—						
	DDT	—	—	—	—	—	—						
	PCB's [2]	50	82	140	160	190	300						

[1] Each sample represents 15 or more mature mollusks.
[2] Calculated as Aroclor 1254®.

SECTION E.—GEORGIA

Monthly collections of the eastern oyster (*C. virginica*) were made at 11 estuarine areas in Georgia during the period February 1967 June 1972. Analyses were done at the Gulf Breeze Laboratory until September 1969, and thereafter at the Marine Institute of the University of Georgia. The approximate locations of monitoring stations are shown in Fig. E-1. A summary of data on organochlorine residues in the monitored species, *C. virginica*, is presented in Table E-1, and the distribution of residues in this species for each sampling station by date of collection in Table E-2. The 15% incidence of DDT residues in Georgia samples was next to the lowest of all States monitored (Washington, lowest at 11%). The maximum level of DDT observed was also next to the lowest of any of the other States monitored. By contrast, the largest dieldrin residue detected in the nationwide program was in Georgia, (230 ppb) and the incidence of dieldrin residues (21%) was well above the average incidence (15%) for all States.

The occurrence of substantial toxaphene residues in the samples collected in St. Simons Sound was unexpected. A special sampling program was initiated in the area that included the placement of trays of oysters in creek beds where oysters did not normally occur. Analyses of these samples pinpointed the industrial source of the toxaphene and precipitated a schedule for control of the effluent discharge by the manufacturer. The magnitude of toxaphene residues at Stations 8 11 illustrates well the importance of dilution (distance) in the abatement of pollution.

Polychlorinated biphenyl residues were analyzed for beginning in 1969. A few samples collected in the Ogeechee and Satilla River basins contained residues of Aroclor 1254®, but the amounts were not quantified.

DDT residue levels were generally low and there was an approximate increase of 13% in the number of samples with negligible residues in 1971 as compared to earlier years. Stations 1 and 2 in the Savannah River basin, however, showed a reversal of this trend in 1972 when oysters contained substantially higher residue levels than in 1971.

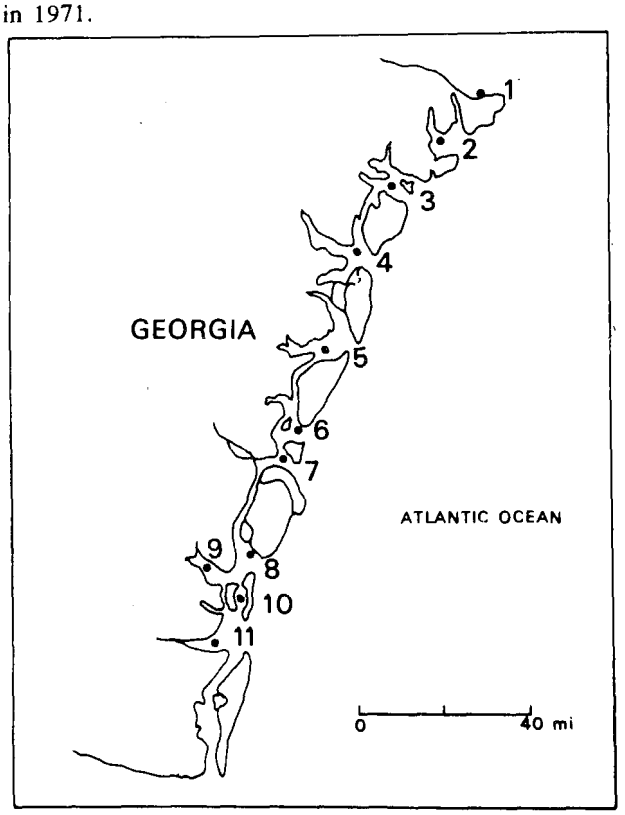


FIGURE E-1.—*Diagram of coastal Georgia showing approximate location of monitoring stations*

1. Lazeretta Creek—Savannah River Basin
2. Wilmington River—Savannah River Basin
3. Ogeechee River—Ogeechee River Basin
4. St. Catherine Sound—Ogeechee River Basin
5. Sapelo Sound—Ogeechee River Basin
6. Doboy Sound—Ogeechee River Basin
7. Egg Island—Altamaha River Basin
8. St. Simons Sound—Satilla River Basin
9. Terry Creek—Satilla River Basin
10. Jekyll Island—Satilla River Basin
11. Satilla River—Satilla River Basin

TABLE E-1—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1967-72—Georgia*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)			
				DDT	Dieldrin	Toxaphene	PCB's [2]
1	Lazeretta Creek	1967-72	64	30 (96)	58 (230)		
2	Wilmington River	1967-72	65	21 (86)	27 (90)		
3	Ogeechee River	1967-72	65	13 (50)	15 (26)		1
4	St. Catherine Sound	1967-72	65	7 (15)	2 (T)		1
5	Sapelo Sound	1967-72	65	12 (50)	6 (12)		2
6	Doboy Sound	1967-72	64	7 (27)	8 (14)		1
7	Egg Island	1967-72	65	3 (52)	22 (23)		
8	St. Simons Sound	1967-72	65	[3]	3 (T)	64 (7,500)	2
9	Terry Creek	1967-70	16	[3]		16 (54,000)	
10	Jekyll Island	1967-72	62	[3]		37 (3,500)	8
11	Satilla River	1967-72	64	3 (15)		8 (1,000)	1
	Occasional stations (2)	1968-69	4	[3]		3 (13,000)	
Total number of samples			664				
Percent of samples positive for indicated compound				15	21	19	2

NOTE: T = >5 but <10 ppb.
[1] Each sample represents 15 or more mature mollusks.
[2] Present but not quantified.
[3] Presence of toxaphene prevented quantification of DDT and its metabolites.

TABLE E-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Georgia*

Year	Compound	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
RESIDUES IN PPB (μg/kg) [Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]													
STATION 1.—LAZERETTA CREEK—64 SAMPLES [1]													
1967	DDE		14	13	21	T	T	53	—	—	—	—	12
	TDE		17	14	29	13	11	25	14	—	—	—	16
	DDT		—	—	T	—	T	18	11	—	—	—	T
	Dieldrin		98	65	56	32	30	30	33	18	42	33	46
1968	DDE	—	13	12	17	—	—	15	—	—		—	T
	TDE	—	16	12	23	—	—	23	—	—		—	T
	DDT	—	—	—	T	—	—	28	—	—		—	—
	Dieldrin	22	42	37	46	—	20	39	22	18		42	56
1969	DDE	—	—	—	—	—	T	—	—	—	—	—	T
	TDE	—	—	—	—	—	13	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	39	23	47	51	16	35	28	23	20	180	230	20
1970	DDE	T	13	—	T	T	—	—	T	—	23	—	—
	TDE	—	—	—	T	T	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	30	31	40	32	17	23	80	T	—	—	T	T

TABLE E-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Georgia*—Continued

YEAR	COMPOUND	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
		RESIDUES IN PPB (μg/kg)											
		[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]											
		STATION 1.—LAZERETTA CREEK—64 SAMPLES[1]—Continued											
1971	DDE	—	—	20	—	—	—	—	—	—	T	T	—
	TDE	—	—	—	—	—	—	—	—	—	T	T	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	19	19	T	17	13	10	—	T	T	—	—	13
1972	DDE	T	T	23	18	T	T						
	TDE		T	14	12	T	T						
	DDI	—	—	T	T	—	T						
	Dieldrin	15	13	T	T	22	T						
		STATION 2.—WILMINGTON RIVER 65 SAMPLES[1]											
1967	DDE		T	T	T	—	—	T	12	—	—	—	—
	TDE		T	T	T	—	—	T	—	—	—	—	—
	DDT		—	—	—	—	—	—	—	—	—	—	—
	Dieldrin		17	19	22	—	—	—	—	—	—	—	—
1968	DDE	—	—	T	T	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	10	21	12	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	10	—	—	—	—	—	—	90	T
1970	DDE	—	11	—	T	—	—	—	T	86	—	—	—
	TDE	—	—	—	T	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	T	T	10	25	—	T	—	—	—	—	—	T
1971	DDE	—	—	10	—	—	—	—	—	—	T	T	T
	TDE	—	—	—	—	—	—	—	—	—	T	T	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	T	12	12	T	T	—	—	—	—	—	T	T
1972	DDE	T	12	15	12	T	T						
	TDE	—	13	T	11	T	T						
	DDT	—	—	—	—	T	T						
	Dieldrin	10	17	15	—	T	T						

TABLE E-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Georgia*—Continued

Year	Compound	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		RESIDUES IN PPB (μg/kg)											
		[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]											
		STATION 3.—OGEECHEE RIVER—65 SAMPLES [1]											
1967	DDE		T	T	T	—	—	—	T	—	—	—	T
	TDE		T	10	—	—	—	—	—	—	—	—	T
	DDT		—	—	—	—	—	—	—	—	—	—	T
	Dieldrin		13	26	10	—	—	—	—	—	—	—	—
1968	DDE	—	—	13	T	—	—	—	—	—	—	—	—
	TDE	—	—	13	11	—	—	—	—	—	—	—	—
	DDT	—	—	24	—	—	—	—	—	—	—	—	—
	Dieldrin	—	18	16	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	T	—	—	—	16	—	—	—	T
	PCB's	—	—	—	—	—	—	—	—	—	—	—	[2]
1970	DDE	—	10	—	—	—	—	—	T	12	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	T	T	T	—	—	—	—	—	—	—	—	—
1971	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	T	T	T	—	—	11	—	—	—	—	—
1972	DDE	—	—	T	—	T	T						
	TDE	—	—	—	—	—	T						
	DDT	—	—	—	—	—	—						
		STATION 4.—ST. CATHERINE SOUND—65 SAMPLES [1]											
1967	DDE		T	T	T	—	—	—	—	—	—	—	—
	TDE		T	—	—	—	—	—	—	—	—	—	—
	DDT		T	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	T	—	—	—	—	—	—	—	—
	TDE	—	—	—	T	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	PCB's	—	—	—	—	—	—	—	—	—	—	—	[2]

TABLE E-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Georgia*—Continued

YEAR	COMPOUND	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
		RESIDUES IN PPB (μg/kg)											
		[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]											
		STATION 4.—ST. CATHERINE SOUND—65 SAMPLES [1]—Continued											
1970	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1971	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	T	—	T	—	—	—	—	—	—	—	—
1972	DDE	—	—	T	—	T	T						
	TDE	—	—	—	—	—	T						
	DDT	—	—	—	—	—	—						
		STATION 5.—SAPELO SOUND—65 SAMPLES [1]											
1967	DDE		T	T	T	—	T	T	—	—	—	—	T
	TDE		T	—	T	—	T	22	—	—	—	—	T
	DDT		T	—	—	—	T	23	—	—	—	—	—
	Dieldrin		12	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	T	—	—	—	—	—	—	—	—	—
	TDE	—	—	13	—	—	—	—	—	—	—	—	—
	DDT	—	—	19	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	T	—	—	—	—	—	—	—	—
	PCB's	—	—	—	—	—	—	—	—	—	—	(2)	(2)
1970	DDE	—	11	—	T	—	—	—	—	—	—	—	—
	TDE	—	—	—	T	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	T	—	—	—	—	—	—	—	—
1971	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	T	T	T	—	—	—	—	—	—	—	—
1972	DDE	—	—	T	—	T	T						
	TDE	—	—	—	—	—	T						
	DDT	—	—	—	—	T	—						

TABLE E-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Georgia*—Continued

Year	Compound	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		RESIDUES IN PPB (μg/kg)											
		[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]											
		STATION 6.—DOBOY SOUND—64 SAMPLES [1]											
1967	DDE		T	T	—	—	—	T	—	—	—	—	—
	TDE		—	T	—	—	—	11	—	—	—	—	—
	DDT		—	—	—	—	—	11	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	14	14	13	—	—	—	—	—	—	—	—
	PCB's	—	—	—	—	—	—	—	—	—	—	(2)	—
1970	DDE	—	10	—	—		—	—	—	—	—	—	—
	TDE	—	—	—	—		—	—	—	—	—	—	—
	DDT	—	—	—	—		—	—	—	—	—	—	—
	Dieldrin	T	—	—	—		—	—	—	—	—	—	—
1971	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	T	T	T	—	T	—	—	—	—	—	—	—
1972	DDE	—	—	T	T	T	—						
	TDE	—	—	T	—	—	—						
	DDT	—	—	—	—	—	—						
		STATION 7.—EGG ISLAND—65 SAMPLES [1]											
1967	DDE		—	—	—	—	—	15	—	—	—	—	—
	TDE		—	—	—	—	—	19	—	—	—	—	—
	DDT		—	—	—	—	—	18	—	—	—	—	—
	Dieldrin		—	—	—	—	—	—	—	—	—	—	20
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	13	15	23	14	—	—	—	—	—	—	—	21
1969	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	16	—	15	15	—	—	—	—	—	—	—	T

TABLE E-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Georgia*—Continued

Year	Compound	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		RESIDUES IN PPB (μg/kg)											
		[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]											
		STATION 7.—EGG ISLAND—65 SAMPLES[1]—Continued											
1970	DDE	—	16	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	T	T	T	—	T	—	—	—	—	T	T	T
1971	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	11	T	T	T	—	—	—	—	—	—	—	—
1972	DDE	—	—	T	—	—	—						
	TDE	—	—	—	—	—	—						
	DDT	—	—	—	—	—	—						
	Dieldrin	—	T	—	—	—	—						
		RESIDUES IN PPM (mg/kg)											
		[Blank = no sample collected; — = no residue detected above 0.1 ppm or no residue detected (PCB's); T = >0.1 but <0.25 ppm]											
		STATION 8.—ST. SIMONS ISLAND—65 SAMPLES[1,3,5]											
1967	Toxaphene		(4)	2.5	1.5	1.5	1.0	1.0	1.1	0.8	0.7	2.0	2.0
1968	Toxaphene	0.8	5.0	6.0	4.3	1.6	2.0	2.0	0.6	T	—	5.4	2.8
1969	Toxaphene	2.0	1.2	2.5	7.5	5.0	1.5	1.0	1.0	1.5	1.6	1.6	1.8
1970	Toxaphene	3.8	3.8	7.2	3.3	1.8	1.1	<1.0	0.8	T	0.6	0.7	1.6
	PCB's	—	—	—	—	—	—	(2)	—	—	—	—	—
1971	Toxaphene	1.3	0.7	1.1	1.6	0.7	0.1	0.6	T	T	0.6	T	0.6
	PCB's	—	—	—	—	(2)	—	—	—	—	—	—	—
1972	Toxaphene	0.6	1.0	1.1	1.0	0.8	0.6						
		RESIDUES IN PPM (mg/kg)											
		[Blank = no sample collected; — = no residues detected above 0.1 ppm; T = >0.1 but <0.25 ppm]											
		STATION 9.—TERRY CREEK—16 SAMPLES[1]											
1967	Toxaphene				12.0			4.7				18.0	13.0
1968	Toxaphene		23.0	6.0	54.0		5.0				6.3	12.0	
1969	Toxaphene	9.0				12.0	17.0		8.0				
1970	Toxaphene				6.2	8.2							

TABLE E-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Georgia*—Continued

Year	Compound	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		RESIDUES IN PPM (mg/kg)											
		[Blank = no sample collected; — = no residue detected above 0.1 ppm; T = >0.1 but <0.26 ppm]											
		STATION 10.—JEKYLL ISLAND—62 SAMPLES [1,6]											
1967	Toxaphene		—	T	0.5	0.4	0.4	—	—	—		1.0	
1968	Toxaphene	0.7	2.1	0.7	0.7	0.5	T	0.4	—	—	—	—	—
	PCB's	—	—	—	—	—	—	—	—	—	—	—	—
1969	Toxaphene	1.0	1.0	1.0	1.0		—	—	—	3.5	T	—	0.7
	PCB's	—	—	—	—		—	—	[2]	—	[2]	—	—
1970	Toxaphene	0.8	—	T	T	—	—	—	—	—	—	—	0.6
	PCB's	—	[2]	[2]	[2]	[2]	—	—	—	—	—	—	—
1971	Toxaphene	0.5	T	0.6	0.8	T	T	—	—	—	0.5	T	T
	PCB's	—	[2]	—	—	[2]	—	—	—	—	—	—	—
1972	Toxaphene	0.3	T	1.0	0.6	T	—						
		RESIDUES IN PPM (mg/kg)											
		[Blank = no sample collected; — = no residue of DDT detected above 0.005 ppm or no residue detected above 0.1 ppm (toxaphene and PCB's); t = >0.1 but <0.25 ppm; T = >0.005 but <0.010 ppm]											
		STATION 11.—SATILLA RIVER—64 SAMPLES [1]											
1967	DDE		—	—	—	—	—	—	—	—	—	—	—
	TDE		—	—	—	—	—	—	—	—	—	—	—
	DDT		—	—	—	—	—	—	—	—	—	—	—
	Toxaphene		—	t	t	t	t	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	
	TDE	—	—	—	—	—	—	—	—	—	—	—	
	DDT	—	—	—	—	—	—	—	—	—	—	—	
	Toxaphene	1.0	0.5	0.7	t	—	—	—	—	—	—	—	
1969	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1970	DDE		15	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1971	DDE	—	—	T	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	PCB's	—	[3]	—	—	—	—	—	—	—	—	—	—
1972	DDE	—	—	—	—	—	—						
	TDE	—	—	T	—	—	—						
	DDT	—	—	—	—	—	—						

[1] Each sample represents 15 or more mature mollusks.
[2] Aroclor 1254® present but not quantified.
[3] Presence of toxaphene prevented quantification of DDT and its metabolites in these samples.
[4] Toxaphene present but not quantified.
[5] One sample each in April 1969, April 1970, and February 1972 contained a trace of dieldrin.
[6] DDT and its metabolites not detected in any samples.

SECTION F.—MAINE

The monthly monitoring of Maine estuaries for persistent synthetic residues was initiated in December 1965 and continued until November 1970. There were 10 principal stations; about 40 other sites were sampled occasionally. Samples were analyzed at the Gulf Breeze Laboratory until June 1969 and, thereafter, at the Fisheries Research Station, Maine Department of Sea and Shore Fisheries.

The soft clam (*Mya arenaria*) and the blue mussel (*Mytilus edulis*) were the principal mollusks monitored and, on occasion, both eastern oysters (*Crassostrea virginica*) and horse mussels (*Modiolus modiolus*) were collected at the same sites. In the laboratory, the uptake of DDT was greater in the soft clam than in other species tested as was the flushing rate, and 90% of DDT residues was lost within 7 days after the toxicant was removed. This may explain why in simultaneous collections of two or more species of mollusks, DDT residues in soft clams examined at 30-day intervals were usually lower than those in the oyster or horse mussel. A summary of data on organochlorine residues in the monitored species, is presented in Table F-1, and the distribution of residues in these species for each sampling station by date of collection in Table F-2.

The Maine samples are characterized by the low incidence (18%) of detectable DDT residues as compared to most other monitored areas, despite the fact that substantial amounts of DDT are reported to have been used agriculturally in some watersheds in Maine. The maximum magnitude of DDT residues detected was, however, larger than that found in seven other States. Analysis of occasional collections of fish and invertebrates other than mollusks revealed DDT residues larger than those in mollusks. Presumably organochlorine pollution in Maine estuaries was usually too low and too transitory to be detected except in animals that retain residues for a long period of time.

Despite the generally low incidence of DDT residues at most stations, there was sufficient continuity in detectable DDT residues at Station 10 on the Piscataqua River to show a gradual decline from an average of about 28 ppb in 1966 to an undetectable level in 1970. A similar trend is clearly shown in samples collected at Station 7, Small Point.

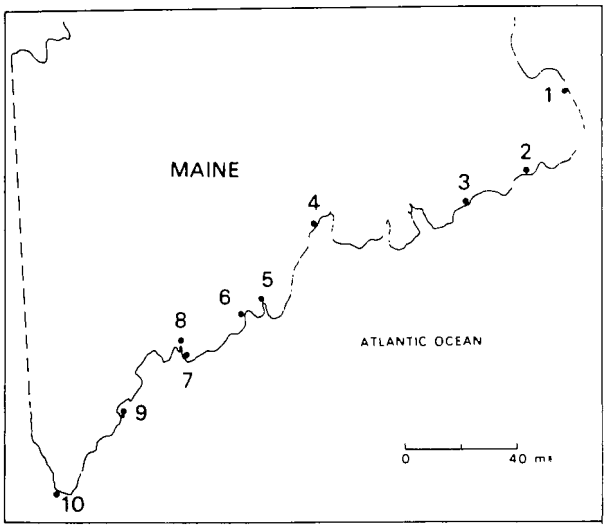


FIGURE F-1.—*Diagram of coastal Maine showing approximate location of monitoring stations*

1. Mill Cove—St. Croix River
2. Machiasport—Machias River
3. Millbridge—Narraguagus River
4. Fort Point—Penobscot River
5. Thomaston—St. George River
6. Medomak—Medomak River
7. Small Point—Kennebec-Androscoggin River
8. Phippsburg—Kennebec-Androscoggin River
9. Biddeford Pool—Saco River
10. Eliot—Piscataqua River

TABLE F-1—*Summary of data on organochlorine residues in the monitored species, 1965-70—Maine*

Station Number	Location	Monitoring Period	Principal Monitored Species	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)	
					DDT	Dieldrin
1	Mill Cove	1965-66	*M. arenaria*	12		
2	Machiasport	1965-70	*M. arenaria*	52	2 (15)	
3	Millbridge	1966-70	*M. arenaria*	37	1 (12)	
4	Fort Point	1965-70	*M. arenaria*	42	1 (15)	
5	Thomaston	1965-70	*M. arenaria*	42	1 (80)	1 (11)
6	Medomak	1967-70	*M. arenaria*	23	2 (11)	
7	Small Point	1968-70	*M. edulis*	18	12 (359)	

TABLE F-1.—*Summary of data on organochlorine residues in the monitored species, 1965-70—Maine*—Continued

Station Number	Location	Monitoring Period	Principal Monitored Species	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (µg/kg)	
					DDT	Dieldrin
8	Phippsburg	1965-69	*M. arenaria*	39	7 (24)	
9	Biddeford Pool	1968-70	*M. edulis*	24	7 (64)	
10	Eliot	1966-70	*M. arenaria*	45	22 (67)	9 (38)
11	Occasional stations (40)	1965-69	Mixed	62	16 (93)	4 (18)
Total number of samples				396		
Percent of samples positive for indicated compound					18	4

[1] Each sample represents 15 or more mature mollusks.

TABLE F-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—Maine*

[Blank = no sample collected; — = no residue detected above 5 ppb; T = >5 but <10 ppb.]

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—MILL COVE—*M. ARENARIA*—12 SAMPLES [1]													
1965	DDE												—
	TDE												—
	DDT												—
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
STATION 2.—MACHIASPORT—*M. ARENARIA*—52 SAMPLES [1]													
1965	DDE												—
	TDE												—
	DDT												—
1966	DDE	—	—	—	—	T	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—		—	—	—	—	—	—	—	—	—	—
	TDE	—		—	—	—	—	—	—	—	—	—	—
	DDT	—		—	—	—	—	—	—	—	—	—	—
1968	DDE	—			—	—	—	—	—	—	T	—	—
	TDE	—			—	—	—	—	—	—	T	—	—
	DDT	—			—	—	—	—	—	—	T	—	—
1969	DDE		—	—	—	—	—	—	—	—	—	—	—
	TDE		—	—	—	—	—	—	—	—	—	—	—
	DDT		—	—	—	—	—	—	—	—	—	—	—

TABLE F-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—Maine*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 2.—MACHIASPORT—*M. ARENARIA*—52 SAMPLES [1]—Continued													
1970	DDE				—		—	—	—	—	—	—	
	TDE				—		—	—	—	—	—	—	
	DDT				—		—	—	—	—	—	—	
STATION 3.—MILLBRIDGE—*M. ARENARIA*—37 SAMPLES [1]													
1966	DDE												—
	TDE												—
	DDT												—
1967	DDE				—	—	—	—	—	—	—	—	—
	TDE				—	—	—	—	—	—	—	—	—
	DDT				—	—	—	—	—	—	—	—	—
1968	DDE			—	—	—	—	—	—	—	—	—	—
	TDE			—	—	—	—	—	—	—	—	—	—
	DDT			—	—	—	—	—	—	—	—	—	—
1969	DDE		—	—	—	—	—	—	—	—	—	—	—
	TDE		—	—	—	—	—	—	—	—	—	—	—
	DDT		—	—	—	—	—	—	—	—	—	—	—
1970	DDE						—	—	—	12	—	—	
	TDE						—	—	—	—	—	—	
	DDT						—	—	—	—	—	—	
STATION 4.—FORT POINT—*M. ARENARIA*—42 SAMPLES [1]													
1965	DDE												—
	TDE												—
	DDT												—
1966	DDE	—	—	—	—	—	—	—	—		—	—	
	TDE	—	—	—	—	—	—	—	—		—	—	
	DDT	—	—	—	—	—	—	—	—		—	—	
1967	DDE			—	—	—	—	—	—	—	—	—	—
	TDE			—	—	—	—	—	—	—	—	—	—
	DDT			—	—	—	—	—	—	—	—	—	—
1968	DDE			—	—	—	—	—		—	—	—	—
	TDE			—	—	—	—	—		—	—	—	—
	DDT			—	—	—	—	—		—	—	—	15
1969	DDE	—	—	—	—	—	—	—	—	—			—
	TDE	—	—	—	—	—	—	—	—	—			—
	DDT	—	—	—	—	—	—	—	—	—			—

TABLE F-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—Maine*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 4.—FORT POINT—*M. ARENARIA*—42 SAMPLES [1]—Continued													
1970	DDE	—	—										
	TDE	—	—										
	DDT	—	—										
STATION 5.—THOMASTON—*M. ARENARIA*, UNLESS OTHERWISE INDICATED—42 SAMPLES [1]													
1965	DDE												—
	TDE												—
	DDT												—
1966	DDE		—	—	—	—	—	—	—		21	—	
	TDE		—	—	—	—	—	—	—		35	—	
	DDT		—	—	—	—	—	—	—		24	—	
	Dieldrin		—	—	—	11	—	—	—		—	—	
1967	DDE	—		—	—	—	—	—	—	—	—	—	—
	TDE	—		—	—	—	—	—	—	—	—	—	—
	DDT	—		—	—	—	—	—	—	—	—	—	—
1968	DDE			—	—	—	—		—	—	—	—	
	TDE			—	—	—	—		—	—	—	—	
	DDT			—	—	—	—		—	—	—	—	
1969	DDE	[2] —			—	—	—	—	—			—	
	TDE	—			—	—	—	—	—			—	
	DDT	—			—	—	—	—	—			—	
1970	DDE	—	—			—		—		—	—		
	TDE	—	—			—		—		—	—		
	DDT	—	—			—		—		—	—		
STATION 6—MEDOMAK—*M. ARENARIA*—23 SAMPLES [1]													
1967	DDE	—			—	—	—	—	—		—	—	—
	TDE	—			—	—	—	—	—		—	—	—
	DDT	—			11	—	T	—	—		—	—	—
1968	DDE				—	—				—	—	—	
	TDE				—	—				—	—	—	
	DDT				—	—				—	—	—	
1969	DDE				—	—	—		—			—	
	TDE				—	—	—		—			—	
	DDT				—	—	—		—			—	
1970	DDE				—			—		—	—		
	TDE				—			—		—	—		
	DDT				—			—		—	—		

TABLE F-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—Maine*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 7.—SMALL POINT—*M. EDULIS*—18 SAMPLES [1]													
1968	DDE							35	21	17	12	25	19
	TDE							44	25	20	14	27	18
	DDT							280	77	68	18	26	26
1969	DDE		11		T	T	T	12	—	—	—		—
	TDE		14		T	T	T	21	—	—	—		—
	DDT		18		15	13	T	13	49	—	—		—
1970	DDE	—	—		—								
	TDE	—	—		—								
	DDT	—	—		—								
STATION 8.—PHIPPSBURG—*M. ARENARIA*—39 SAMPLES [1]													
1965	DDE												—
	TDE												—
	DDT												—
1966	DDE	—	—	—	—	—	—	—	—			—	—
	TDE	—	—	—	—	—	—	—	—			—	—
	DDT	—	—	—	—	—	—	—	—			—	—
1967	DDE	—	—	—	T	—	—	—	—		—	—	—
	TDE	—	—	—	—	—	—	—	—		—	—	—
	DDT	—	—	—	11	11	T	—	—		—	—	—
1968	DDE		—	—	—	—	T	—	—	—	—	—	—
	TDE		—	—	—	—	—	—	—	—	—	—	—
	DDT		—	—	—	T	19	—	—	—	—	T	11
1969	DDE	—		—	—			—	—		—		
	TDE	—		—	—			—	—		—		
	DDT	—		—	—			—	—		—		
STATION 9.—BIDDEFORD POOL—*M. EDULIS*—24 SAMPLES [1]													
1968	DDE			—		—	T	—	T	T	T	T	
	TDE			—		—	T	—	T	T	T	T	
	DDT			—		—	21	—	54	22	15	13	
1969	DDE			T	—	T		—	—	—		—	—
	TDE			T	—	—		—	—	—		—	—
	DDT			21	—	12		—	—	—		—	—
1970	DDE	—			—	—		—	—	—	—	—	
	TDE	—			—	—		—	—	—	—	—	
	DDT	—			—	—		—	—	—	—	—	

TABLE F-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—Maine*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 10.—ELIOT—*M. ARENARIA*, UNLESS OTHERWISE INDICATED—45 SAMPLES [1]													
1966	DDE	T	—	12	14	13	13	T	—	T	T	T	—
	TDE	T	—	16	21	19	21	11	—	T	11	T	—
	DDT	21	T	32	32	23	16	T	—	T	T	T	—
	Dieldrin	32	—	38	—	27	23	—	—	—	—	—	T
1967	DDE	—		—	T	T	—	—	—	T	T	—	T
	TDE	—		—	11	14	T	—	—	15	T	—	T
	DDT	—		—	20	18	30	—	—	T	22	—	15
	Dieldrin	—		16	Lost	10	T	—	—	—	—	—	—
1968	DDE			—	T		—	—	T	—	—	—	
	TDE			T	12		—	—	12	—	—	—	
	DDT			18	15		—	—	T	—	—	—	
	Dieldrin			10	—		—	—	—	—	—	—	
1969	DDE			[3] 11	[3] 12	T		—	—	—			—
	TDE			23	22	T		—	—	—			—
	DDT			18	14	T		—	—	—			—
1970	DDE				—	—	—		—	—	—	—	
	TDE				—	—	—		—	—	—	—	
	DDT				—	—	—		—	—	—	—	

[1] Each sample represents 15 or more mature mollusks.
[2] *M. edulis.*
[3] *M. demissus.*

SECTION G.—MARYLAND

Eastern oysters, *Crassostrea virginica*, were collected in upper Chesapeake Bay and its tributaries at irregular intervals (usually twice yearly) from August 1966 to November 1970. The sampling was made possible because of oyster surveys being conducted for other programs. All samples from the 10 locations in Maryland were analyzed at the Gulf Breeze Laboratory. The approximate station locations are shown in Fig. G-1. A summary of data on organochlorine residues in the monitored species, *C. virginica*, is presented in Table G-1, and the distribution of residues in this species for each sampling station by date of collection in Table G-2.

Maryland was fifth among all States, in the incidence of DDT residues (81%), but the magnitude of residues in oysters was surprisingly low in view of the size of the Susquehanna River watershed and the extent of its agricultural development. More selective monitoring might show that the major pesticide burden of the river is precipitated with silt in the headwaters of the Bay and does not enter the trophic web of the estuarine system extensively. DDT residues detected at monitoring stations probably reflected pollution primarily in the adjacent and usually small drainage basins.

Despite the small number of samples, the decline in average DDT residues from 26 ppb in 1966 to 10 ppb in 1970 together with a more than 150% increase in samples containing less than 11 ppb suggests a real change in average pollution levels.

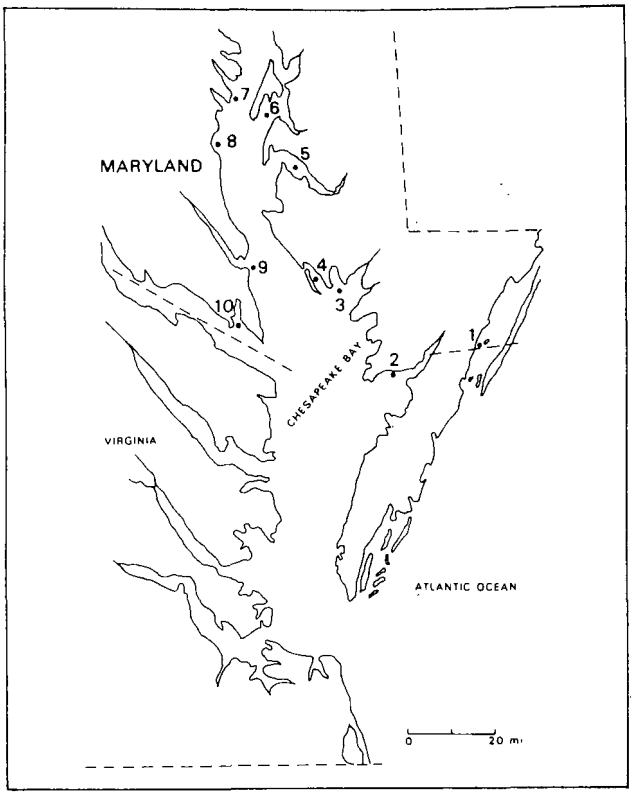


FIGURE G-1.—*Diagram of coastal Maryland showing approximate location of monitoring stations*

1. Franklin City—Chincoteague Bay
2. Pocomoke Sound
3. Tangier Sound
4. Honga River
5. Choptank River
6. Eastern Bay
7. Tollys Bar—Chesapeake Bay
8. Herring Bay—Chesapeake Bay
9. Cedar Point—Chesapeake Bay
10. St. Marys River

TABLE G-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1966-70—Maryland*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)	
				DDT	Dieldrin
1	Franklin City	1966-70	8	8 (43)	
2	Pocomoke Sound	1966-69	6	5 (47)	
3	Tangier Sound	1966-70	10	5 (48)	
4	Honga River	1966-70	10	8 (43)	
5	Choptank River	1966-70	8	4 (30)	
6	Eastern Bay	1966-70	10	8 (70)	
7	Tollys Bar	1967-70	8	8 (44)	7 (22)
8	Herring Bay	1966-70	10	9 (46)	4 (18)
9	Cedar Point	1966-70	10	9 (70)	
10	St. Marys River	1966-70	8	7 (33)	
Total number of samples			88		
Percent of samples positive for indicated compound				81	13

[1] Each sample represents 15 or more mature mollusks.

TABLE G-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Maryland*

[Blank = no sample collected; — = no residue detected above 5 ppb; T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—FRANKLIN CITY—8 SAMPLES [1]													
1966	DDE								10			T	
	TDE								14			—	
	DDT								T			—	
1967	DDE			11				10					
	TDE			—				—					
	DDT			—				13					
1968	DDE				26			T				T	
	TDE				17			T				—	
	DDT				—			16				T	
1969		No Samples Collected											
1970	DDE				14								
	TDE				—								
	DDT				—								
STATION 2.—POCOMOKE SOUND—6 SAMPLES [1]													
1966	DDE								—			T	
	TDE								17			T	
	DDT								T			—	
1967	DDE			T					T				
	TDE			T					T				
	DDT			—					T				
1968	DDE							11					
	TDE							12					
	DDT							24					
1969	DDE			—									
	TDE			—									
	DDT			—									
STATION 3.—TANGIER SOUND—10 SAMPLES [1]													
1966	DDE								13			T	
	TDE								24			T	
	DDT								11			—	
1967	DDE		—					T					
	TDE		—					—					
	DDT		—					10					

TABLE G-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Maryland*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 3.—TANGIER SOUND—10 SAMPLES[1]—Continued													
1968	DDE				T			17			—		
	TDE				T			10			—		
	DDT				—			—			—		
1969	DDE			—									
	TDE			—									
	DDT			—									
1970	DDE				—							—	
	TDE				—							—	
	DDT				—							—	
STATION 4.—HONGA RIVER—10 SAMPLES[1]													
1966	DDE								T			12	
	TDE								12			20	
	DDT								11			11	
1967	DDE		T					T					
	TDE		12					T					
	DDT		—					T					
1968	DDE				T			T			T		
	TDE				—			T			—		
	DDT				—			28			10[2]		
1969	DDE			—									
	TDE			—									
	DDT			—									
1970	DDE				—							T	
	TDE				—							10	
	DDT				—							—	
STATION 5.—CHOPTANK RIVER—8 SAMPLES[1]													
1966	DDE								T			T	
	TDE								11			13	
	DDT								—			12	
1967	DDE		T					T					
	TDE		11					T					
	DDT		—					—					
1968	DDE										—		
	TDE										—		
	DDT										—		

TABLE G-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Maryland*—Continued

Year	Compound	Residues in PPB (μg/kg)										
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 5.—CHOPTANK RIVER—8 SAMPLES[1]—Continued													
1969	DDE			—									
	TDE			—									
	DDT			—									
1970	DDE				—							—	
	TDE				—							—	
	DDT				—							—	
STATION 6.—EASTERN BAY—10 SAMPLES[1]													
1966	DDE								—				14
	TDE								—				17
	DDT								—				—
1967	DDE		11					T					
	TDE		15					11					
	DDT		—					T					
1968	DDE				—			11				11	
	TDE				—			11				—	
	DDT				—			48				16	
1969	DDE			10									
	TDE			T									
	DDT			—									
1970	DDE				11							10	
	TDE				T							10	
	DDT				—							—	
STATION 7.—TOLLYS BAR—8 SAMPLES[1]													
1967	DDE		18					13					
	TDE		19					17					
	DDT		T					11					
	Dieldrin		13					13					
1968	DDE				14			12			15		
	TDE				11			14			13		
	DDT				—			T			16		
	Dieldrin				15			—			11		
1969	DDE			15									
	TDE			14									
	DDT			—									
	Dieldrin			16									

TABLE G-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Maryland*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 7.—TOLLYS BAR—8 SAMPLES [1]—Continued													
1970	DDE			16								T	
	TDE			17								10	
	DDT			—								—	
	Dieldrin			22								15	
STATION 8.—HERRING BAY—10 SAMPLES [1]													
1966	DDE								10			T	
	TDE								15			—	
	DDT								T			—	
1967	DDE		12					10					
	TDE		11					17					
	DDT		—					T					
	Dieldrin		—					13					
1968	DDE				T			12			12		
	TDE				T			14			11		
	DDT				—			20			11		
1969	DDE			10									
	TDE			11									
	DDT			—									
	Dieldrin			13									
1970	DDE				14								—
	TDE				16								—
	DDT				—								—
	Dieldrin				18								12
STATION 9.—CEDAR POINT—10 SAMPLES [1]													
1966	DDE							18				20	
	TDE							27				24	
	DDT							25				15	
1967	DDE		22					15					
	TDE		20					16					
	DDT		T					13					
1968	DDE				T			21			T		
	TDE				—			13			T		
	DDT				—			16			24		
1969	DDE			T	11								
	TDE			T	12								
	DDT			—	—								

TABLE G-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Maryland*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 9.—CEDAR POINT—10 SAMPLES [1]—Continued													
1970	DDE											—	
	TDE											—	
	DDT											—	
STATION 10.—ST. MARYS RIVER—8 SAMPLES [1]													
1966	DDE							—				12	
	TDE							—				16	
	DDT							—				T	
1967	DDE		15					T					
	TDE		17					T					
	DDT		—					T					
1968	DDE				T			11				T	
	TDE				—			11				—	
	DDT				—			11				12	
1969		No Samples Collected											
1970	DDE			15									
	TDE			11									
	DDT			—									

[1] Each sample represents 15 or more mature mollusks.

SECTION H.—MISSISSIPPI

Mississippi Sound and tributaries were monitored for organochlorine residues in eastern oysters, *C. virginica*, during the period August 1965 - June 1972. All samples from the eight sampling stations were analyzed at the Gulf Breeze Laboratory. Approximate station locations are shown in Fig. H-1. A summary of data on organochlorine residues in the monitored species, *C. virginica*, is presented in Table H-1, and the distribution of residues in this species for each sampling station by date of collection in Table H-2.

Only four States had a lower incidence of DDT residues in oysters, and the maximum residue detected in Mississippi (135 ppb) was lower than that in 12 of the other 14 States. Maximum DDT residues appeared to be more directly related to runoff from urban and industrialized centers rather than from agricultural areas.

In 1971, there was a more than 70% increase in the number of DDT residues of less than 10 ppb as compared to earlier years. This trend was reversed in the first 6 months of 1972 when 44% of the residues were more than 10 ppb as compared to only 25% in 1971.

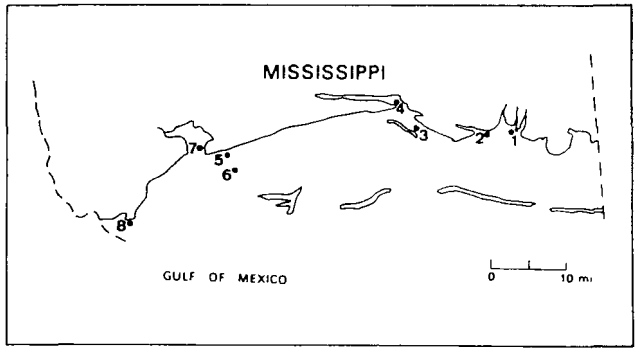


FIGURE H-1.—*Diagram of coastal Mississippi showing approximate location of monitoring stations*

1. Pascagoula—Pascagoula River
2. Graveline—Graveline Bay
3. Deer Island—Biloxi Bay
4. Biloxi Bay—Biloxi Bay
5. Pass Christian (Inshore)—Mississippi Sound
6. Pass Christian (Offshore)—Mississippi Sound
7. Bay St. Louis—St. Louis Bay
8. St. Joseph Point—Mississippi Sound

TABLE H-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1965-72—Mississippi*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)	
				DDT	Dieldrin
1	Pascagoula	1965-72	78	47 (74)	
2	Graveline	1965-72	79	56 (99)	
3	Deer Island	1965-69	49	33 (105)	
4	Biloxi Bay	1965-72	78	71 (135)	8 (19)
5	Pass Christian (Inshore)	1965-66	13	7 (53)	
6	Pass Christian (Offshore)	1965-72	78	29 (42)	3 (16)
7	Bay St. Louis	1966-72	66	31 (124)	7 (20)
8	St. Joseph Point	1969-72	29	11 (69)	1 (18)
Total number of samples			470		
Percent of samples positive for indicated compound				61	4

[1] Each sample represents 15 or more mature mollusks.

TABLE H-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Mississippi*

[Blank = no sample collected; — = no residue detected above 5 ppb; T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—PASCAGOULA—78 SAMPLES [1]													
1965	DDE								T	T	T	14	—
	TDE								T	—	T	55	—
	DDT								T	—	—	T	—
1966	DDE	T	17		13	19	—	T	T	—	—	T	11
	TDE	T	41		10	19	—	T	—	—	—	—	12
	DDT	—	—		—	—	—	—	—	—	—	—	—
1967	DDE	13	12	11	14	T	—	—	—	T	T	T	—
	TDE	14	47	T	13	T	—	—	—	T	—	—	—
	DDT	T	10	—	—	—	—	—	—	17	—	—	—
1968	DDE	T	T	T	T	10	—	—	—	—	—	—	—
	TDE	—	T	T	T	11	—	—	—	—	—	—	—
	DDT	—	T	—	—	T	—	—	—	—	—	—	—
1969	DDE	T	—	11	T	15	T	T	—		11	12	
	TDE	13	—	12	T	19	12	T	—		14	T	
	DDT	—	—	—	—	T	—	—	—		T	—	
1970	DDE	13	12	15	16	10	T	—	—	—	—	T	—
	TDE	40	12	18	14	13	64	—	—	—	—	T	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—

TABLE H-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Mississippi*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—PASCAGOULA—78 SAMPLES [1]—Continued													
1971	DDE	—	—	—	—	T	—		—	—		—	T
	TDE	—	—	—	—	12	55		—	—		—	T
	DDT	—	—	—	—	—	—		—	—		—	—
1972	DDE	T	T	—	10	11	—						
	TDE	11	T	—	10	—	—						
	DDT	—	—	—	—	—	—						
STATION 2.—GRAVELINE—79 SAMPLES [1]													
1965	DDE								T	T	T	T	T
	TDE								T	T	T	—	20
	DDT								T	—	—	—	—
1966	DDE	10	T	12	29	21	22	16	12	18	T	13	13
	TDE	27	T	10	10	60	68	19	31	69	13	17	36
	DDT	T	—	—	T	T	—	—	—	—	—	—	—
1967	DDE	14	12	23	24	T	T	T	—	T	12	14	15
	TDE	11	10	66	36	T	18	T	—	12	23	18	23
	DDT	13	—	10	T	—	—	—	—	21	29	10	T
1968	DDE	15	16	13	11	16	22	10	—	—	—	—	T
	TDE	22	23	19	18	23	25	14	—	T	—	—	—
	DDT	T	12	—	T	T	T	—	—	—	—	—	—
1969	DDE	—	—	11	T	15	T	—	—		—	11	17
	TDE	—	—	14	T	15	12	—	—		—	13	20
	DDT	—	—	—	—	T	—	—	—		—	—	10
1970	DDE		—	14	14	15	10	T	—	—	—	—	—
	TDE		—	16	17	14	11	10	—	—	—	—	—
	DDT		—	10	T	—	—	T	—	—	—	—	—
1971	DDE	15	—	—	—	12	—		—	—		—	T
	TDE	17	—	—	—	23	—		—	—		—	T
	DDT	—	—	—	—	—	—		—	—		—	—
1972	DDE	T	T	—	15	T	16						
	TDE	T	T	—	—	T	—						
	DDT	—	—	—	—	—	—						
STATION 3.—DEER ISLAND—49 SAMPLES [1]													
1965	DDE								10	T	—	T	T
	TDE								21	T	—	17	17
	DDT								T	—	—	—	—

TABLE H-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Mississippi*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 3.—DEER ISLAND—49 SAMPLES [1]—Continued													
1966	DDE	14	20	15	22	27	23	17	T	—	T	—	T
	TDE	27	43	25	45	62	65	38	T	—	T	—	11
	DDT	T	16	T	12	16	—	—	—	—	—	—	—
1967	DDE	10	15	14	15	11	—	—	—	—	—	—	T
	TDE	12	T	14	11	17	—	—	—	—	—	—	T
	DDT	T	—	—	—	—	—	—	—	12	—	—	—
1968	DDE	T	11	T	12	12	—	—	—	—	—	—	—
	TDE	13	13	T	12	12	—	—	—	—	—	—	—
	DDT	—	T	—	—	—	—	—	—	—	—	—	—
1969	DDE	T	T	11	T	T	—	T	T				
	TDE	T	—	13	13	T	—	27	34				
	DDT	—	—	—	—	—	—	—	22				
STATION 4.—BILOXI BAY—78 SAMPLES [1]													
1965	DDE								14	T	—	T	T
	TDE								23	11	—	23	18
	DDT								T	—	—	—	—
1966	DDE	14	16	16	29	32	—	20	19	—	T	15	T
	TDE	43	30	33	73	87	—	47	48	—	T	27	15
	DDT	14	T	11	15	16	—	—	—	—	—	T	—
1967	DDE	16	19	23	28	15	13	T	T	—	—	T	T
	TDE	31	40	43	50	33	21	43	25	17	17	19	22
	DDT	15	23	15	20	—	T	—	—	T	T	—	—
	Dieldrin	—	—	—	13	—	—	—	—	—	—	—	—
1968	DDE	12	14	16	20	30	17	T	—	—	—	T	16
	TDE	32	30	39	34	94	61	52	25	24	T	43	49
	DDT	—	T	—	T	T	—	—	—	—	—	—	—
1969	DDE	T	18	17	14	20	17	T	—		14	14	20
	TDE	28	46	47	46	67	54	22	25		42	28	53
	DDT	—	T	T	11	T	—	—	—		18	—	12
	Dieldrin	—	—	—	—	—	—	—	—		—	16	18
1970	DDE			18	19	28	—	—	—	—	—	—	—
	TDE			60	50	78	—	77	40	—	12	12	17
	DDT			15	T	14	—	—	—	—	—	—	—
	Dieldrin			19	16	15	—	11	—	16	—	—	—
1971	DDE	T	24	16	24	T	—		22	—		—	T
	TDE	26	65	43	69	85	—		81	—		19	27
	DDT	—	—	—	—	—	—		—	—		—	—

TABLE H-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Mississippi*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 4.—BILOXI BAY—78 SAMPLES [1]—Continued													
1972	DDE	T	10	12	18	17	T						
	TDE	20	28	35	58	63	30						
	DDT	—	—	—	—	—	—						
STATION 5.—PASS CHRISTIAN (INSHORE)—13 SAMPLES [1]													
1965	DDE								T	T	—	T	—
	TDE								T	—	—	—	—
	DDT								—	—	—	—	—
1966	DDE	T	—	—	T	11	19	—	—				
	TDE	T	—	—	T	12	34	—	—				
	DDT	—	—	—	—	11	—	—	—				
STATION 6.—PASS CHRISTIAN (OFFSHORE)—78 SAMPLES [1]													
1965	DDE								T	—	—	T	—
	TDE								T	—	—	T	—
	DDT								—	—	—	T	—
1966	DDE	T	—	—	11	14	—	—	—	—	—	—	—
	TDE	T	—	—	12	15	—	—	—	—	—	—	—
	DDT	—	—	—	T	13	—	—	—	—	—	—	—
1967	DDE	—	—	T	—	—	—	—	—	—	—	—	T
	TDE	—	—	T	—	—	—	—	—	—	—	—	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1968	DDE	T	T	T	T	T	—	—	—	—	—	—	—
	TDE	—	T	T	11	11	—	—	—	—	—	—	—
	DDT	—	T	—	—	—	—	—	—	—	—	—	—
1969	DDE		—	—	—	T	T	—	—		—	T	T
	TDE		—	—	—	T	13	—	—		—	T	T
	DDT		—	—	—	T	—	—	—		—	—	—
1970	DDE		12	13	15	11	T	—	—	—	—	—	—
	TDE		T	22	22	16	T	—	—	—	—	—	—
	DDT		—	T	—	T	—	—	—	—	—	—	—
	Dieldrin		11	16	—	—	—	—	—	15	—	—	—
1971	DDE	—	—	—	—	T	—		—	—		—	T
	TDE	—	—	—	—	14	—		—	—		—	T
	DDT	—	—	—	—	—	—		—	—		—	—
1972	DDE	T	T	12	12	T	11						
	TDE	T	T	10	16	T	—						
	DDT	—	—	—	T	—	—						

TABLE H-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Mississippi*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 7.—BAY ST. LOUIS—66 SAMPLES [1]													
1966	DDE									—	—	—	—
	TDE									—	—	—	—
	DDT									—	—	—	—
1967	DDE	T	T	T	T	T	—	—	—	—	—	11	T
	TDE	T	T	14	T	T	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	10	—	—	—
1968	DDE	T	T	T	T	10	—	—	—	—	—	—	—
	TDE	T	T	T	T	11	—	—	—	—	—	—	—
	DDT	—	T	—	—	T	—	—	—	—	—	—	—
1969	DDE	—	T	T	—	T	T	—	—		—	T	10
	TDE	T	—	12	—	T	11	—	—		—	11	12
	DDT	—	—	—	—	T	—	—	—		—	T	11
	Dieldrin	T	14	11	T	—	—	—	—		—	—	—
1970	DDE		T	12	17	34	32	—	—	—	—	—	—
	TDE		13	15	21	76	12	—	—	—	—	—	—
	DDT		—	—	T	14	—	—	—	—	—	—	—
	Dieldrin		17	20	—	14	—	—	—	—	—	—	—
1971	DDE	—	—	—	—	—	—		—	—		—	T
	TDE	—	—	—	—	13	—		—	—		—	—
	DDT	—	—	—	—	—	—		—	—		—	—
1972	DDE	T	T	—	11	T	—						
	TDE	T	T	—	11	—	—						
	DDT	—	—	—	T	—	—						
STATION 8.—ST. JOSEPH POINT—29 SAMPLES [1]													
1969	DDE											—	—
	TDE											—	—
	DDT											—	—
1970	DDE		T	T	T	14	15	—	—	—	—	—	—
	TDE		—	—	—	22	54	—	—	—	—	—	—
	DDT		—	—	—	—	—	—	—	—	—	24	—
	Dieldrin		18	—	—	—	—	—	—	—	—	—	—
1971	DDE	—	—	—	—	—	—		—	—		—	—
	TDE	—	—	—	—	—	—		—	—		—	—
	DDT	—	—	—	—	—	—		—	—		24	—
1972	DDE	T	T	—	—	T	T						
	TDE	—	15	—	—	T	—						
	DDT	—	—	—	—	—	—						

[1] Each sample represents 15 or more mature mollusks.

SECTION I.—NEW JERSEY

Samples of eastern oysters, *Crassostrea virginica,* were collected at five principal stations in the New Jersey waters of Delaware Bay during the period June 1966 - June 1972. All samples were analyzed at the Gulf Breeze Laboratory. The approximate station locations are shown in Fig. I-1. A summary of data on organochlorine residues in the monitored species, *C. virginica,* is presented in Table I-1, and the distribution of residues in this species for each sampling station by date of collection in Table I-2.

Oyster samples collected in Delaware Bay were characterized by a 100% incidence of DDT residues and a relatively high incidence (24%) of dieldrin residues as compared to other areas monitored.

The maximum DDT residue observed, 272 ppb, is low compared to that in many other estuaries; most residues, from New Jersey were less than half this amount. The fact that DDE was the principal component of these residues suggests that the pesticide had been metabolized in other links of the trophic web before its acquisition by the oyster.

DDT residues appear to have been somewhat higher in the 1968-69 period than earlier, but the 1971 data show a clear-cut trend towards decreased residue levels.

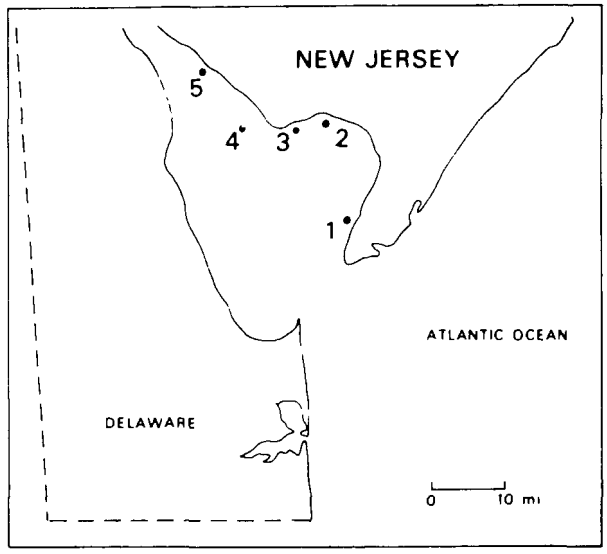


FIGURE I-1.—*Diagram of coastal New Jersey showing approximate location of monitoring stations*

1. Drum Beds—Delaware River
2. Maurice River—Delaware River
3. Dividing Creek—Delaware River
4. Lease 564/496D—Delaware River
5. Cohansey—Delaware River

TABLE I-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1966-72—New Jersey*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)		
				DDT	Dieldrin	PCB's [2]
1	Drum Beds	1966-72	49	49 (213)	3 (12)	2
2	Maurice River	1966-72	50	50 (143)	1 (T)	1
3	Dividing Creek	1966-71	7	7 (125)	1 (12)	
4	Lease 564/496D	1966-72	52	52 (278)	28 (26)	2
5	Cohansey	1966-72	49	49 (245)	16 (23)	1
	Occasional Stations (7)	1966-71	12	12 (166)	3 (29)	
Total number of samples			219			
Percent of samples positive for indicated compound				100	24	3

NOTE: T = >5 but <10 ppb.

[1] Each sample represents 15 or more mature mollusks.

[2] Present but not quantified.

TABLE I-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—New Jersey*

[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—DRUM BEDS—49 SAMPLES[1]													
1966	DDE						19		33	24		28	17
	TDE						18		38	24		26	14
	DDT						—		15	11		10	—
1967	DDE			43	39	30	31	27	18	27	37	42	
	TDE			34	28	27	30	40	25	33	46	50	
	DDT			11	T	—	—	21	16	13	22	14	
1968	DDE	43	50	55		50	110	110	55	44	75	52	
	TDE	43	43	54		57	98	83	38	35	48	44	
	DDT	10	T	T		—	T	13	13	T	11	—	
1969	DDE			49	19	48	51	73	52	63	67	56	58
	TDE			35	18	15	45	44	30	34	32	41	28
	DDT			—	—	—	—	—	T	—	T	T	T
1970	DDE			99			100	110	34	37		46	38
	TDE			42			52	47	12	15		21	16
	DDT			11			—	—	—	—		—	—
	Dieldrin			—			—	12	—	—		—	—
1971	DDE			35	59		70	38		27			53
	TDE			10	35		30	26		14			28
	DDT			—	—		—	—		—			—
1972	DDE				[2] 10		[2] 52						
	TDE				20		29						
	DDT				52		—						
	Dieldrin				T		T						
	PCB's				[3]		[3]						
STATION 2.—MAURICE RIVER—50 SAMPLES[1]													
1966	DDE						11		T	14		13	T
	TDE						15		12	15		16	T
	DDT						—		—	—		—	—
1967	DDE			12	12	13	15	12	T	18	26	19	
	TDE			T	T	19	28	23	10	30	40	31	
	DDT			17	—	—	—	T	—	13	13	—	
1968	DDE	19	24	22	21		45	37	17	20	16	24	
	TDE	27	31	32	43		68	38	21	24	17	19	
	DDT	T	—	—	—		—	16	T	10	T	T	

TABLE I-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—New Jersey*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		STATION 2.—MAURICE RIVER—50 SAMPLES [1]—Continued											
1969	DDE		21		16	77	75	13	22	12	T	19	13
	TDE		21		15	26	68	14	23	18	11	14	T
	DDT		—		—	—	—	—	T	—	—	—	—
1970	DDE			16		24	25	32	17	13	18		13
	TDE			T		22	36	34	16	15	19		10
	DDT			—		—	—	—	—	—	—		—
1971	DDE		12		T		26	23		14	T		
	TDE		T		—		29	29		13	—		
	DDT		—		—		—	—		—	—		
1972	DDE			14			[2] 17						
	TDE			—			17						
	DDT			—			—						
	Dieldrin			—			T						
	PCB's			—			(3)						
		STATION 3.—DIVIDING CREEK—7 SAMPLES [1]											
1966	DDE									T			
	TDE									13			
	DDT									—			
1967	DDE				26			41				22	
	TDE				28			66				33	
	DDT				—			18				11	
	Dieldrin				—			12				—	
1968	DDE						49						
	TDE						64						
	DDT						T						
1969	DDE				60								
	TDE				35								
	DDT				—								
1970		No Samples Collected											
1971	DDE									13			
	TDE									13			
	DDT									—			
		STATION 4.—LEASE 564/496D—52 SAMPLES [1]											
1966	DDE						34		29	41		51	58
	TDE						42		39	53		53	110
	DDT						—		T	12		67	15

TABLE I-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—New Jersey*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 4.—LEASE 564/496D—52 SAMPLES [1]—Continued													
1967	DDE			47	41	48	41	44	29	38	26	39	39
	TDE			48	39	86	81	72	47	56	41	56	56
	DDT			T	—	—	—	18	17	19	10	—	—
	Dieldrin			10	—	18	20	18	—	12	—	—	—
1968	DDE	35	38	37	23		110	77	51	69	67	57	
	TDE	50	54	60	45		140	82	46	57	57	56	
	DDT	T	T	T	—		18	23	16	T	T	T	
	Dieldrin	11	14	15	—		—	14	—	—	—	11	
1969	DDE		100		66	95	27	82	47	72	84	72	
	TDE		87		51	74	34	68	39	45	48	56	
	DDT		13		—	—	—	17	T	13	14	14	
	Dieldrin		13		12	18	21	—	—	19	13	11	
1970	DDE	130		140		180	75	35	62	95	120	42	12
	TDE	61		67		98	92	18	33	36	33	36	43
	DDT	15		16		—	—	—	—	—	—	—	—
	Dieldrin	14		16		20	26	—	—	—	—	—	12
1971	DDE		150		180		180	190		49			56
	TDE		45		87		62	78		25			18
	DDT		—		T		—	—		—			—
	Dieldrin		13		19		19	T		—			—
1972	DDE			[2] 67			[2] 46						
	TDE			42			35						
	DDT			11			—						
	Dieldrin			T			14						
	PCB's			[3]			[3]						
STATION 5.—COHANSEY—49 SAMPLES [1]													
1966	DDE						12		17		20	22	11
	TDE						24		37		35	33	23
	DDT						—		—		—	—	—
1967	DDE			29	35	41	54	24	29	17	17	32	
	TDE			45	53	19	150	59	66	39	38	59	
	DDT			T	T	22	25	10	13	—	T	—	
	Dieldrin			—	—	23	18	12	T	—	—	11	
1968	DDE	20	30	22		49	81	68	32	33	52	28	
	TDE	41	62	66		12	150	110	60	50	70	32	
	DDT	—	—	—		—	14	11	15	T	T	—	
	Dieldrin	—	12	14		13	19	12	—	—	—	—	

TABLE I-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—New Jersey*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		STATION 5.—COHANSEY—49 SAMPLES[1]—Continued											
1969	DDE		33		59	57	36	37	29	24	42	38	
	TDE		46		43	76	49	46	45	44	48	42	
	DDT		—		—	—	—	T	T	—	—	T	
	Dieldrin		—		—	16	—	—	—	—	—	—	
1970	DDE			45	41	55		30	27	53	36	42	
	TDE			46	51	98		30	34	54	42	36	
	DDT			—	—	—		T	—	—	—	—	
	Dieldrin			—	11	21		—	—	—	—	—	
1971	DDE		52		28	38		27		21			22
	TDE		42		24	61		30		20			17
	DDT		—		—	T		—		—			—
	Dieldrin		13		—	16		—		—			—
1972	DDE			23		[2] 40							
	TDE			114		49							
	DDT			—		—							
	Dieldrin			—		T							
	PCB's			—		[3]							

[1] Each sample represents 15 or more mature mollusks.
[2] DDT values are approximate because of presence of unidentified PCB's.
[3] Present but not quantified.

SECTION J.—NEW YORK

Several different species of mollusks (*Crassotrea virginica, Modiolus domissus, Mytilus edulis, Mercenavia mercenaria,* and *Mya arenaria*) were collected at 16 principal sites in New York's coastal waters to monitor organochlorine pollution during the period March 1966 - June 1972. Samples were analyzed at the Gulf Breeze Laboratory until February 1969 and thereafter by the New York Conservation Department. Analyses of aliquots of some of the samples collected during the period October 1968 - July 1970 have been reported by the cooperating agency (9) and do not differ significantly from the data reported here.

Approximate station locations are shown in Fig. J-1. A summary of data on organochlorine residues in the monitored species is presented in Table J-1, and the distribution of residues in these species for each sampling station by date of collection in Table J-2.

The hard clam, *M. mercenaria,* was the principal species collected because of its ubiquity and despite its recognized inefficiency in storing organochlorine residues. This lack of sensitivity to low levels of DDT pollution is especially well documented in the analytical record of samples collected in Conscience Bay, Station 6. Hard clams were the only mollusk of four species collected there in which DDT residues were undetected. DDT pollution apparently disappeared at this station during the period July 1968 - March 1969, but this was because of the substitution of hard clams for the blue mussel as monitors.

These data emphasize the fact that in areas where hard clams did show DDT residues, there were probably significant levels of DDT in the water or food supply. This parallels the situation in Delaware, where hard clams collected in Delaware Bay (Cape Henlopen) consistently had DDT residues while residues were usually not detected in hard clams collected in inner bays. There was generally good agreement in the magnitude of residues in two or more species, other than the hard clam, collected at the same station on the same day.

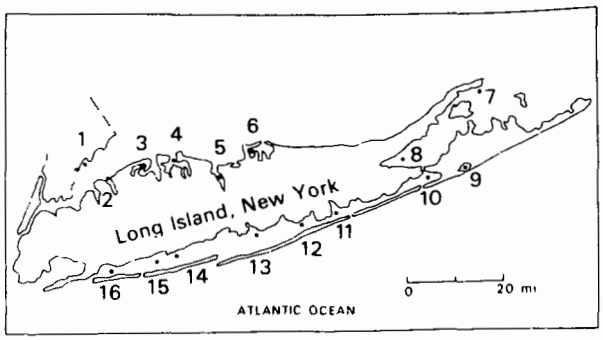


FIGURE J-1.—*Diagram of coastal New York showing approximate location of monitoring stations*

1. Mamaroneck
2. Hempstead Harbor
3. Oyster Bay Harbor
4. Huntington Bay
5. Nissequogue River
6. Conscience Bay
7. Southold—Gardiners Bay
8. Flanders Bay
9. Mecox Bay
10. Shinnecock Bay
11. Moriches Bay
12. Bellport—Great South Bay
13. Sayville—Great South Bay
14. Amityville—South Oyster Bay
15. East Bay
16. West Bay

The New York samples ranked fifth among the States in incidence and sixth in magnitude of DDT residues. More samples (43%) contained dieldrin residues than in any other area monitored. PCB's were present in some samples in 1972, but they were not identified or quantified.

Despite the large number of samples collected over a period of 7 years, no clearly defined trends in pollution levels can be identified. This may be the result of having used a variety of species. The overall impression is one of no significant change in DDT residue levels in mollusks.

TABLE J-1.—*Summary of data on organochlorine residues in the monitored species, 1966-72—New York*

Station Number	Location	Monitoring Period	Principal Monitored Species	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)	
					DDT	Dieldrin
1	Mamaroneck	1966-69	*M. mercenaria*	36	34 (96)	27 (29)
2	Hempstead Harbor	1966-72	*M. mercenaria*	74	70 (201)	61 (132)
3	Oyster Bay	1966-72	*M. mercenaria*	73	54 (99)	48 (86)
4	Huntington Bay	1966-72	*M. edulis*	74	72 (588)	52 (104)
5	Nissequogue River	1966-72	*M. edulis*	74	70 (138)	58 (117)
6	Conscience Bay	1966-72	*M. edulis*	73	61 (112)	52 (75)
7	Southold	1969-72	*C. virginica*	34	32 (149)	26 (78)
8	Flanders Bay	1966-72	*M. mercenaria*	69	63 (199)	15 (107)
9	Mecox Bay	1966-72	*C. virginica*	67	65 (596)	14 (22)

TABLE J-1.—*Summary if data on organochlorine residues in the monitored species, 1966-72—New York*—Continued

Station Number	Location	Monitoring Period	Principal Monitored Species	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)	
					DDT	Dieldrin
10	Shinnecock Bay	1966-72	*M. mercenaria*	73	43 (188)	19 (46)
11	Moriches Bay	1966-72	*M. mercenaria*	71	49 (83)	13 (49)
12	Bellport Bay	1966-72	*M. mercenaria*	71	51 (132)	10 (53)
13	Sayville	1966-72	*M. mercenaria*	74	41 (107)	16 (59)
14	Amityville	1966-72	*M. mercenaria*	73	49 (64)	13 (42)
15	East Bay	1966-72	*M. mercenaria*	57	43 (98)	13 (38)
16	West Bay	1966-72	*M. mercenaria*	57	51 (111)	19 (20)
	Occasional stations (8)	1967-72	Mixed	9	9 (159)	3 (31)
Total number of samples				1,059		
Percent of samples positive for indicated compound					81	43

[1] Each sample represents 15 or more mature mollusks.

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*

[Blank = no sample collected; — = no residue detected above 5 ppb; T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—MAMARONECK—*M. MERCENARIA*—36 SAMPLES [1]													
1966	DDE				—	T	—	—	—	—	T	16	T
	TDE				—	T	—	13	T	12	18	49	29
	DDT				—	—	—	—	—	—	—	14	13
	Dieldrin				—	—	—	—	—	—	—	29	12
1967	DDE	T	19	12	11	11	11	T	T	T	12	T	T
	TDE	35	50	31	27	27	28	20	15	26	28	32	30
	DDT	24	27	11	11	11	15	T	T	—	T	14	T
	Dieldrin	16	21	16	16	15	15	15	14	14	13	14	15
1968	DDE	T	T	T	11	10	T	—	—	—	—	—	T
	TDE	27	25	27	30	27	26	22	20	20	18	23	19
	DDT	T	—	—	T	12	15	T	—	—	—	—	—
	Dieldrin	11	12	14	11	12	—	10	12	12	11	15	14
1969	DDE	—	10	—									
	TDE	18	24	13									
	DDT	—	—	—									
	Dieldrin	11	11	—									

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 2.—HEMPSTEAD HARBOR—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—74 SAMPLES[1]													
1966	DDE			—	—	—	T	—	[3] 24	T	11	15	13
	TDE			—	—	—	29	—	48	17	29	39	35
	DDT			—	—	—	13	—	26	—	—	17	14
	Dieldrin			—	—	—	—	—	—	—	—	17	15
1967	DDE	T	14	13	12	12	14	T	T	12	13	12	10
	TDE	17	36	35	30	33	39	23	25	29	30	32	30
	DDT	—	13	13	11	15	28	15	16	—	T	11	11
	Dieldrin	—	15	16	17	15	19	15	17	15	16	14	16
1968	DDE	11	13	13	13	10	T	—	—	—	T	—	T
	TDE	29	31	31	34	26	27	17	18	17	24	22	23
	DDT	11	—	10	10	10	—	T	—	—	—	—	—
	Dieldrin	12	16	14	15	20	16	—	—	11	50	93	66
1969	DDE	—	10	[2] 15	[2] 16	18	15	T	—	[2] 34	[3] 30	[3] 23	[3] 26
	TDE	12	29	33	34	18	19	22	10	93	62	57	57
	DDT	—	T	34	38	12	T	T	—	74	49	47	46
	Dieldrin	47	70	22	86	85	13	33	38	132	28	40	31
1970	DDE	[3] 41	11	[3] 24	[3] 13	[3] 12	[2] 18	10	10	[3] 21	[3] 18	[3] 18	[3] 20
	TDE	71	33	51	28	33	48	22	35	71	40	48	50
	DDT	76	15	62	29	54	70	17	18	28	35	43	51
	Dieldrin	29	30	30	25	30	33	20	18	26	22	19	25
1971	DDE	[3] 10	[3] 13	[2] 13	[2] 15	[3] 20		—	[2] 16		[3] 13	[2] 22	[2] 23
	TDE	22	26	33	38	27		16	37		31	46	58
	DDT	18	31	34	44	23		10	30		17	32	51
	Dieldrin	10	14	16	31	—		13	21		19	23	20
1972	DDE	[3] 19	[3] 17	[2] 23	[2] 34	[3] 9	—						
	TDE	41	—	48	67	15	T						
	DDT	22	24	44	53	9	—						
	Dieldrin	19	14	19	21	—	T						
STATION 3.—OYSTER BAY HARBOR—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—73 SAMPLES[1]													
1966	DDE			—	—	—	—	—	—	—	—	T	T
	TDE			—	—	—	—	—	T	—	—	22	11
	DDT			—	—	13	—	—	—	—	—	T	—
	Dieldrin			—	—	—	—	—	—	—	—	13	11
1967	DDE	13	T	T	T	T	T	—	T	T	T	T	T
	TDE	50	15	19	14	20	22	26	17	13	T	13	15
	DDT	18	—	—	—	—	13	T	12	—	—	—	—
	Dieldrin	10	—	14	13	14	14	11	14	—	12	11	—

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 3.—OYSTER BAY HARBOR—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—73 SAMPLES [1]—Continued													
1968	DDE	T	T	—	T	T	—	—	—	—	—	—	—
	TDE	16	T	—	T	11	—	T	—	—	—	—	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	11	—	—	—	—	—	—	—	—	10
1969	DDE	—	T	—	[3] 11	[3] 24	—	—	—	—	[4] 27	T	—
	TDE	—	T	—	25	28	11	15	—	—	55	13	T
	DDT	—	—	—	16	32	—	T	—	—	17	—	—
	Dieldrin	T	T	12	17	24	86	16	—	17	—	15	18
1970	DDE		[4] 27	[3] 10	—	[3] 21	T	[3] 16	T		[4] 22	[4] 27	[4] 24
	TDE		52	19	—	44	16	37	18		48	55	50
	DDT		20	11	—	34	T	24	T		20	15	16
	Dieldrin		31	12	37	23	11	19	16		29	30	26
1971	DDE	[4] 19	[4] 19	[4] 22	—	[3] 13		—	—	[4] 19	[4] 23	[4] 19	[4] 23
	TDE	38	38	45	T	32		T	—	30	41	34	44
	DDT	16	T	15	—	18		—	—	13	10	17	18
	Dieldrin	18	25	27	16	18		10	—	21	20	14	17
1972	DDE	[3] 18	[3] 19	—	—	[4] 10	[3] 13						
	TDE	36	31	T	—	24	22						
	DDT	24	18	—	—	10	11						
	Dieldrin	12	15	—	10	11	19						
STATION 4.—HUNTINGTON BAY—*M. EDULIS*, UNLESS OTHERWISE INDICATED—74 SAMPLES [1]													
1966	DDE			[5] T	[5] —	[5] 32	98	[6] 53	40	[5] —	[5] —	[5] 21	[5] 29
	TDE			16	—	75	280	190	110	—	T	64	71
	DDT			—	—	81	210	60	25	—	—	17	16
	Dieldrin			—	—	—	—	18	13	—	—	12	T
1967 [5]	DDE	20	27	21	19	19	16	T	13	12	T	T	17
	TDE	88	65	54	57	54	63	50	40	39	27	22	56
	DDT	44	20	17	12	24	26	13	11	—	—	—	12
	Dieldrin	21	—	11	11	—	12	12	12	11	—	—	12
1968 [5]	DDE	T	—	12	16	15	12	—	—	—	T	T	10
	TDE	27	40	49	48	46	35	35	49	43	47	60	48
	DDT	—	T	—	T	14	—	T	—	—	—	T	11
	Dieldrin	11	11	—	12	—	—	—	—	—	—	11	T
1969	DDE	[5] —	[5] 11	[5] T	18	[5] T	[6] 30	29	[5] 15	34	24	28	30
	TDE	26	28	32	35	24	104	100	23	127	73	84	80
	DDT	—	—	—	21	21	46	50	—	47	28	55	50
	Dieldrin	T	T	T	11	14	104	26	T	18	—	23	34

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 4.—HUNTINGTON BAY—*M. EDULIS*, UNLESS OTHERWISE INDICATED—74 SAMPLES [1]—Continued													
1970	DDE	38	15	23	—	20	13	21	20	19	20	24	21
	TDE	90	58	60	22	50	59	146	70	40	66	89	73
	DDT	61	40	49	12	40	40	55	29	44	28	28	29
	Dieldrin	18	16	17	38	22	21	26	21	26	22	17	17
1971	DDE	21	18	15	11	21		15	26		33	21	19
	TDE	70	57	50	37	51		63	74		14	56	60
	DDT	35	30	24	22	22		22	27		T	23	28
	Dieldrin	17	18	22	14	—		21	14		13	—	14
1972	DDE	—	14	17	15	10	14						
	TDE	T	33	40	36	20	41						
	DDT	—	20	15	18	—	15						
	Dieldrin	—	13	15	12	—	60						
STATION 5.—NISSEQUOGUE RIVER—*M. EDULIS*, UNLESS OTHERWISE INDICATED—74 SAMPLES [1]													
1966	DDE			21	21	24	18	T	19	17	22	42	40
	TDE			33	47	45	49	17	43	38	48	76	59
	DDT			23	37	50	34	13	30	29	22	20	30
	Dieldrin			16	20	23	—	—	—	—	—	31	17
1967	DDE	—	23	24	21	23	21	18	14	20	19	17	18
	TDE	33	53	59	50	18	51	42	38	49	42	35	44
	DDT	13	27	29	33	45	42	33	32	33	27	18	32
	Dieldrin	13	17	27	27	27	22	18	19	15	15	14	17
1968	DDE	14	16	13	17	17	13	11	—	[5] —	[5] —	[5] —	[5] —
	TDE	32	33	36	40	44	49	44	31	—	—	—	T
	DDT	20	18	21	23	42	51	47	25	—	—	—	—
	Dieldrin	14	18	16	18	—	—	—	—	—	—	—	12
1969	DDE	[6] —	[5] T	[3] T	12	[3] T	[3] —	[3] 15	[6] —	10	14	18	20
	TDE	—	T	14	30	12	T	22	T	34	35	49	42
	DDT	—	—	T	22	11	T	14	—	17	30	36	25
	Dieldrin	T	T	T	16	12	117	—	T	24	21	20	20
1970	DDE	16	17	[3] T	—	21	14	18	15	[3] T	14	11	[3] T
	TDE	37	44	18	16	46	37	48	46	17	38	29	14
	DDT	25	33	T	T	38	26	34	30	—	22	17	—
	Dieldrin	27	17	10	16	23	20	24	25	T	24	12	T
1971	DDE	14	T	[3] T	[3] —	[3] —		10	[3] 17		[3] 14	11	[3] —
	TDE	30	16	17	15	12		38	T		10	34	T
	DDT	24	12	T	T	—		20	—		—	13	—
	Dieldrin	14	T	17	20	T		27	—		13	10	—

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 5.—NISSEQUOGUE RIVER—*M. EDULIS*, UNLESS OTHERWISE INDICATED—74 SAMPLES [1]—Continued													
1972	DDE	[3] —	15	[3] T	[3] —	T	T						
	TDE	12	33	12	T	20	14						
	DDT	—	18	T	—	10	T						
	Dieldrin	10	13	T	—	T	12						
STATION 6.—CONSCIENCE BAY—*M. EDULIS*, UNLESS OTHERWISE INDICATED—73 SAMPLES [1]													
1966	DDE			[3] T	[3] —	[5] —	[5] 18	[5] —	[5] —	20	21	24	[6] 17
	TDE			18	—	—	26	—	—	35	34	46	40
	DDT			15	12	—	15	—	—	22	22	30	17
	Dieldrin			—	12	—	—	—	—	—	—	25	—
1967	DDE	18	23	18	18	20	22	21	21	20	18	17	16
	TDE	48	43	33	34	42	44	48	44	36	31	29	31
	DDT	24	23	18	21	29	41	36	35	24	21	18	20
	Dieldrin	17	20	20	30	24	22	21	16	13	15	14	13
1968	DDE	14	16	14	15	T	12	[5] —	[5] —	[5] —	[5] —	[9] —	[9] —
	TDE	23	24	22	27	24	24	—	—	—	—	—	—
	DDT	14	13	17	19	21	21	—	—	—	—	—	—
	Dieldrin	15	14	14	14	—	—	—	—	—	—	—	T
1969	DDE	[5] —	[5] —	[5] —	16	15	[6] 16	[8] 18	[6] 14	[8] 13	17	23	21
	TDE	—	—	—	23	24	36	22	20	14	29	39	36
	DDT	—	—	—	14	32	48	20	—	T	15	26	17
	Dieldrin	—	T	T	14	15	—	T	—	15	26	18	13
1970	DDE		17	22	[8] —	25	21	24	26	[8] —	24	23	18
	TDE		37	37	11	49	45	59	59	17	46	37	26
	DDT		25	36	—	36	29	28	27	—	24	15	14
	Dieldrin		16	19	20	26	20	23	23	T	16	T	10
1971	DDE	[3] T	[3] T	[3] —	16	[3] 10		[6] T	[3] —	[3] —	T	12	[5] T
	TDE	12	15	13	29	15		24	13	13	16	23	11
	DDT	T	10	—	13	—		10	—	T	T	11	—
	Dieldrin	T	14	22	18	—		10	10	17	T	T	11
1972	DDE	[9] —		—	[5] —	—	13						
	TDE	10		18	T	11	21						
	DDT	—		14	—	—	—						
	Dieldrin	—		T	—	75	49						

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 7.—SOUTHOLD—*C. VIRGINICA*, UNLESS OTHERWISE INDICATED—34 SAMPLES [1]													
1969	DDE				[6] 22	[5] T	[5] —	[6] 22		[5] T	[5] T		[6] 20
	TDE				48	11	T	38		14	T		41
	DDT				28	—	—	89		—	—		63
	Dieldrin				—	78	T	—		—	—		T
1970	DDE		[6] 16	29	27	27	[5] 10	21	19		17	22	20
	TDE		32	30	32	35	13	18	19		21	21	21
	DDT		21	26	23	22	—	22	23		18	15	12
	Dieldrin		—	21	20	26	T	11	27		14	15	14
1971	DDE	[5] T	17	17	18	[5] —		10	[5] —	[5] —	[5] —	[5] 15	[5] —
	TDE	16	19	19	17	—		10	T	T	—	18	16
	DDT	—	14	11	12	—		13	—	—	—	13	—
	Dieldrin	21	12	14	16	14		—	—	12	—	T	T
1972	DDE	16	17	18	16	[5] 10	21						
	TDE	27	17	22	21	18	28						
	DDT	16	T	T	11	—	31						
	Dieldrin	14	11	T	10	T	39						
STATION 8.—FLANDERS BAY—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—69 SAMPLES [1]													
1966	DDE			56	28	22	24	—	11	T	T	10	17
	TDE			54	25	21	44	—	23	14	—	15	23
	DDT			89	29	15	—	—	—	—	—	—	—
1967	DDE	15	18	25	23	[3] 23	[3] 20	17	17	16	17	16	15
	TDE	20	27	42	32	58	53	46	47	36	42	37	33
	DDT	—	—	—	—	12	12	—	T	—	—	—	—
1968	DDE	13	10	12	17	10	19	10	T	10	—	T	10
	TDE	25	17	25	41	24	59	44	38	38	18	39	34
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	T	12	[6] 33	15	14	10	12	[6] 20	—		13
	TDE	12	32	25	84	33	28	19	23	49	T		28
	DDT	—	—	—	27	—	—	—	—	75	—		—
	Dieldrin	—	—	—	—	17	107	11	—	14	93		—
1970	DDE			14	[6] T	13	[3] 14	[3] 14			[3] T	T	[5] T
	TDE			26	22	30	29	29			19	T	14
	DDT			—	12	—	—	T			—	—	—
	Dieldrin			11	—	—	—	T			T	—	—

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 8.—FLANDERS BAY—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—69 SAMPLES [1]—Continued													
1971	DDE	—	T	—	T	[6] 16		[3] —	T		13	[3] —	—
	TDE	10	16	—	16	50		16	12		24	—	11
	DDT	—	—	—	—	15		—	—		—	—	—
	Dieldrin	10	T	12	13	T		—	—		T	—	—
1972	DDE	T	—	—	—	—	—						
	TDE	12	—	—	10	T	—						
	DDT	—	—	—	—	—	—						
	Dieldrin	T	—	—	—	—	—						
STATION 9.—MECOX BAY—*C. VIRGINICA*, UNLESS OTHERWISE INDICATED—67 SAMPLES [1]													
1966	DDE			300	120	[3] 22	[3] T	[3] 27	[3] 21	[3] —	46	42	83
	TDE			240	120	41	13	45	29	—	46	37	83
	DDT			56	22	20	—	19	—	—	12	—	—
1967	DDE	63	67	77	T	[3] 37	190	[3] 15	49	64	69	93	110
	TDE	66	60	62	11	67	180	22	52	74	74	100	73
	DDT	—	—	—	—	29	27	T	18	17	11	15	T
1968	DDE		100	53	130	150	130	68	32	34	37	75	30
	TDE		81	48	87	120	85	48	29	18	32	50	18
	DDT		—	—	20	38	48	T	—	—	—	—	—
1969	DDE	44	62	26	60	[3] 20	[3] 21	[3] 19	[3] T	[3] 10	[3] —	[3] 12	
	TDE	31	45	20	39	31	29	21	10	10	—	23	
	DDT	—	—	—	T	24	10	—	—	—	—	—	
	Dieldrin	—	—	—	—	12	—	—	22	—	—	—	
1970	DDE		[3] 19	[3] 21	16	32	[2] 16	21		22	36	[3] T	[3] T
	TDE		27	27	14	45	36	25		38	48	10	12
	DDT		52	49	10	—	32	T		13	13	—	—
	Dieldrin		—	—	—	T	10	—		—	16	—	10
1971	DDE			30	41	34		T	14	37			35
	TDE			24	32	34		10	15	40			46
	DDT			—	T	T		—	—	15			T
	Dieldrin			—	12	T		—	10	19			—
1972	DDE	45	37	26	34	26	35						
	TDE	52	39	26	40	27	38						
	DDT	13	T	—	T	—	12						
	Dieldrin	10	T	10	—	T	—						

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 10.—SHINNECOCK BAY—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—73 SAMPLES													
1966	DDE			—	—	—	[3] T	—	T	—	—	—	T
	TDE			—	—	—	T	—	14	—	—	—	13
	DDT			—	—	—	—	—	—	—	—	—	—
1967	DDE	T	T	T	59	T	T	—	T	—	T	—	—
	TDE	12	12	12	50	T	T	—	T	—	T	—	—
	DDT	—	—	—	—	—	T	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	10	—	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	—	T	[3] 20	—	—	[2]12	[2] 14	[3] 10	[3] —	[2] 13
	TDE	—	T	—	T	38	T	T	21	20	16	—	24
	DDT	—	—	—	—	21	—	—	12	T	T	—	22
	Dieldrin	—	—	—	—	14	T	—	—	T	46	—	—
1970	DDE	[2] 11	[3] —	[3] —	[3] —	[3] 10	[2] 18	[2] 10	[2] 12		[3] —	[3] —	[3] T
	TDE	18	13	T	—	19	34	18	22		—	T	11
	DDT	—	—	—	—	T	21	24	20		T	—	—
	Dieldrin	14	—	12	—	—	12	T	T		12	—	—
1971	DDE		[2] 10	[2] T	[3] —	[3] —		[3] —	—	—	[3] —	[3] —	[3] —
	TDE		17	18	10	10		—	—	T	—	—	T
	DDT		12	14	T	—		—	—	—	—	—	—
	Dieldrin		10	10	—	—		—	44	18	T	T	—
1972	DDE	[3] —	[3] —	[3] —	[2] 11	[2] —	[2] 11						
	TDE	T	T	—	22	11	165						
	DDT	—	—	—	12	T	12						
STATION 11.—MORICHES BAY—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—71 SAMPLES [1]													
1966	DDE			—	—	—	T	—	—	—	—	[3] 15	T
	TDE			—	—	—	10	—	—	—	—	33	14
	DDT			—	—	—	—	—	—	—	—	—	—
1967	DDE	T	T	T	T	T	T	T	T	T	T	T	—
	TDE	13	14	18	13	18	17	10	T	17	T	18	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1968	DDE	T	—	T	T	—	—	—	—	—	—	—	—
	TDE	10	—	14	T	—	—	—	T	—	12	T	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	—	—	—	—	—	—	—	—		—
	TDE	—	11	—	—	13	—	T	T	—	—		T
	DDT	—	—	—	—	—	—	—	—	—	24		—

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 11.—MORICHES BAY—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—71 SAMPLES [1]—Continued													
1970	DDE		—	T	—	T	T	[3] T	[2] 20		[2] 25	[2] 25	[2] 13
	TDE		T	13	—	14	20	21	40		29	19	13
	DDT		—	T	—	—	—	11	23		17	17	T
	Dieldrin		—	—	—	—	T	—	—		14	T	—
1971	DDE		T	[2] 20	[2] 18	—		—	—	—	[2] 22	[2] 10	[2] 21
	TDE		10	27	25	11		—	—	T	15	26	34
	DDT		—	15	14	—		—	—	—	17	T	25
	Dieldrin		T	T	16	—		—	17	18	—	T	T
1972	DDE	[2] 17	[2] 16	[2] 17	[2] 20	[2] 17	[2] 25						
	TDE	27	20	27	26	13	33						
	DDT	19	T	—	15	—	22						
	Dieldrin	T	—	—	T	—	49						
STATION 12.—BELLPORT BAY—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—71 SAMPLES [1]													
1966	DDE			57	—	—	12	T	T	T	14	16	10
	TDE			44	—	10	27	18	13	16	28	30	20
	DDT			31	—	—	T	—	—	—	—	—	—
1967	DDE	20	21	24	19	12	12	11	10	15	T	13	14
	TDE	42	40	45	36	30	31	25	19	26	20	22	27
	DDT	—	—	—	—	T	—	—	T	—	—	—	—
1968	DDE	15	13	15	30	10	T	T	—	T	—	—	—
	TDE	28	24	27	50	23	14	12	—	T	—	—	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	T	—	[6] 25	T	—	T	—	—	—	—	
	TDE	—	10	—	50	10	15	18	—	—	—	—	
	DDT	—	—	—	33	—	—	—	—	—	—	—	
1970	DDE			T	—	11	10	T	T	—	—	—	
	TDE			13	—	26	17	21	17	—	11	—	
	DDT			—	—	—	T	—	—	—	—	—	
	Dieldrin			—	—	—	T	—	—	37	T	—	
1971	DDE	—	T	T	—	—		—	—	—	—	—	—
	TDE	10	12	11	13	10		—	—	—	—	—	T
	DDT	—	—	—	—	—		—	—	—	—	—	—
	Dieldrin	—	—	12	T	T		T	—	10	—	—	53
1972	DDE	—	—	—	—	—	T						
	TDE	—	T	T	T	—	—						
	DDT	—	—	—	—	—	—						
	Dieldrin	—	16	—	—	—	—						

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 13.—SAYVILLE—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—74 SAMPLES [1]													
1966	DDE			—	—	—	T	—	—	—	—	—	T
	TDE			—	—	T	10	—	—	—	—	—	10
	DDT			—	—	T	T	—	—	—	—	—	—
1967	DDE	T	T	11	T	T	T	T	T	T	—	T	T
	TDE	14	19	24	16	24	14	T	10	T	—	16	19
	DDT	T	—	—	—	T	—	—	T	—	—	—	—
1968	DDE	—	—	—	—	—	—	T	—	—	—	—	—
	TDE	—	—	—	—	—	—	T	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	—	—	—	—	—	—	—	—	[4] 11	T
	TDE	—	—	—	—	—	—	—	—	T	T	24	13
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	—	—	—	—	—	59	—	19	12
1970	DDE	—	—	—	—	—	T	—	T	T	10	—	T
	TDE	T	13	11	—	T	16	T	14	16	12	T	20
	DDT	—	—	—	—	—	T	10	—	T	T	—	10
	Dieldrin	—	T	—	—	—	T	—	—	—	T	10	T
1971	DDE	T	—	—	—	—		—	—		—	—	—
	TDE	12	T	11	11	10		—	—		—	—	16
	DDT	—	—	—	—	—		—	—		—	—	12
	Dieldrin	T	T	—	—	—		13	—		41	T	11
1972	DDE	[4] 22	[4] 22	—	17	—	—						
	TDE	56	38	—	16	—	—						
	DDT	29	T	T	10	—	T						
	Dieldrin	15	—	—	11	—	—						
STATION 14.—AMITYVILLE—*M. MERCENARIA*—73 SAMPLES [1]													
1966	DDE			—	—	—	—	—	—	—	—	T	T
	TDE			—	—	—	18	—	—	—	—	10	17
	DDT			—	—	—	—	—	—	—	—	—	—
1967	DDE	T	T	T	T	T	T	—	—	—	—	T	—
	TDE	20	15	16	16	18	14	17	13	T	11	14	—
	DDT	T	—	—	—	T	—	—	T	—	—	—	—
1968	DDE	—	—	—	T	—	—	T	—	T	—	—	—
	TDE	—	—	—	13	—	—	11	T	T	—	13	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	—

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 14.—AMITYVILLE—*M. MERCENARIA*—73 SAMPLES [1]—Continued													
1969	DDE	—	—	—	—	—	—	—	—	—	—	20	—
	TDE	—	T	—	T	14	—	T	—	11	T	11	—
	DDT	—	—	—	—	—	—	—	—	—	T	33	—
	Dieldrin	—	—	—	—	T	—	—	—	T	—	T	T
1970	DDE		—	—	—	10	T	T	T	—	—	—	—
	TDE		11	—	T	20	19	15	15	10	T	T	10
	DDT		—	—	—	11	—	—	—	—	—	—	—
	Dieldrin		—	T	—	T	—	—	—	—	18	—	T
1971	DDE		—	—	—	—		—	—	—	—	—	—
	TDE		11	12	T	16		—	T	T	10	—	10
	DDT		—	—	—	—		—	—	T	—	—	—
	Dieldrin		—	—	10	10		—	—	T	—	42	—
1972	DDE	T	—	—	—	—	—						
	TDE	16	T	T	T	—	—						
	DDT	T	—	—	—	—	—						
	Dieldrin	11	—	—	—	—	—						
STATION 15.—EAST BAY—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—57 SAMPLES [1]													
1966	DDE			—	—	—	—	—	—	—	—	T	T
	TDE			—	—	T	—	—	—	—	—	18	19
	DDT			—	—	T	—	—	—	—	—	T	—
1967	DDE	T	—	T	T	—	T	—	—	—	T	T	T
	TDE	21	12	13	15	19	19	15	T	T	13	T	14
	DDT	T	—	—	—	T	T	T	T	—	—	—	T
1968	DDE			T	T	T	—	—	—	—	—	—	—
	TDE			16	10	12	—	—	T	—	12	—	T
	DDT			—	—	—	—	—	—	—	—	—	—
1969	DDE	—	—	—	—	—	—	—	—	—	—		T
	TDE	16	10	—	—	16	14	14	T	T	T		16
	DDT	—	—	—	—	—	—	—	—	—	T		—
	Dieldrin	—	—	—	—	T	10	16	17	T	14		14
1970	DDE	—		—	—							[2] 10	
	TDE	T		12	T							21	
	DDT	—		—	—							18	
	Dieldrin	—		T	—							16	

TABLE J-2.—*Distribution of organochlorine residues in the monitored species for each sampling station by date of collection—New York*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 15.—EAST BAY—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—57 SAMPLES [1]—Continued													
1971	DDE		[2] 12		[6] T	[2] 18		[3] —				—	T
	TDE		29		21	57		19				15	26
	DDT		29		11	23		12				10	23
	Dieldrin		15		11	38		11				—	—
1972	DDE	—			[3] T	[6] —	[6] —						
	TDE	T			14	T	—						
	DDT	—			T	—	—						
STATION 16.—WEST BAY—*M. MERCENARIA*, UNLESS OTHERWISE INDICATED—57 SAMPLES [1]													
1966	DDE				—	T	—	—	—	—	—	T	T
	TDE				—	15	—	—	13	—	—	26	22
	DDT				—	T	—	—	—	—	—	14	13
1967	DDE	T	T	T	—	T	10	T	T	—	T	T	T
	TDE	20	17	24	24	27	32	—	17	T	20	14	19
	DDT	13	T	12	18	17	14	—	13	—	—	—	13
	Dieldrin	—	—	11	12	10	—	—	—	—	—	—	—
1968	DDE			—	T	T	T	—	—	—	—	—	—
	TDE			20	18	13	17	T	15	14	16	15	14
	DDT			—	T	—	—	—	—	—	—	—	—
1969	DDE	—	T	—	—	11	—	—	—	—	—		10
	TDE	19	20	T	—	28	13	18	19	14	21		21
	DDT	—	—	—	—	33	T	—	—	—	12		43
	Dieldrin	—	—	—	—	T	T	18	17	20	12		17
1970	DDE	—		T	—							[2] T	
	TDE	13		20	19							15	
	DDT	—		11	40							15	
	Dieldrin	—		14	T							T	
1971	DDE		[2] 11		[6] T	[2] 15			[6] —			—	10
	TDE		23		22	47			15			14	25
	DDT		28		12	44			—			T	20
	Dieldrin		T		10	16			—			T	10
1972	DDE			—	—	[6] 30	[4] 13						
	TDE	10		18	T	59	28						
	DDT	—		14	—	22	T						
	Dieldrin	—		T	—	—	—						

[1] Each sample represents 15 or more mature mollusks.
[2] *M. edulis*.
[3] *M. arenaria*.
[4] *C. virginica*.
[5] *M. mercenaria*.
[6] *M. demissus*.

SECTION K.—NORTH CAROLINA

The monthly collection of eastern oysters, *Crassostrea virginica,* to monitor pollution was initiated in July 1966 and continued until July 1972. During the program, 17 stations were sampled routinely for periods ranging from 3 to 6 years. All samples were analyzed by the Gulf Breeze Laboratory.

Approximate station locations are shown in Fig. K-1. A summary of data on organochlorine residues in the monitored species, *C. virginica,* is presented in Table K-1, and the distribution of residues in this species for each sampling station by date of collection in Table K-2.

North Carolina samples are noteworthy for the continuity of collections of a single species of mollusk at short intervals over a relatively long period of time. For this reason the data present a good picture of annual and seasonal trends of a persistent synthetic pollutant in this estuarine environment.

The incidence of DDT residues (75%) and maximum magnitude (566 ppb) are about the median of the 15 States monitored. The 1% incidence of dieldrin residues was somewhat lower than most other states. PCB compounds were not detected.

Although there are exceptions from one estuary to another, the magnitude of DDT residues in oysters showed little seasonal variation during the period 1967-69 when maximum levels of DDT pollution were detected. The overall decline in DDT residues (Part I. Table 7 and Fig. 2) is notable and undoubtedly associated with the decreased agricultural use of this chemical in North Carolina.

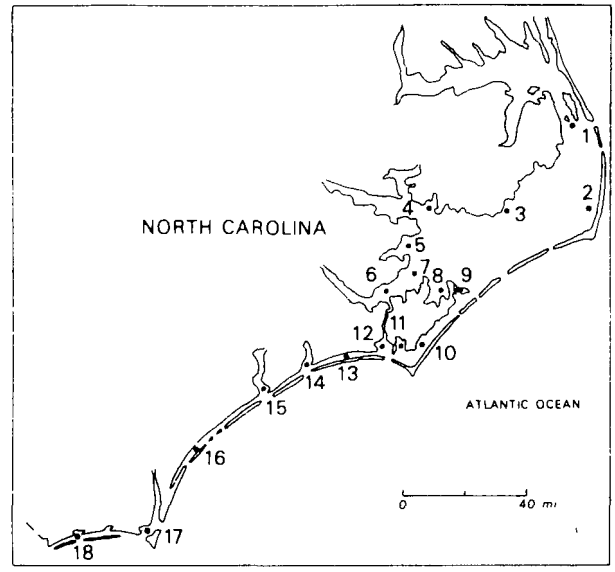


FIGURE K-1.—*Diagram of coastal North Carolina showing approximate location of monitoring stations*

1. Wanchese—Croatan Sound
2. Salvo—Pamlico Sound
3. Wysocking Bay
4. Rose Bay
5. Bay River
6. Neuse River
7. Point of Marsh—Neuse River
8. West Bay
9. Back Bay—Core Sound
10. Jarrett Bay—Core Sound
11. North River
12. Newport River
13. Bogue Sound
14. White Oak River
15. New River
16. Wrightsville Beach—Wrightsville Sound
17. Southport—Cape Fear River
18. Shallotte River

TABLE K-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1966-72—North Carolina*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)	
				DDT	Dieldrin
1 *	Wanchese	1966-72	72	49 (264)	
2 **	Salvo	1966-72	71	58 (566)	
3	Wysocking Bay	1966-70	43	35 (64)	
4 *	Rose Bay	1966-72	71	46 (121)	3 (14)
5 *	Bay River	1966-72	71	69 (310)	2 (12)
6	Neuse River	1966-70	43	43 (176)	
7 *	Point of Marsh	1966-72	71	53 (139)	2 (19)
8	West Bay	1967-72	58	34 (74)	
9	Back Bay	1966-67	9	8 (103)	
10 *	Jarrett Bay	1966-72	66	42 (106)	
11 *	North River	1966-72	64	48 (172)	2 (10)
12 *	Newport River	1966-72	68	54 (121)	3 (13)

TABLE K-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1966-72—North Carolina*—Continued

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (µg/kg)	
				DDT	Dieldrin
13	Bogue Sound	1967-72	51	33 (71)	
14	White Oak River	1966-70	43	30 (60)	
15 *	New River	1966-72	72	61 (118)	
16	Wrightsville Beach	1966-70	43	35 (57)	
17 *	Southport	1966-72	72	32 (116)	
18	Shallotte River	1966-70	43	38 (51)	
Total number of samples			1,031		
Percent of samples positive for indicated compound				75	1

* Data from these stations summarized in Part I. Table 7. and Fig. 2.
[1] Each sample represents 15 or more mature mollusks.

TABLE K-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—North Carolina*

[Blank = no sample collected; — = no residue detected above 5 ppb; T = >5 but <10 ppb]

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—WANCHESE—72 SAMPLES [1]													
1966	DDE							19	20	12	22	20	20
	TDE							12	17	T	17	18	32
	DDT							13	—	T	22	—	11
1967	DDE	14	25	28	16	26	21	21	19	20	24	43	28
	TDE	16	20	32	17	32	31	35	15	15	11	57	10
	DDT	15	10	17	—	29	21	64	17	13	17	64	53
1968	DDE	140	140	85	78	62	T	30	—	43	35	29	21
	TDE	56	51	59	16	32	T	13	—	22	27	16	15
	DDT	68	29	37	42	86	—	12	—	60	49	57	—
1969	DDE	40	17	13	10	37	11	T	10	—	T	T	12
	TDE	15	16	18	10	44	21	21	10	—	T	13	15
	DDT	T	T	11	—	43	T	19	13	—	—	—	T
1970	DDE	T	T	13	T	17	—	—	—	—	—	—	—
	TDE	11	T	15	—	19	—	—	—	—	11	—	—
	DDT	—	—	T	—	—	—	—	—	—	—	—	—
1971	DDE	—	—	—	—	—	T	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1972	DDE	—	—	—	T	—	T						
	TDE	—	—	—	—	—	10						
	DDT	—	—	—	—	—	—						

TABLE K-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—North Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		STATION 2.—SALVO—71 SAMPLES [1]											
1966	DDE							12	14	14	14	32	23
	TDE							—	17	—	17	28	17
	DDT							—	—	—	—	28	15
1967	DDE	26	24	31	35	24	21	17	24		120	74	87
	TDE	11	13	21	25	21	30	21	19		56	45	29
	DDT	T	T	T	11	19	21	29	44		390	190	66
1968	DDE	35	85	66	85	65	34	58	28	95	45	58	66
	TDE	16	26	20	24	37	29	38	21	40	33	37	35
	DDT	20	50	36	21	28	19	87	36	240	76	120	80
1969	DDE	100	73	100	59	120	39	27	—	15	13	10	15
	TDE	31	31	38	27	51	38	37	—	21	15	19	13
	DDT	87	35	50	22	67	16	28	—	T	T	—	11
1970	DDE	23	34	24	19	27	14	—	12	11	—	10	19
	TDE	25	27	20	16	33	20	—	17	—	—	—	16
	DDT	14	20	13	10	19	—	—	14	—	—	—	11
1971	DDE	16	15	—	17	21	—	12	—	—	—	—	—
	TDE	17	16	—	18	21	—	14	—	—	—	—	—
	DDT	T	—	—	—	11	—	—	—	—	—	—	—
1972	DDE	—	—	—	T	13	13						
	TDE	—	—	—	—	T	22						
	DDT	—	—	—	—	—	—						
		STATION 3.—WYSOCKING BAY—43 SAMPLES [1]											
1966	DDE							17	—	17	T	T	T
	TDE							17	—	18	13	12	11
	DDT							—	—	20	—	—	—
1967	DDE	T	14	15	17	16	12	15	T	—	—	T	10
	TDE	12	17	17	22	29	22	35	T	—	—	T	15
	DDT	T	10	—	—	14	13	14	T	—	—	T	T
1968	DDE	T	T	T	18	13	15	—	T	T	—	T	—
	TDE	T	T	—	20	20	13	—	T	T	—	13	—
	DDT	—	—	—	—	T	13	—	—	21	—	—	—
1969	DDE	T	-	T	12	T	T	T	T	--	T	T	14
	TDE	14	--	—	16	T	T	T	--	--	T	12	19
	DDT	—	—	—	T	—	—	T	12	—	—	—	12
1970	DDE	T											
	TDE	T											
	DDT	T											

TABLE K-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—North Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		STATION 4.—ROSE BAY—71 SAMPLES[1]											
1966	DDE							28	16	19	18	16	20
	TDE							34	13	22	23	23	36
	DDT							21	—	32	16	T	14
1967	DDE	12	T	23	15	16	15	35	T	T	—	14	12
	TDE	18	14	32	26	32	30	63	15	10	—	30	15
	DDT	T	T	T	11	16	40	23	13	T	—	19	T
1968	DDE	T	T	13	15	21	14	T	—	T	T	T	16
	TDE	T	T	12	15	27	25	T	—	T	17	17	17
	DDT	—	—	—	22	13	T	—	—	T	T	T	29
1969	DDE	11	16	11	T	12	T	T	T	12	T	—	10
	TDE	15	18	12	—	17	T	T	13	17	T	—	14
	DDT	20	—	—	—	T	—	T	69	—	—	—	T
1970	DDE	17	13	14	T	T		11	—	—	—	—	—
	TDE	19	19	19	—	15		19	—	—	—	—	—
	DDT	15	10	10	—	—		12	—	—	—	—	—
	Dieldrin	—	—	14	—	—		—	—	—	—	—	—
1971	DDE	—	—	—	—	T	—	—	—	—	—	—	—
	TDE	—	—	—	—	13	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	10	—	12	—	—	—	—	—	—	—
1972	DDE	—	—	—	—	—	—						
	TDE	—	—	—	—	—	—						
	DDT	—	—	—	—	—	—						
		STATION 5.—BAY RIVER—71 SAMPLES[1]											
1966	DDE							36	55	52	23	30	26
	TDE							36	78	73	46	61	60
	DDT							29	25	34	19	11	20
1967	DDE	36	30	81	39	32	29	22	15	T	28	25	24
	TDE	69	49	100	56	65	56	46	48	T	43	34	46
	DDT	23	16	35	20	27	19	27	27	—	51	—	16
1968	DDE	35	44	37	52	75	22	18	23	13	T	24	16
	TDE	35	43	24	49	71	33	24	28	15	—	36	21
	DDT	26	32	28	47	55	T	13	18	12	—	29	T
1969	DDE	19	29	36	43	54	45	37	18	T	13	T	20
	TDE	25	39	36	56	59	80	71	16	T	T	20	35
	DDT	T	20	37	37	32	17	26	18	14	—	—	21

TABLE K-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—North Carolina*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 5.—BAY RIVER—71 SAMPLES[1]—Continued													
1970	DDE	24	40	36	42	55	10	15	11	13	—		23
	TDE	33	43	29	31	94	27	23	12	22	16		39
	DDT	17	33	18	14	22	—	18	—	14	—		13
1971	DDE	12	16	—	22	110	16	52	49	18	17	13	T
	TDE	15	13	—	21	170	19	97	96	34	26	11	—
	DDT	—	10	—	—	30	—	27	22	—	18	12	—
	Dieldrin	—	—	—	—	10	—	—	—	—	—	—	—
1972	DDE	16	41	—	71	85	43						
	TDE	11	37	—	48	130	91						
	DDT	—	21	—	37	87	43						
	Dieldrin	—	—	—	—	12	—						
STATION 6.—NEUSE RIVER—43 SAMPLES[1]													
1966	DDE							29	18	32	36	24	29
	TDE							46	24	48	57	55	60
	DDT							13	15	49	30	13	18
1967	DDE	17	16	24	29	21	16	19	14	20	25	24	24
	TDE	29	28	41	50	42	30	37	33	32	47	49	46
	DDT	—	T	T	T	T	16	25	14	14	13	T	T
1968	DDE	19	20	15	30	49	32	26	29	13	T	19	25
	TDE	24	25	15	44	110	68	42	56	22	T	32	25
	DDT	11	T	—	27	17	15	19	39	T	—	T	20
1969	DDE	20	17	23	16	37	29	30	13	T	10	T	16
	TDE	17	13	26	23	56	75	40	23	T	17	19	35
	DDT	10	T	11	—	20	21	20	11	—	—	—	16
1970	DDE	27											
	TDE	45											
	DDT	17											
STATION 7.—POINT OF MARSH—71 SAMPLES[1]													
1966	DDE							33	26	15	29	16	20
	TDE							37	26	20	31	33	40
	DDT							38	28	25	19	16	16
	Dieldrin							T	—	—	—	—	—
1967	DDE	15	17	22	20	20	33	13	22	11	12	11	18
	TDE	29	24	28	32	39	82	26	24	19	19	18	25
	DDT	15	10	—	11	45	24	27	33	10	27	15	10

TABLE K-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—North Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 7.—POINT OF MARSH—71 SAMPLES [1]—Continued													
1968	DDE	11	—	12	18	11	15	10	15	—	T	14	11
	TDE	15	—	18	23	18	20	13	19	—	T	21	16
	DDT	T	—	—	21	10	16	11	21	—	—	—	—
1969	DDE	13	T	12	T	T	T	10	37	—	T	T	T
	TDE	14	14	17	13	T	11	16	27	—	T	11	10
	DDT	—	T	T	—	—	—	18	48	—	T	12	—
	Dieldrin	—	—	—	—	—	—	—	19	—	—	—	—
1970	DDE	11	22	20	T	T		—	—	—	—	T	—
	TDE	21	29	21	12	17		—	—	—	—	T	—
	DDT	T	15	—	—	—		—	—	—	—	—	—
1971	DDE	—	T	T	T	T	—	—	—	—	—	—	T
	TDE	—	T	T	32	—	—	—	—	—	—	—	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	T
1972	DDE	11	—	—	T	T	—						
	TDE	20	—	—	—	T	—						
	DDT	—	—	—	—	—	—						
STATION 8.—WEST BAY—58 SAMPLES [1]													
1967	DDE		18	25			22	14	T	—	16	T	15
	TDE		25	39			22	19	T	—	22	10	24
	DDT		T	11			13	11	T	—	17	12	15
1968	DDE	T	11	25	T	16	11	—	T	—	T	—	10
	TDE	—	11	30	T	13	T	—	T	—	—	—	16
	DDT	—	—	19	—	T	—	—	15	—	—	—	—
1969	DDE	16	T	20	16	11	T	12	—	—	—	T	T
	TDE	10	T	17	19	T	12	16	—	—	—	16	17
	DDT	T	—	12	12	10	T	T	—	—	—	—	T
1970	DDE	—				—	—	—	—	—	—	T	T
	TDE	—				—	—	—	—	—	—	T	16
	DDT	—				—	—	—	—	—	—	—	—
1971	DDE	—	T	—	—	T	—	—	—	—	T	—	
	TDE	—	T	—	—	—	—	—	—	—	T	—	
	DDT	—	—	—	—	—	—	—	—	—	T	—	
1972	DDE		—	13	14	11	—						
	TDE		—	10	T	T	—						
	DDT		—	—	—	—	—						

TABLE K-2.—*Distribution of organochlorine residues in C. virginica for each sampling station by date of collection—North Carolina*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 9.—BACK BAY—9 SAMPLES[1]													
1966	DDE							10	—	15	17	24	10
	TDE							12	—	11	32	74	14
	DDT							14	—	12	15	T	—
1967	DDE	10			26	10							
	TDE	10			23	13							
	DDT	—			17	T							
STATION 10.—JARRETT BAY—66 SAMPLES[1]													
1966	DDE							22	—	13	14	T	12
	TDE							22	—	11	16	T	18
	DDT							15	—	19	16	—	14
1967	DDE	11	17	14	29	19	17	10	12	12	14	10	21
	TDE	T	12	19	24	22	18	12	T	T	18	17	17
	DDT	—	T	—	17	14	10	T	12	T	39	13	12
1968	DDE	18	13	47	T	10	T	—	13	T	18	—	T
	TDE	14	10	44	T	—	—	—	T	T	13	—	15
	DDT	T	T	15	—	—	—	—	T	T	66	—	T
1969	DDE	12	T	12	21	13	T	10	—	—	—	10	12
	TDE	T	11	16	22	12	10	15	—	—	—	18	22
	DDT	—	T	T	—	T	—	—	—	—	—	—	11
1970	DDE	T				—	—	—	—	—	—	—	—
	TDE	T				—	—	—	—	—	—	—	—
	DDT	T				—	—	—	—	—	—	—	—
1971	DDE	—	T	—	—	11	—	—	—	—	T	—	
	TDE	—	T	—	—	10	—	—	—	—	T	—	
	DDT	—	—	—	—	—	—	—	—	—	T	—	
1972	DDE			—	12	T	—						
	TDE			—	—	—	—						
	DDT			—	—	—	—						
STATION 11.—NORTH RIVER—64 SAMPLES[1]													
1966	DDT							64	43	43	47	36	35
	TDE							50	33	41	47	33	29
	DDT							58	36	31	30	10	—
	Dieldrin							10	—	—	—	—	—
1967	DDE	27	26	45	11	52	53	34	21	16	17	10	20
	TDE	15	18	40	12	46	49	28	20	12	12	10	16
	DDT	10	16	27	—	29	31	15	26	T	10	—	T
	Dieldrin	—	—	10	—	—	—	—	—	—	—	—	—

TABLE K-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—North Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 11.—NORTH RIVER—64 SAMPLES [1]—Continued													
1968	DDE	18	14	T	35	34	T	32	21	T	22	38	27
	TDE	13	—	—	25	26	—	27	19	T	17	20	—
	DDT	T	—	—	43	36	—	57	26	T	36	27	—
1969	DDE	18	13	16	32	16	T	—	—	12	53	—	T
	TDE	T	—	T	15	12	—	—	—	T	73	—	T
	DDT	—	—	12	14	—	—	—	—	11	34	—	T
1970	DDE	T				11	—	—	—	—	—	—	T
	TDE	T				11	—	—	—	—	—	—	—
	DDT	T				—	—	—	—	—	—	—	—
1971	DDE	—		—	12	T	T	—	—	—	—		
	TDE	—		—	—	—	—	—	—	—	—		
	DDT	—		—	—	—	—	—	—	—	—		
1972	DDE			20	10	T	—						
	TDE			—	—	—	—						
	DDT			11	—	—	—						
STATION 12.—NEWPORT RIVER—68 SAMPLES [1]													
1966	DDE							20	17	14	16	24	14
	TDE							21	13	19	26	44	22
	DDT							—	T	T	—	11	—
	Dieldrin							T	—	—	—	—	—
1967	DDE	14	18	25	20	21	19	T	T	14	16	12	16
	TDE	21	24	85	27	29	30	12	15	25	18	17	26
	DDT	T	T	11	T	—	T	10	T	15	23	—	T
1968	DDE	11	16	17	25	—	T	T	T	11	18	19	15
	TDE	14	16	23	31	—	—	T	T	13	24	29	18
	DDT	—	—	T	—	—	—	—	—	T	23	11	T
1969	DDE	21	21	20	27	17	12	T	—	T	13	15	18
	TDE	21	22	21	29	23	19	T	—	T	16	15	17
	DDT	—	10	T	13	T	T	—	—	—	25	17	17
	Dieldrin	—	—	—	—	—	—	—	—	—	T	—	—
1970	DDE	16	12	12	18	T	—	T		—	—	T	
	TDE	14	T	10	23	T	—	—		—	—	T	
	DDT	13	—	T	10	—	—	—		—	—	—	
1971	DDE	—	11		—	13	—	—	17	—	—	—	—
	TDE	—	11		—	18	—	—	16	—	—	—	—
	DDT	—	—		—	—	—	—	—	—	—	—	—

TABLE K-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—North Carolina*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 12.—NEWPORT RIVER—68 SAMPLES [1]—Continued													
1972	DDE	—		30	T	T	T						
	TDE	—		24	—	—	—						
	DDT	—		36	—	—	—						
	Dieldrin	—		13	—	—	—						
STATION 13.—BOGUE SOUND—51 SAMPLES [1]													
1967	DDE			31	20	15	T	10	10	13	15	14	15
	TDE			26	23	17	T	12	12	14	—	13	T
	DDT			12	T	T	—	11	16	24	T	12	T
1968	DDE	12	T	16	15	T	—	T	T	13	13	21	18
	TDE	T	—	—	T	—	—	—	T	13	13	17	11
	DDT	—	—	—	—	—	—	—	T	21	T	13	11
1969	DDE	20	29	17	25	19	T	15	—	—	12	13	12
	TDE	15	23	11	20	19	11	16	—	—	14	17	—
	DDT	T	19	T	12	11	11	37	—	—	T	15	—
1970	DDE					—							
	TDE					—							
	DDT					—							
1971	DDE			—	—	T	—	—	—	—	—	—	—
	TDE			—	—	—	—	—	—	—	—	—	—
	DDT			—	—	—	—	—	—	—	—	—	—
1972	DDE	—	—	T	—	—	—						
	TDE	—	—	—	—	—	—						
	DDT	—	—	—	—	—	—						
STATION 14.—WHITE OAK RIVER—43 SAMPLES [1]													
1966	DDE							T	20	T	29	T	T
	TDE							—	25	T	31	T	T
	DDT							—	T	—	—	—	—
1967	DDE	—	—	T	—	T	—	T	—	T	T	T	T
	TDE	—	—	—	—	T	—	13	—	T	—	T	T
	DDT	—	—	—	—	—	—	10	—	T	—	—	—
1968	DDE	T	T	13	T	—	—	—	T	11	T	T	14
	TDE	T	T	13	T	—	—	—	T	11	—	T	T
	DDT	T	—	—	—	—	—	—	T	T	—	—	T
1969	DDE	T	12	14	T	T	—	—	—	—	T	T	—
	TDE	—	—	T	—	T	—	—	—	—	T	T	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—

TABLE K-2.—*Distribution of organochlorine residues in C. virginica for each sampling station by date of collection—North Carolina*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 14.—WHITE OAK RIVER—43 SAMPLES[1]—Continued													
1970	DDE	T											
	TDE	—											
	DDT	—											
STATION 15.—NEW RIVER—72 SAMPLES[1]													
1966	DDE							25	T	23	21	28	36
	TDE							21	T	27	26	34	44
	DDT							28	—	T	—	—	14
1967	DDE	39	16	30	45	20	16	11	19	16	28	21	28
	TDE	49	18	30	59	23	13	13	28	20	34	26	37
	DDT	16	T	T	14	—	—	—	11	—	—	—	21
1968	DDE	42	16	35	39	25	15	T	10	14	15	15	14
	TDE	38	14	31	29	25	15	—	T	14	12	12	T
	DDT	31	T	T	—	—	—	—	T	T	—	—	—
1969	DDE	25	18	21	28	13	15	11	13	14	19	23	27
	TDE	19	19	27	35	13	15	15	13	12	22	26	27
	DDT	—	T	T	T	—	—	—	T	—	11	—	11
1970	DDE	21	28	29	26	26	T	—	—	—	—	—	17
	TDE	19	24	22	31	20	—	—	—	—	—	—	—
	DDT	—	T	T	12	—	—	—	—	—	—	—	—
1971	DDE	11	T	15	T	T	—	—	—	—	12	—	20
	TDE	—	T	12	—	—	—	—	—	—	11	—	22
	DDT	—	—	—	—	—	—	—	—	—	T	—	T
1972	DDE	19	17	24	11	T	—						
	TDE	18	21	21	—	—	—						
	DDT	—	—	—	—	—	—						
STATION 16.—WRIGHTSVILLE BEACH—43 SAMPLES[1]													
1966	DDE							11	14	15	15	19	12
	TDE							12	18	12	16	24	10
	DDT							—	—	—	13	14	—
1967	DDE	11	16	19	14	T	T	T	10	—	T	—	11
	TDE	14	18	15	14	T	T	14	18	—	12	—	13
	DDT	—	T	T	—	T	—	T	14	—	22	—	T
1968	DDE	T	T	10	13	T	—	—	10	T	T	T	T
	TDE	T	—	—	10	T	—	—	—	—	—	12	—
	DDT	T	—	—	—	—	—	—	T	—	—	—	—

TABLE K-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—North Carolina*—Continued

YEAR	COMPOUND	RESIDUES IN PPB (μG/KG)											
		JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
STATION 16.—WRIGHTSVILLE BEACH—43 SAMPLES[1]—Continued													
1969	DDE	T	T	T	T	T	—	—	—	—	T	T	12
	TDE	—	—	—	T	T	—	—	—	—	T	11	16
	DDT	—	—	—	—	—	—	—	—	—	—	—	12
1970	DDE	T											
	TDE	—											
	DDT	—											
STATION 17.—SOUTHPORT—72 SAMPLES[1]													
1966	DDE							11	21	11	29	T	T
	TDE							T	16	10	25	T	T
	DDT							12	—	—	—	—	—
1967	DDE	T	13	11	T	—	T	10	14	11	T	—	T
	TDE	—	12	T	T	—	—	10	T	—	—	—	T
	DDT	—	T	—	—	—	—	17	30	15	—	—	—
1968	DDE	18	13	T	T	11	—	—	10	11	T	17	17
	TDE	13	T	T	T	14	—	—	—	T	11	—	T
	DDT	T	—	—	—	—	—	—	13	T	—	21	10
1969	DDE	T	—	12	T	11	—	—	—	—	—	—	—
	TDE	—	—	17	—	12	—	—	—	—	—	—	—
	DDT	—	—	87	—	T	—	—	—	—	—	—	—
1970	DDE	—	T	—	—	—	—	—	T	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1971	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1972	DDE	—	—	—	—	—	—						
	TDE	—	—	—	—	—	—						
	DDT	—	—	—	—	—	—						
STATION 18.—SHALLOTTE RIVER—43 SAMPLES[1]													
1966	DDE							—	T	T	T	19	T
	TDE							—	—	T	—	18	T
	DDT							—	—	—	—	11	—
1967	DDE	T	16	11	14	19	T	T	T	10	T	12	T
	TDE	—	13	T	15	22	T	11	T	T	—	11	T
	DDT	—	T	—	—	10	—	10	T	T	—	T	—

TABLE K-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—North Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 18.—SHALLOTTE RIVER—43 SAMPLES [1]—Continued													
1968	DDE	13	14	16	15	—	—	—	T	T	13	—	17
	TDE	11	10	11	12	—	—	—	—	—	T	—	10
	DDT	T	T	—	—	—	—	—	—	—	11	—	T
1969	DDE	T	T	12	11	15	T	14	T	T	11	T	T
	TDE	—	—	10	T	19	—	14	T	—	T	13	—
	DDT	—	—	19	—	T	—	16	16	12	T	—	—
1970	DDE	T											
	TDE	—											
	DDT	—											

[1] Each sample represents 15 or more mature mollusks.

SECTION L.—SOUTH CAROLINA

Monthly collections of eastern oysters, *Crassostrea virginica,* to identify estuarine pollution were made from August 1965 through November 1969. The 17 stations (Fig. L-1) were monitored for periods ranging from 1 to 5 years. All samples were analyzed at the Gulf Breeze Laboratory. A summary of data on organochlorine residues in the monitored species, *C. virginica,* is presented in Table L-1, and the distribution of residues in this species for each sampling station by date of collection in Table L-2.

South Carolina samples are characterized by the uniformly low level of DDT residues and moderately low incidence of positive samples. Samples from only three other States indicated generally lower levels of DDT contamination.

In those areas with adequate numbers of samples for annual comparison, there was an obvious decline at most stations in the magnitude and incidence of DDT residues in 1968-69 as compared to earlier years (Part I. Table 6).

South Carolina was the only State in which mirex residues were detected in mollusks. These residues were observed only in the period March May 1969. They were found at nine stations widely distributed along the South Carolina coast. Largest residues were found in samples collected in the Charleston area, i.e., Stations 8 and 9.

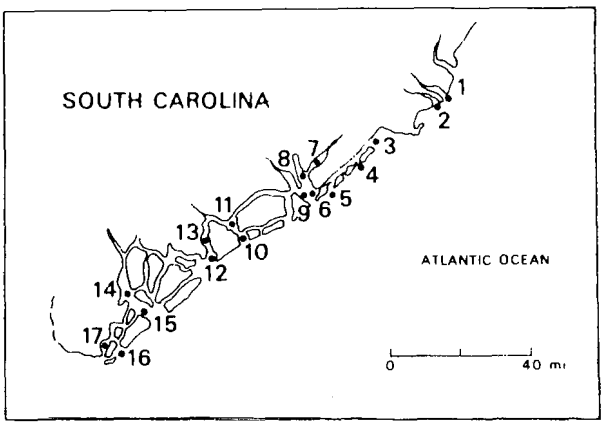


FIGURE L-1.—*Diagram of coastal South Carolina showing approximate location of monitoring stations*

1. North Santee Bay—Santee River
2. South Santee Bay—Santee River
3. Bull Creek
4. Price Creek
5. Inlet Creek
6. Hog Island Channel—Ashley, Cooper, and Wando Rivers
7. Wando River—Ashley, Cooper, and Wando Rivers
8. Ashley River—Ashley, Cooper, and Wando Rivers
9. Fort Johnson—Ashley, Cooper, and Wando Rivers
10. Steamboat Creek—North Edesto River
11. Toogoodoo Creek—North Edesto River
12. Big Bay Creek—South Edesto River
13. St. Pierre Creek—South Edesto River
14. Whale Branch—Broad River
15. Skull Creek—Broad River
16. May Creek
17. New River

TABLE L-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1965-69—South Carolina*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)					
				DDT		Dieldrin		Mirex	
1	North Santee Bay	1965-68	41	10	(19)	4	(19)		
2	South Santee Bay	1965-68	40	14	(80)	3	(19)		
3	Bull Creek	1969	12	2	(10)	2	(13)	2	(35)
4	Price Creek	1965-68	42	25	(81)	2	(12)		
5	Inlet Creek	1965-68	42	21	(52)				
6	Hog Island Channel	1965-68	41	33	(73)				
7	Wando River	1965-68	42	31	(44)				
8	Ashley River	1965-69	54	45	(154)	8	(90)	1	(190)
9	Fort Johnson	1969	12	4	(10)			1	(540)
10	Steamboat Creek	1965-69	54	26	(32)			1	(38)
11	Toogoodoo Creek	1965-69	53	40	(98)			1	(38)
12	Big Bay Creek	1965-69	54	32	(91)			1	(T)
13	St. Pierre Creek	1969	12	7	(88)			1	(38)
14	Whale Branch	1965-68	41	21	(79)				
15	Skull Creek	1965-68	39	12	(30)	1	(35)		
16	May Creek	1969	12	3	(15)	1	(11)	3	(37)
17	New River	1969	12	1	(16)	1	(21)	1	(27)
	Occasional stations (6)	1965-68	7	5	(201)	2	(15)		
Total number of samples			610						
Percent positive for indicated compound				54		4		2	

NOTE: T = >5 but <10 ppb.
[1] Each sample represents 15 or more mature mollusks.

TABLE L-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—South Carolina*

[Blank = no sample collected; — = no residue detected above 5 ppb; T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—NORTH SANTEE BAY—41 SAMPLES [1]													
1965	DDE							T	T	T	—	—	—
	TDE							T	T	T	—	—	—
	DDT							—	—	—	T	—	—
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—		—	T	T	T	—	—	—	—
	TDE	—	—	—		—	—	—	—	—	—	—	—
	DDT	—	—	—		—	—	T	T	—	—	—	—
	Dieldrin	—	—	15		—	—	—	—	—	—	—	—

TABLE L-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—South Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—NORTH SANTEE BAY—41 SAMPLES[1]—Continued													
1968	DDE	T	—	—	T	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	19	—	—
	Dieldrin	15	—	12	19	—	—	—	—	—	—	—	—
STATION 2.—SOUTH SANTEE BAY—40 SAMPLES[1]													
1965	DDE							T	T	T	—	—	—
	TDE							T	T	—	—	—	—
	DDT							—	—	—	T	—	—
1966	DDE	—	—	—	—	T	—	—	—	—		—	—
	TDE	—	—	—	—	—	—	—	—	—		—	—
	DDT	—	—	—	—	—	—	—	—	—		—	—
1967	DDE	—	—	T		—	—	T	T	T	—	—	—
	TDE	—	—	—		—	—	T	—	—	—	—	—
	DDT	—	—	—		—	—	13	T	T	—	—	—
1968	DDE	T	46	—	10	T	—	—	T	—	—	—	—
	TDE	T	—	—	T	—	—	—	—	—	—	—	—
	DDT	T	34	—	—	—	—	—	12	—	—	—	—
	Dieldrin	—	13	10	19	—	—	—	—	—	—	—	—
STATION 3.—BULL CREEK—12 SAMPLES[1]													
1969	DDE	—	—	—	T	T	—	—	—	—	—	—	—
	TDE	—	—	—	T	T	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	13	12	—	—	—	—	—	—	—
	Mirex	—	—	22	—	35	—	—	—	—	—	—	—
STATION 4.—PRICE CREEK—42 SAMPLES[1]													
1965	DDE							T	—	T	—	—	—
	TDE							T	—	—	—	—	—
	DDT							—	—	—	—	—	—
1966	DDE	T	—	T	—	—	—	—	—	19	T	—	T
	TDE	—	—	—	—	—	—	—	—	36	—	—	T
	DDT	—	—	—	—	—	—	—	—	26	—	—	—
	Dieldrin	—	—	12	—	—	—	—	—	—	—	—	—
1967	DDE	T	11	12	T	T	T	T	13	T	—	10	T
	TDE	—	T	—	—	—	10	T	12	—	—	T	T
	DDT	—	T	—	—	T	11	10	11	T	—	T	—

TABLE L-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—South Carolina*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 4.—PRICE CREEK—42 SAMPLES [1]—Continued													
1968	DDE	T	T	T	11	T	—	T	—	—	T	—	—
	TDE	10	T	—	T	—	—	—	—	—	—	—	—
	DDT	T	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	10	—	—	—	—	—	—	—	—	—	—
STATION 5.—INLET CREEK—42 SAMPLES [1]													
1965	DDE							T	T	—	—	T	T
	TDE							T	T	—	—	—	T
	DDT							—	T	—	—	—	—
1966	DDE	T	—	—	—	14	—	—	T	—	T	T	T
	TDE	T	—	—	—	14	—	—	—	—	—	—	T
	DDT	T	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	T	T	T	14	T	T	T	16	—	—	—	T
	TDE	—	—	T	13	—	—	15	21	—	—	—	—
	DDT	—	—	—	12	T	—	17	15	—	—	—	—
1968	DDE	11	—	12	—	—	—	—	—	—	—	—	—
	TDE	11	—	T	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
STATION 6.—HOG ISLAND CHANNEL—41 SAMPLES [1]													
1965	DDE							T	T	T	T	T	—
	TDE							T	T	—	T	—	—
	DDT							T	—	—	T	—	—
1966	DDE	T	13	T	14	13	—	20	—	14	10	—	T
	TDE	T	T	—	—	15	T	15	—	—	—	—	—
	DDT	T	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	15	20	20	16	14	11	10	19	T	—	T	T
	TDE	14	13	—	—	32	11	13	28	T	—	T	T
	DDT	11	11	—	—	12	20	14	26	11	—	16	—
1968	DDE	T	13	12	20	—	—	T	—	—	T	T	
	TDE	—	10	—	16	—	—	10	—	—	—	—	
	DDT	—	—	—	14	—	—	T	—	—	—	—	
STATION 7.—WANDO RIVER—42 SAMPLES [1]													
1965	DDE							T	T	T	T	—	T
	TDE							T	T	T	T	—	T
	DDT							T	T	—	T	—	—

TABLE L-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—South Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 7.—WANDO RIVER—42 SAMPLES [1]—Continued													
1966	DDE	10	—	T	11	10	—	—	—	10	10	T	T
	TDE	10	—	—	—	12	—	—	—	13	12	11	14
	DDT	T	—	—	—	—	—	—	—	T	—	—	T
1967	DDE	10	12	16	T	T	T	—	10	T	T	T	11
	TDE	17	13	17	—	T	T	12	20	10	T	—	16
	DDT	—	—	—	—	—	T	10	14	10	—	—	T
1968	DDE	14	13	11	14	13	—	T	—	—	—	—	—
	TDE	18	T	T	T	14	—	10	—	—	—	—	—
	DDT	12	—	—	—	—	—	T	—	—	—	—	—
STATION 8.—ASHLEY RIVER—54 SAMPLES [1]													
1965	DDE							16	T	T	T	18	32
	TDE							16	T	T	15	27	22
	DDT							14	T	—	10	28	22
1966	DDE	33	34	38	35	25	16	13	11	—	T	25	18
	TDE	25	29	28	33	23	39	17	—	—	T	31	28
	DDT	28	30	16	22	14	—	—	—	—	11	31	21
	Dieldrin	—	13	21	20	90	—	—	—	—	—	—	—
1967	DDE	36	36	51	26	42	11	16	18	T	14	23	33
	TDE	21	28	39	21	69	—	20	23	10	19	26	37
	DDT	24	28	35	18	43	19	18	32	11	18	42	49
	Dieldrin	—	—	11	—	—	—	—	—	—	—	—	—
1968	DDE	31	15	66	35	32	T	13	15	T	T	T	60
	TDE	30	13	26	31	28	—	16	15	11	—	—	51
	DDT	28	—	25	23	25	—	14	11	13	—	T	28
	Dieldrin	T	—	19	—	—	—	—	—	—	—	—	—
1969	DDE	12	13	16	—	15	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	13	—	—	—	—	—	—	—
	Dieldrin	—	—	—	—	—	—	—	—	—	—	23	—
	Mirex	—	—	190	—	—	—	—	—	—	—	—	—
STATION 9.—FORT JOHNSON—12 SAMPLES [1]													
1969	DDE	—	—	T	T	—	—	—	—	—	T	—	T
	TDE	—	—	—	—	—	—	—	—	—	T	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Mirex	—	—	540	—	—	—	—	—	—	—	—	—

TABLE L-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—South Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		STATION 10.—STEAMBOAT CREEK—54 SAMPLES[1]											
1965	DDE							T	T	T	—	T	—
	TDE							T	T	—	—	—	—
	DDT							—	T	—	—	—	—
1966	DDE	T	T	—	14	—	11	—	T	—	T	—	—
	TDE	—	—	—	11	—	11	—	—	T	T	—	—
	DDT	—	—	—	—	—	—	—	—	T	—	—	—
1967	DDE	T	11	10	—	13	T	—	—	T	—	—	—
	TDE	T	T	10	—	14	T	—	—	T	—	—	T
	DDT	—	—	T	—	T	16	—	—	T	—	—	T
1968	DDE	T	T	—	T	T	—	T	—	—	—	—	—
	TDE	T	—	—	—	—	—	—	—	—	—	—	—
	DDT	T	—	—	—	—	—	—	—	—	—	—	—
1969	DDE	—	T	—	—	—	—	—	—	—	—	T	T
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Mirex	—	—	38	—	—	—	—	—	—	—	—	—
		STATION 11.—TOOGOODOO CREEK—53 SAMPLES[1]											
1965	DDE							32	14	18	16	20	16
	TDE							43	20	19	20	33	16
	DDT							T	T	T	—	T	—
1966	DDE	27	18	15	38	24	19	—	15	13	18	11	22
	TDE	25	20	13	36	33	20	—	16	13	18	12	26
	DDT	—	T	T	24	16	—	—	—	11	10	—	T
1967	DDE	10	20	21	25	21	14	T	—		12	T	30
	TDE	T	17	18	23	22	15	—	—		—	—	26
	DDT	—	—	T	—	14	T	—	—		—	—	12
1968	DDE	17	20	16	18	25	21	T	18	11	T	T	13
	TDE	14	16	—	16	20	16	T	19	10	—	—	T
	DDT	T	—	—	—	—	—	—	17	T	—	—	—
1969	DDE	—	—	—	—	—	—	—	—	—	—	T	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Mirex	—	—	38	—	—	—	—	—	—	—	—	—
		STATION 12.—BIG BAY CREEK—54 SAMPLES[1]											
1965	DDE							T	T	T	T	—	T
	TDE							T	T	—	T	—	T
	DDT							—	—	—	—	—	—

TABLE L-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—South Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 12—BIG BAY CREEK—54 SAMPLES [1]—Continued													
1966	DDE	11	—	—	—	T	T	12	T	—	—	—	T
	TDE	T	—	—	—	T	T	T	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	11	14	14	12	12	T	13	—	T	—	—	T
	TDE	T	12	14	11	15	T	14	—	—	—	—	—
	DDT	T	T	11	12	13	21	31	—	—	—	—	—
1968	DDE	T	T	T	13	12	—	—	T	—	—	—	—
	TDE	11	—	T	T	—	—	—	T	—	—	—	—
	DDT	T	—	—	—	—	—	—	T	—	—	—	—
1969	DDE	—	—	—	T	T	24	—	—	—	12	14	17
	TDE	—	—	—	—	—	23	—	—	—	13	T	13
	DDT	—	—	—	—	33	44	—	—	—	27	13	13
	Mirex	—	—	T	—	—	—	—	—	—	—	—	—
STATION 13.—ST. PIERRE CREEK—12 SAMPLES [1]													
1969	DDE	—	T	—	T	T	22	—	—	—	15	13	T
	TDE	—	—	—	—	—	21	—	—	—	15	T	—
	DDT	—	—	—	—	30	45	—	—	—	29	13	—
	Mirex	—	—	38	—	—	—	—	—	—	—	—	—
STATION 14.—WHALE BRANCH—41 SAMPLES [1]													
1965	DDE							T	T	—	T	T	T
	TDE							T	T	—	T	—	T
	DDT							T	T	—	—	—	T
1966	DDE	—	—	—	T	T	—	—	—		11	33	T
	TDE	—	—	—	T	—	—	—	—		11	20	—
	DDT	—	—	—	—	—	—	—	—		—	26	—
1967	DDE	13	T	—	11	T	—	T	—	11	—	—	—
	TDE	T	—	—	T	T	—	T	—	—	—	—	—
	DDT	T	—	—	T	—	—	T	—	T	—	—	—
1968	DDE	11	T	—	14	—	—	—	T	T	—	—	—
	TDE	T	T	—	14	—	—	—	12	—	—	—	—
	DDT	—	—	—	—	—	—	—	15	—	—	—	—
STATION 15.—SKULL CREEK—39 SAMPLES [1]													
1965	DDE							T	T		T	T	—
	TDE							T	T		T	—	—
	DDT							—	T		—	—	—

TABLE L-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—South Carolina*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 15.—SKULL CREEK—39 SAMPLES[1]—Continued													
1966	DDE	—	—	—	—	12	T	—	—	—	—	T	—
	TDE	—	—	—	—	12	T	—	—	—	—	T	—
	DDT	—	—	—	—	T	—	—	—	—	—	—	—
1967	DDE	—	—	—	11	—	T		—	—	—		—
	TDE	—	—	—	14	—	T		—	—	—		T
	DDT	—	—	—	T	—	16		—	—	—		T
1968	DDE	T	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	17	—	—
	Dieldrin	—	—	—	—	—	—	—	—	—	—	—	35
STATION 16.—MAY CREEK—12 SAMPLES[1]													
1969	DDE	—	—	—	15	T	—	—	T	—	—	—	—
	TDE	—	—	—	—	T	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	11	—	—	—	—	—	—	—	—
	Mirex	—	—	23	37	27	—	—	—	—	—	—	—
STATION 17.—NEW RIVER—12 SAMPLES[1]													
1969	DDE	—	—	—	T	—	—	—	—	—	—	—	—
	TDE	—	—	—	11	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	21	—	—	—	—	—	—	—	—
	Mirex	—	—	—	—	27	—	—	—	—	—	—	—

[1] Each sample represents 15 or more mature mollusks.

SECTION M.—TEXAS

The eastern oyster, *Crassostrea virginica*, was used to monitor pollution in Texas estuarine waters during the period July 1965 June 1972. All samples were analyzed at the Gulf Breeze Laboratory. Approximate locations of the 13 sampling stations are shown in Fig. M-1. A summary of data on organochlorine residues in the monitored species, *C. virginica*, is presented in Table M-1, and the distribution of residues in this species for each sampling station by date of collection in Table M-2. In some instances, more than one reef was sampled at different times in a particular bay. In these instances, the data have been integrated to reflect bay conditions as a whole. At some times, floods resulting from tropical storms decimated oyster reefs and inerrupted routine monitoring. On at least one occasion, sample preparation reagents were contaminated with chlordane leading to spurious analytical results. Consequently, all findings of chlordane have been omitted from the data tabulations.

In conjunction with oyster monitoring in Texas, many samples of fish and other vertebrates were analyzed throughout the monitoring program. These analyses indicated, as might be expected, more kinds of pollutants and of greater magnitude than those found in oysters. PCB's, for example, were commonly found in fish samples but were detected in only five collections of oysters. In the Arroyo Colorado, Station 12, findings of consistently large DDT residues in oysters were paralleled by DDT residues about 10 times larger in fish. A causal relationship between DDT residues in the eggs and reproductive failure of the spotted sea trout, *Cynoscion nebulosus*, there in 1969, has been postulated (*5*).

Although the incidence of DDT residues was higher in eight other States, samples from monitoring stations in Texas bays that receive runoff from the agricultural areas were consistently contaminated with DDT. The maximum DDT residue detected, 1,249 ppb, was in an isolated sample; more typically the residues in contaminated areas were in the range of 100 500 ppb of DDT. Toxaphene of presumably agricultural origin was detected in only one sample.

There is a clearly defined trend of declining DDT residues in oysters. In 1971, there was a more than 50% increase in the number of samples containing negligible DDT residues (i.e., <11 ppb) over previous years and a 75% decrease in the number of samples in the 100 - 1,000 ppb range.

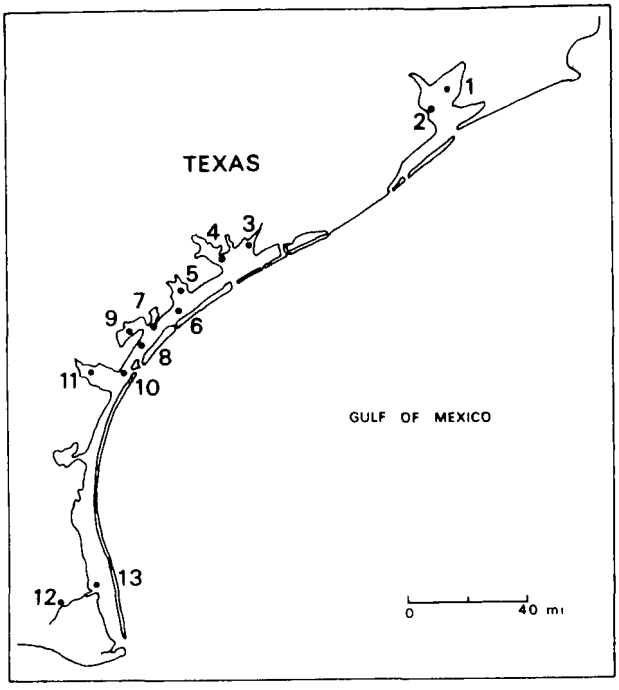


FIGURE M-1.—*Diagram of coastal Texas showing approximate location of monitoring stations*

1. Trinity Bay—Trinity-San Jacinto River basins
2. Galveston Bay—Trinity-San Jacinto River basins
3. Tres Palacios Bay—Lavaca River Basin
4. Lavaca Bay—Lavaca River Basin
5. San Antonio Bay, North—Guadalupe-San Antonio River Basin
6. San Antonio Bay, South—Guadalupe-San Antonio River Basin
7. St. Charles Bay—San Antonio-Nueces Coastal Area
8. Aransas Bay—San Antonio-Nueces Coastal Area
9. Copano Bay—San Antonio-Nueces Coastal Area
10. Red Fish Bay—San Antonio-Nueces Coastal Area
11. Nueces Bay—Nueces River Basin
12. Arroyo Colorado—Rio Grande Coastal Area
13. Lower Laguna Madre—Rio Grande Coastal Area

TABLE M-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1965-72—Texas*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in ppb (μg/kg)							
				DDT		Dieldrin		Endrin		Toxaphene [2]	PCB's [2]
1	Trinity Bay	1965-69	47	28	(51)	1	(20)				
2	Galveston Bay	1965-72	71	60	(88)	31	(87)				
3	Tres Palacios Bay	1965-72	74	71	(974)	6	(18)				
4	Lavaca Bay	1965-72	66	59	(400)	4	(24)				2
5	San Antonio Bay, North	1965-72	59	38	(78)	8	(27)				
6	San Antonio Bay, South	1965-72	75	40	(488)	3	(56)	1	(10)		

TABLE M-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1965-72—Texas*—Continued

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)										PCB's [2]
				DDT		Dieldrin		Endrin		Toxaphene [2]	
7	St Charles Bay	1966-72	66	33	(93)	11	(80)					
8	Aransas Bay	1965-67	19	18	(83)	2	(48)					
9	Copano Bay	1967-71	51	24	(96)							
10	Red Fish Bay	1966-72	67	52	(82)							2
11	Nueces Bay	1965-68	20	20	(450)	4	(33)	3	(18)			
12	Arroyo Colorado	1965-71	48	48	(710)	45	(46)	18	(32)	1		
13	Lower Laguna Madre	1965-67	24	15	(57)	1	(46)					
	Occasional stations (16)	1965-72	41	24	(1,249)	16	(64)					1
Total number of samples			728									
Percent of samples positive for indicated compound				73		18		3				<1

[1] Each sample represents 15 or more mature mollusks.
[2] Present but not quantified.

TABLE M-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Texas*

[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB s); T = >5 but <10 ppb.]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—TRINITY BAY—47 SAMPLES [1]													
1965	DDE							T	—	T	T	T	11
	TDE							T	—	—	T	10	16
	DDT							—	—	—	—	—	—
1966	DDE	12	18	15		18	—	—	—	—	—	T	T
	TDE	17	27	28		33	—	—	—	—	—	T	12
	DDT	—	—	—		—	—	—	—	—	—	—	—
1967	DDE	T	T	12	—	—	T	—	—	—	T	T	T
	TDE	T	12	18	—	—	T	—	—	—	T	T	—
	DDT	—	—	—	—	—	T	—	—	—	T	—	—
1968	DDE	T	T	T	T	T	—	—	T	—	10	T	
	TDE	11	T	T	11	T	—	—	—	—	T	—	
	DDT	—	—	—	—	—	—	—	—	—	—	—	
1969	DDE		—			T	—	—	—	—			T
	TDE		—			13	—	—	—	—			—
	DDT		—			—	—	—	—	—			—
	Dieldrin		—			20	—	—	—	—			—

TABLE M-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Texas*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 2.—GALVESTON BAY—71 SAMPLES[1]													
1965	DDE							—	T	T		T	T
	TDE							—	—	—		11	17
	DDT							—	—	—		—	—
1966	DDE	15	18	13		11	—	—	—	—	T	T	T
	TDE	36	43	32		33	—	—	—	—	T	24	24
	DDT	T	—	—		—	—	—	—	—	—	—	—
	Dieldrin	—	—	—		—	—	—	—	—	—	12	—
1967	DDE	T	11	15	13	—	T	—	T	T	11	11	13
	TDE	26	29	44	34	15	18	—	10	T	21	23	32
	DDT	T	—	—	—	—	—	—	—	—	11	—	—
	Dieldrin	12	14	15	—	—	—	—	—	—	—	14	19
1968	DDE	10	16	T	14	20	T	T	T	T	14	T	—
	TDE	24	43	36	37	46	41	30	13	15	46	34	49
	DDT	—	T	—	—	—	—	—	—	—	13	—	—
	Dieldrin	13	25	20	—	—	—	—	—	—	—	19	14
1969	DDE		T			24	—	T	10	—			19
	TDE		44			64	23	13	11	T			19
	DDT		—			—	—	—	—	—			—
	Dieldrin		30			19	—	—	—	—			—
1970	DDE		13	15	T	12	T	—	—	—	—	11	—
	TDE		35	34	34	32	18	20	—	—	17	31	32
	DDT		—	10	T	—	—	—	—	—	—	—	—
	Dieldrin		18	19	11	14	—	—	—	13	—	24	18
1971	DDE	T	11		—				—	—	—	T	T
	TDE	39	38		48				—	—	—	17	17
	DDT	—	—		—				—	—	—	T	11
	Dieldrin	16	23		30				65	46	—	—	26
1972	DDE	T	T	T	T	—							
	TDE	18	T	26	15	T							
	DDT	—	—	—	—	—							
	Dieldrin	42	26	87	24	36							
STATION 3.—TRES PALACIOS BAY—74 SAMPLES[1]													
1965	DDE							11	11	T	T	21	93
	TDE							T	T	—	—	T	29
	DDT							—	—	—	—	T	65

TABLE M-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Texas*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 3.—TRES PALACIOS BAY—74 SAMPLES [1]—Continued													
1966	DDE	78	78	250	190	300	47	210	97	21	11	23	26
	TDE	36	36	44	53	89	22	97	29	T	—	T	T
	DDT	43	53	80	59	130	12	23	—	—	—	—	—
1967	DDE	42	42	67	51	34	58	18	12	18	72	150	240
	TDE	14	17	31	19	14	24	—	—	—	41	52	57
	DDT	—	T	11	T	15	11	—	—	—	T	71	38
1968	DDE	270	220	230	320	300	91	62	43	20	19	22	24
	TDE	66	57	23	590	77	62	95	10	42	13	19	15
	DDT	83	21	81	64	22	T	17	—	T	—	—	—
	Dieldrin	—	18	—	—	—	—	—	—	—	—	—	—
1969	DDE	25	24	83		91	55	95	58	43			25
	TDE	19	18	35		44	62	15	56	T			40
	DDT	—	T	27		21	15	—	—	—			12
	Dieldrin	—	—	—		—	—	—	—	10			—
1970	DDE	70	36	110	230		44	33	41	—	50		16
	TDE	48	29	51	44		38	55	72	—	—		12
	DDT	31	—	19	56		31	—	—	—	—		—
	Dieldrin	—	13	17	13		—	—	—	—	—		—
1971	DDE	—	43	14	13		10	13	15	T	—	14	T
	TDE	—	30	12	12		23	27	44	—	—	15	T
	DDT	—	—	—	—		—	—	—	—	—	—	—
1972	DDE	100	59										
	TDE	25	20										
	DDT	15	T										
	Dieldrin	—	T										
STATION 4.—LAVACA BAY—66 SAMPLES [1]													
1965	DDE								T	—	T	13	22
	TDE								—	—	—	T	T
	DDT								—	—	—	T	10
1966	DDE	33	40	43	56	51	140	39	26	17	T	T	11
	TDE	12	17	T	27	25	30	16	11	—	—	—	—
	DDT	11	18	T	16	21	23	—	—	—	—	—	—
1967	DDE	22	16	20	25	14	T	25	14	14	T	19	26
	TDE	13	T	12	14	T	—	13	—	—	—	—	12
	DDT	—	—	—	T	14	—	34	—	—	—	—	T

TABLE M-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Texas*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 4.—LAVACA BAY—66 SAMPLES [1]—Continued													
1968	DDE	39	38	39	69	46	140						24
	TDE	16	17	22	62	39	120						13
	DDT	26	40	18	49	34	140						—
	Dieldrin	—	—	—	—	—	24						—
1969	DDE	20	19	33		41	33	—	12	—	26		40
	TDE	T	—	11		19	22	—	—	—	18		33
	DDT	—	—	—		14	16	—	—	—	15		48
1970	DDE	—	120		30	[2]		37	[2]	—	—		16
	TDE	—	42		16	[2]		53	[2]	—	—		T
	DDT	—	26		11	[2]		—	[2]	—	—		—
	Dieldrin	—	—		10	—		—	—	—	—		—
	PCB's	—	—		—	[3]		—	[3]	—	—		—
1971	DDE	48		43	43	22	T	T	—		12	T	13
	TDE	29		15	—	—	—	—	—		25	—	—
	DDT	T		—	—	—	—	—	—		—	—	—
	Dieldrin	—		—	—	—	—	—	—		—	—	14
1972	DDE	18											
	TDE	T											
	DDT	T											
	Dieldrin	21											
STATION 5.—SAN ANTONIO BAY (NORTH)—59 SAMPLES [1]													
1965	DDE							T	T	T	T	11	30
	TDE							T	T	T	T	T	25
	DDT							—	T	—	T	T	16
	Dieldrin							—	—	—	—	—	11
1966	DDE	31	30	32	29	33	29	16	—	T	13	15	19
	TDE	24	27	30	23	27	22	10	—	—	—	T	14
	DDT	14	16	15	—	18	13	—	—	—	—	—	—
	Dieldrin	—	—	17	—	—	—	—	—	—	—	—	—
1967	DDE	17	20	22	29	13	12	T	—	11	14		
	TDE	13	18	18	30	T	—	—	—	T	13		
	DDT	—	—	—	T	—	—	—	—	14	—		
	Dieldrin	—	10	—	—	—	—	—	—	—	—		
1968		No Samples Collected											
1969	DDE							—	—	—	T		18
	TDE							—	—	—	T		12
	DDT							—	—	—	—		T

TABLE M-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Texas*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 5.—SAN ANTONIO BAY (NORTH)—59 SAMPLES [1]—Continued													
1970	DDE	—	—	18	14	17	—	—	—	—	—	—	—
	TDE	—	—	—	—	33	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	—	—	—	27	—	—	—	17	—
1971	DDE	T	—	—		—	—	—	—	10	—	T	13
	TDE	12	—	—		—	—	—	—	—	—	11	—
	DDT	—	—	—		—	—	—	—	19	—	—	—
	Dieldrin	—	—	—		—	—	—	—	—	—	12	17
1972	DDE	12	12			T							
	TDE	—	—			—							
	DDT	—	—			—							
	Dieldrin	T	—			—							
STATION 6.—SAN ANTONIO BAY (SOUTH)—75 SAMPLES [1]													
1965	DDE							—	T	T	T	12	17
	TDE							—	—	T	T	10	T
	DDT							—	—	T	T	T	T
1966	DDE	13	13	14	19	14	T	—	—	—	T	15	10
	TDE	T	—	—	14	10	—	—	—	—	—	T	T
	DDT	T	—	—	—	10	—	—	—	—	—	T	—
1967	DDE	17	20	20	14	T	—	—	—	T			
	TDE	11	10	11	—	—	—	—	—	T			
	DDT	10	—	11	—	—	—	—	—	T			
1968	DDE				21	T	—	—	110	—	—	—	T
	TDE				19	—	—	—	310	—	—	—	T
	DDT				13	—	—	—	68	—	—	—	—
	Dieldrin				56	—	—	—	14	—	—	—	—
	Endrin				10	—	—	—	—	—	—	—	—
1969	DDE	T	16	20	16	T	—		—	—	T	T	12
	TDE	—	—	—	14	T	—		—	—	T	T	15
	DDT	—	—	—	T	—	—		—	—	—	—	T
	Dieldrin	14	—	—	—	—	—		—	—	—	—	—
1970	DDE	—	—	—	—	11	—	—	—	—	—	13	—
	TDE	—	—	—	—	25	—	—	—	—	—	41	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1971	DDE	—	—	—	—	T	—	—	—	—	—	T	13
	TDE	—	—	—	—	—	—	—	—	—	—	—	10
	DDT	—	—	—	—	—	—	—	—	—	—	—	T

TABLE M-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Texas*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 6.—SAN ANTONIO BAY (SOUTH)—75 SAMPLES [1]—Continued													
1972	DDE	13	16			T							
	TDE	—	—			—							
	DDT	—	—			—							
STATION 7.—ST. CHARLES BAY—66 SAMPLES [1]													
1966	DDE	11	T	11		11	—	—	—	—	T	T	15
	TDE	—	—	—		—	—	—	—	—	—	—	T
	DDT	—	—	—		T	—	—	—	—	—	—	T
	Dieldrin	—	—	—		—	—	—	—	—	—	—	11
1967	DDE	15	20	17	23	16	—	—	—	12	15	16	17
	TDE	T	T	T	52	47	—	—	—	—	—	T	12
	DDT	T	T	—	—	23	—	—	—	—	—	—	10
	Dieldrin	13	12	—	28	39	78	80	—	—	—	—	15
1968 [4]	DDE	22	30	—	24	19	—	T	—	—	—	—	—
	TDE	—	20	—	19	16	—	—	—	—	—	—	—
	DDT	—	43	—	11	23	—	—	—	—	—	—	—
1969	DDE	—	—	14	12	T	T	—	—	—	—	—	T
	TDE	—	—	T	21	17	15	—	—	—	—	—	—
	DDT	—	—	—	—	T	—	—	—	—	—	—	—
	Dieldrin	49	—	—	—	—	—	27	—	T	—	—	—
1970	DDE							—	—		—	—	—
	TDE							—	—		—	—	—
	DDT							—	—		—	—	—
1971 [4]	DDE	—	—	—	—	—	—	—		T	T		13
	TDE	—	—	—	—	—	—	—		T	T		T
	DDT	—	—	—	—	—	—	—		21	10		15
1972 [4]	DDE	14	23	27	16								
	TDE	T	T	10	—								
	DDT	14	15	—	—								
STATION 8.—ARANSAS BAY—19 SAMPLES [1]													
1965	DDE								T	21			
	TDE								T	T			
	DDT								—	—			
1966	DDE	12	16	20	16	16	10	T	T	T	27	15	15
	TDE	T	45	57	—	43	35	30	33	20	26	42	43
	DDT	T	—	T	—	14	—	—	—	—	—	—	T

TABLE M-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Texas*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 8.—ARANSAS BAY—19 SAMPLES[1]—Continued													
1967	DDE	23	24	27	—	T							
	TDE	54	49	56	—	—							
	DDT	T	T	—	—	—							
	Dieldrin	—	—	—	28	48							
STATION 9.—COPANO BAY—51 SAMPLES[1, 4]													
1967	DDE						—	—	—	—	T	—	21
	TDE						—	—	96	—	20	—	30
	DDT						—	—	—	—	—	—	T
1968	DDE	14	18	—	21	T	12	T	—	—	—	—	—
	TDE	23	28	—	24	—	—	—	—	—	—	—	—
	DDT	—	—	—	T	—	—	—	—	—	—	—	—
1969	DDE	—	T	—	50	15	17	—	—	—	—	—	15
	TDE	T	—	—	21	27	23	—	—	—	—	—	T
	DDT	—	—	—	T	18	T	—	—	—	—	—	T
1970	DDE	17	15	25	14	—	—	—	—	—	—	—	—
	TDE	11	T	T	14	—	—	—	—	—	—	—	—
	DDT	T	T	—	T	—	—	—	—	—	—	—	—
1971	DDE	10	13	15	17	10	—	—	—				
	TDE	10	11	10	39	10	—	—	—				
	DDT	—	—	—	—	—	—	—	—				
STATION 10.—RED FISH BAY—67 SAMPLES[1, 4]													
1966	DDE					29		24	12	T	T	T	T
	TDE					25		21	18	T	11	T	T
	DDT					12		12	—	—	—	—	—
1967	DDE	T	15	17	—	T	14	T	—				
	TDE	T	21	32	—	14	39	19	—				
	DDT	—	T	T	—	—	13	14	—				
1968	DDE		23	25	18		12	10	10	11	—	—	10
	TDE		14	26	43		18	21	18	15	21	—	17
	DDT		—	12	21		—	—	T	23	T	—	T
1969	DDE	15	T	10	15	—	T	—	—	12	—	11	17
	TDE	27	T	19	27	18	T	—	—	—	—	15	22
	DDT	13	—	T	19	—	—	—	—	—	—	T	13

TABLE M-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Texas*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 10.—RED FISH BAY—67 SAMPLES [1]—Continued													
1970	DDE	16	14	18	17	10	12	14	12	—	—	—	T
	TDE	20	19	29	25	23	38	25	18	—	—	—	29
	DDT	T	10	13	12	—	—	—	10	—	—	—	15
	PCB's	—	—	—	—	—	—	—	—	—	(3)	—	—
1971	DDE	—	—	17	—	T	—	—	13	T	T	T	12
	TDE	—	—	14	—	—	—	—	13	—	T	T	11
	DDE	—	—	—	—	—	—	—	17	26	17	22	26
1972	DDE	14	20	16	18	—	T						
	TDE	11	15	16	12	—	14						
	DDT	22	30	45	42	—	34						
	PCB's	—	—	—	—	—	(3)						
STATION 11.—NUECES BAY—20 SAMPLES [1]													
1965	DDE									T			
	TDE									—			
	DDT									—			
1966	DDE			34	22	32		120	18	18	T	T	
	TDE			30	17	26		200	20	16	—	12	
	DDT			12	—	14		130	—	—	—	—	
	Dieldrin			—	—	—		33	—	—	—	—	
1967	DDE	31	30	43	46	34	29	20	32	57	51		
	TDE	36	61	110	110	48	52	20	20	25	22		
	DDT	T	22	20	26	22	49	17	37	35	15		
	Dieldrin	—	11	13	19	—	—	—	—	—	—		
	Endrin	—	18	12	11	—	—	—	—	—	—		
1968	DDE			45									
	TDE			28									
	DDT			15									
STATION 12.—ARROYO COLORADO—48 SAMPLES [1]													
1965	DDE									170	24	55	64
	TDE									520	33	80	80
	DDT									20	T	17	16
	Dieldrin									19	T	29	34
	Endrin									—	—	32	19
1966	DDE	80	120	120	74	96	230	300	270	98	12	180	63
	TDE	110	140	130	70	69	140	230	93	57	—	50	58
	DDT	21	19	17	—	26	31	53	24	—	—	19	12
	Dieldrin	32	23	24	18	16	30	45	27	14	—	18	20
	Endrin	18	17	14	—	22	23	28	13	—	—	14	12

TABLE M-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Texas*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 12.—ARROYO COLORADO—48 SAMPLES[1]—Continued													
1967	DDE	120	140	210	170	110	160	160	79				
	TDE	73	110	180	75	49	63	92	49				
	DDT	27	—	28	19	26	24	23	16				
	Dieldrin	23	16	42	46	19	33	30	16				
	Endrin	11	29	19	—	—	—	12	—				
1968	DDE			48								160	
	TDE			150								68	
	DDT			—								49	
	Dieldrin			—								33	
1969	DDE	260	330	220	320	180	260	280	86	100	110	210	54
	TDE	110	100	48	100	35	63	55	28	33	30	T	21
	DDT	15	57	35	110	77	48	22	—	—	T	—	24
	Dieldrin	14	16	17	18	14	25	18	17	12	T	16	—
1970	DDE	23	120	140	110	130	96						
	TDE	35	20	25	29	25	54						
	DDT	32	19	22	T	T	60						
	Dieldrin	21	18	23	25	13	25						
	Endrin	T	T	—	—	—	12						
1971	DDE				65	280	380	220					
	TDE				14	61	46	78					
	DDT				—	—	—	—					
	Dieldrin				11	27	24	38					
	Toxaphene				—	—	—	[3]					
STATION 13.—LOWER LAGUNA MADRE—24 SAMPLES[1]													
1965	DDE									T	T	—	—
	TDE									—	—	—	—
	DDT									—	—	—	—
1966	DDE	—	—	—	—	27	T	T	13	—	12	—	T
	TDE	—	—	—	—	19	—	—	—	—	—	—	—
	DDT	—	—	—	—	11	—	—	—	—	—	—	—
	Dieldrin	—	—	—	—	—	46	—	—	—	—	—	—
1967	DDE	11	15	13	13	10	12	T	—				
	TDE	—	—	—	—	—	T	—	—				
	DDT	—	—	—	—	14	T	—	—				

[1] Each sample represents 15 or more mature mollusks.
[2] DDT present but not quantified due to presence of PCB's in sample.
[3] Present but not quantified.
[4] Dieldrin data omitted because of possible sample contamination.

SECTION N.—VIRGINIA

The eastern oyster, *Crassostrea virginica*, was monitored at 10 principal stations in estuarine areas of Virginia during the period July 1965 February 1972. Samples were analyzed at the Gulf Breeze Laboratory until June 1968, and thereafter at the Virginia Institute of Marine Science. The approximate station locations are shown in Fig. N-1. A summary of data on organochlorine residues in the monitored species, *C. virginica*, is presented in Table N-1, and the distribution of residues in this species for each sampling station by data of collection in Table N-2.

The 87% incidence of DDT residues in Virginia samples and the maximum residue of 678 ppb were fourth highest of the States monitored. The higher residues were clearly associated with intensive truck farming (Station 2) and a combination of urban and industrial development (Station 9).

The presence of PCB's was noted in 1970 samples, but not until 1971 was equipment acquired to identify and quantify these compounds. The residue of 2,800 ppb of Aroclor 1254® detected in oysters in the Elizabeth River, a highly industrialized area, has prompted a special study to pinpoint the source of this pollution.

Trends in DDT residues in Virginia oysters differ somewhat from other areas in that, while the larger residues (those above 100 ppb) decreased by 66% in 1971, 100% of the 1971 samples contained residues in excess of 11 ppb as compared to 82% in earlier years. It appears that DDT residues are more widely dispersed but at relatively lower levels, presumably through the processes of recycling.

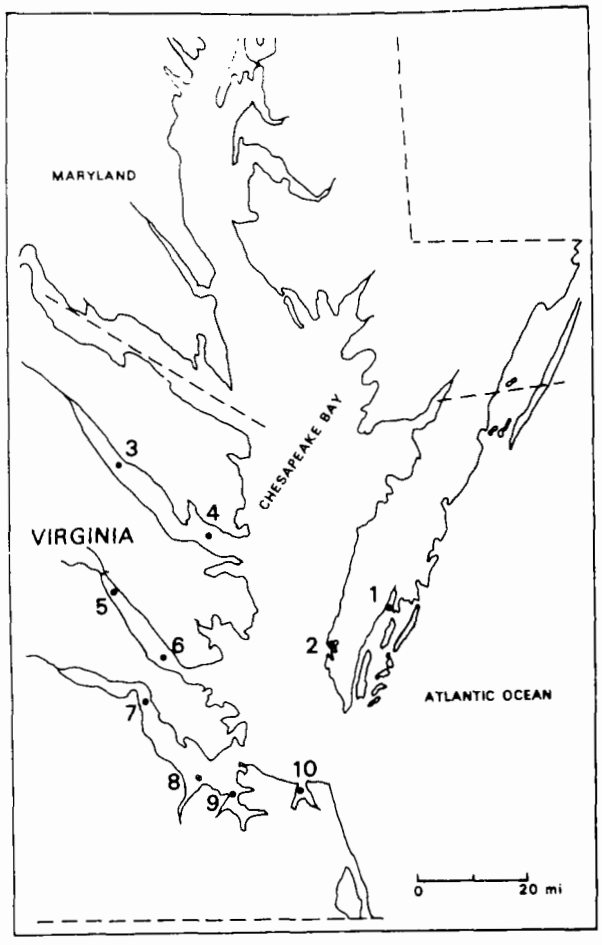


FIGURE N-1.—*Diagram of coastal Virginia showing approximate location of monitoring stations*

1. Machipongo River
2. Cherrystone Inlet—Chesapeake Bay
3. Bowlers Rock—Rappahannock River
4. Urbanna—Rappahannock River
5. Bell Rock—York River
6. Pages Rock—York River
7. Deep Water Shoals—James River
8. Nansemond Ridge—James River
9. Hospital Point—Elizabeth River
10. Lynnhaven Bay

TABLE N-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1965-72—Virginia*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μG/KG)		
				DDT	Dieldrin	PCB's [2]
1	Machipongo River	1965-72	67	56 (127)	2 (11)	1 (390)
2	Cherrystone Inlet	1965-72	68	67 (678)		2 (510)
3	Bowlers Rock	1965-72	70	62 (60)		2 (400)
4	Urbana	1965-72	69	59 (45)		2 (270)
5	Bell Rock	1965-72	69	35 (54)		2 (450)
6	Pages Rock	1965-72	68	50 (100)	1 (T)	2 (400)
7	Deep Water Shoals	1965-72	69	69 (144)	38 (40)	3 (1,000)

TABLE N-1.—*Summary of data on organochlorine residues in the monitored species* (C. virginica), *1965-72—Virginia*—Continued

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)					
				DDT		Dieldrin		PCB's [2]	
8	Nansemond Ridge	1965-72	64	63	(128)	29	(22)	2	(1,000)
9	Hospital Point	1966-72	58	58	(300)	38	(24)	3	(2,800)
10	Lynnhaven Bay	1965-70	62	61	(113)	4	(16)		
	Occasional stations (4)	1965-67	5	5	(241)				
Total number of samples			669						
Percent positive for indicated compound				87		17		3	

NOTE: T = >5 but < 10 ppb.
[1] Each sample represents 15 or more mature mollusks.

TABLE N-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Virginia*
[Blank = no sample collected; — = no residue detected above 5 ppb or no residue detected (PCB's); T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—MACHIPONGO RIVER—67 SAMPLES [1]													
1965	DDE							34	15	18	T	13	15
	TDE							20	13	28	T	T	T
	DDT							73	T	24	—	—	—
	Dieldrin							11	—	—	—	—	—
1966	DDE	12	T	11	14	19	17	19	—	T	T	—	T
	TDE	—	—	—	T	14	18	17	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	34	T	T	17	14	T	T	—	—	T	11
	TDE	—	59	—	—	12	13	—	T	—	—	—	—
	DDT	—	10	—	—	—	T	—	T	—	—	—	—
1968	DDE	T	—	T	—	T	18	15	T	—	T	11	T
	TDE	—	—	—	—	—	10	20	T	—	T	T	—
	DDT	—	—	—	—	—	—	10	—	—	—	—	—
1969	DDE	T	T	T	T	T	13	—	11		T	20	T
	TDE	—	T	—	—	T	13	—	16		T	27	T
	DDT	—	T	—	—	—	T	—	—		—	16	—
	Dieldrin	—	—	—	—	—	—	—	—		T	—	—
1970	DDE	15	10	—	—	11	24		T	T	T	11	
	TDE	14	T	—	—	T	35		13	T	T	T	
	DDT	—	11	—	—	—	17		T	—	—	—	
1971	DDE						10		18		17		
	TDE						—		T		T		
	DDT						—		—		—		
	PCB's						—		—		[2] 390		

TABLE N-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Virginia*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—MACHIPONGO RIVER—67 SAMPLES [1]—Continued													
1972	DDE		12										
	TDE		—										
	DDT		—										
STATION 2.—CHERRYSTONE INLET—68 SAMPLES [1]													
1965	DDE							60	35	16	24	25	45
	TDE							89	60	14	42	32	55
	DDT							230	71	T	35	20	35
1966	DDE	45	42	33	43	36	32	90	41	14	25	19	36
	TDE	49	46	45	40	36	37	110	86	35	66	62	73
	DDT	23	26	25	15	11	16	130	59	T	20	11	14
1967	DDE	49	31	32	37	45	37	34	44	55	26	24	20
	TDE	74	52	36	53	75	68	61	63	81	42	29	18
	DDT	17	14	—	12	10	21	110	110	120	22	13	T
1968	DDE	19	27	35	59	42	40	146	63	20	33	33	T
	TDE	18	20	46	58	55	52	210	172	76	31	31	15
	DDT	T	—	T	21	12	17	322	42	12	T	T	T
1969	DDE	24	11	21	17	12	T	15	35	17	24	16	35
	TDE	16	14	22	16	13	14	10	31	22	34	10	39
	DDT	—	—	—	—	—	—	—	20	11	13	—	17
1970	DDE	32	33	—	20	28			24	T	22	18	19
	TDE	30	42	—	21	29			26	T	34	19	22
	DDT	T	16	—	—	—			23	T	T	—	T
1971	DDE						30		22		29		
	TDE						30		14		23		
	DDT						—		14		T		
	PCB's						—		—		[2] 350		
1972	DDE		43										
	TDE		18										
	DDT		—										
	PCB's		[3] 510										
STATION 3.—BOWLERS ROCK—70 SAMPLES [1]													
1965	DDE							16	T	T	T	T	11
	TDE							23	T	T	T	T	12
	DDT							21	T	T	T	—	—
1966	DDE	11	10	—	13	11	13	13	T	—	—	T	—
	TDE	T	T	—	T	11	15	15	T	—	—	T	—
	DDT	—	—	—	—	—	T	—	—	—	—	—	—

TABLE N-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Virginia*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		STATION 3.—BOWLERS ROCK—70 SAMPLES [1]—Continued											
1967	DDE	—	T	T	10	T	T	10	—	—	T	T	T
	TDE	—	—	T	11	T	11	14	15	—	T	T	T
	DDT	—	—	—	—	—	—	11	18	—	T	T	—
1968	DDE	—	T	T	T	T	13	10	10	—	17	T	12
	TDE	—	—	T	—	—	14	12	15	—	17	T	T
	DDT	—	—	—	—	—	—	—	T	—	T	—	—
1969	DDE	T	T	11	12	10	T	T	T	T	11	T	11
	TDE	10	T	10	14	10	T	T	T	10	18	14	13
	DDT	—	—	—	—	T	—	—	T	T	14	T	T
1970	DDE	16	10	T	T	10	10	T	T	—	T	—	T
	TDE	14	T	—	11	10	17	12	T	T	12	T	T
	DDT	T	32	—	—	—	T	—	—	T	T	—	—
1971	DDE					11				T			14
	TDE					T				—			12
	DDT					—				—			—
	[2] PCB's					T				—			400
1972	DDE		17										
	TDE		12										
	DDT		—										
		STATION 4.—URBANA—69 SAMPLES [1]											
1965	DDE							T	10	T	T	T	13
	TDE							13	16	T	—	—	10
	DDT							14	19	T	—	—	—
1966	DDE	10	T	—	—	10	T	15	—	T	—	—	T
	TDE	T	—	—	—	—	—	—	—	—	—	—	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	T
1967	DDE	12	10	11	T	T	T	T	T	—	T	11	11
	TDE	—	T	T	T	T	T	T	T	—	11	T	12
	DDT	—	—	—	—	—	—	—	T	—	12	—	T
1968	DDE	T	T	T	11	T	11	14	T	—	T	T	T
	TDE	T	—	T	T	—	—	13	T	—	T	12	T
	DDT	—	—	—	—	—	—	T	—	—	—	T	—
1969	DDE	T	T	—	T	T	T	T	T	T	11	T	T
	TDE	T	11	—	—	T	T	T	13	T	18	10	10
	DDT	T	T	—	—	—	—	—	—	—	16	—	—
1970	DDE		T	—	T	10	15	T	—	10	—	T	T
	TDE		10	—	T	T	17	T	T	17	—	T	T
	DDT		16	—	—	—	T	T	—	14	—	—	T

TABLE N-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Virginia*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		STATION 4.—URBANA—69 SAMPLES [1]—Continued											
1971	DDE					10				T		10	
	TDE					T				—		T	
	DDT					—				—		—	
	[3] PCB's					T				—		270	
1972	DDE		10										
	TDE		T										
	DDT		—										
		STATION 5.—BELL ROCK—69 SAMPLES [1]											
1965	DDE							11	T	T	T	11	16
	TDE							24	T	13	10	13	21
	DDT							19	T	10	T	—	13
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	T
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	T	—	T	—	—	—	—	—	—	—	T
	TDE	—	T	—	—	—	—	—	—	—	12	—	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	T	T	—	T	T	11
	TDE	—	—	—	—	—	—	10	T	—	T	T	T
	DDT	—	—	—	—	—	—	—	—	—	—	—	11
1969	DDE	—	—	—	—	—	T	—	T	T	11		17
	TDE	—	—	—	—	—	T	—	T	14	20		T
	DDT	—	—	—	—	—	—	—	—	—	10		—
1970	DDE	T	T	—	T	T	10	10	—	—	—	—	T
	TDE	T	12	11	11	T	18	13	T	T	—	—	T
	DDT	—	—	—	—	—	—	T	—	—	—	—	—
1971	DDE					T				—		T	
	TDE					—				T		T	
	DDT					—				—		—	
	PCB's					—				—		[3] 390	
1972	DDE		T										
	TDE		T										
	DDT		—										
	PCB's		[3] 450										
		STATION 6.—PAGES ROCK—68 SAMPLES [1]											
1965	DDE							10	T	11	11	17	19
	TDE							15	12	17	14	20	16
	DDT							17	14	14	12	24	13

TABLE N-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Virginia*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
		STATION 6.—PAGES ROCK—68 SAMPLES [1]—Continued											
1966	DDE	14	—	—	T	10	11	—	T	—	T	—	T
	TDE	13	—	—	—	T	11	—	T	—	T	—	T
	DDT	T	—	—	—	—	—	—	11	—	—	—	—
1967	DDE	—	—	T	10	—	T	T	—	T	T	T	13
	TDE	—	—	—	T	—	T	T	—	16	14	11	14
	DDT	—	—	—	—	—	—	—	T	14	T	—	—
1968	DDE	T	T	—	T	—	12	13	T	—	T	T	10
	TDE	—	T	—	—	—	—	14	10	—	10	T	11
	DDT	—	—	—	—	—	—	—	—	—	—	—	T
1969	DDE	—	—	T	—	T	13	—		14	18		T
	TDE	—	—	T	—	T	T	—		12	16		T
	DDT	—	—	T	—	—	—	—		T	T		—
	Dieldrin	—	—	—	—	—	—	—		—	T		—
1970	DDE	—	T	—	T	T	T	T	T	—	10	90	T
	TDE	T	20	—	T	T	29	12	T	—	T	10	T
	DDT	—	—	—	—	—	11	T	—	—	—	—	—
1971	DDE					T				—			T
	TDE					—				—			T
	DDT					—				—			—
	PCB's					[2] T				—			[3] 400
1972	DDE		T										
	TDE		T										
	DDT		—										
		STATION 7.—DEEP WATER SHOALS—69 SAMPLES [1]											
1965	DDE							21	18	10	13	30	40
	TDE							52	31	17	22	41	56
	DDT							63	35	T	13	17	23
	Dieldrin							14	T	—	—	11	13
1966	DDE	37	11	24	26	30	40	37	23	11	18	19	17
	TDE	43	15	24	32	45	63	57	41	21	29	28	32
	DDT	15	—	—	—	14	20	30	22	T	16	15	10
	Dieldrin	—	—	—	23	34	38	16	17	—	12	—	—
1967	DDE	21	26	19	31	19	20	20	T	17	13	21	19
	TDE	29	30	21	41	25	32	37	25	33	20	23	20
	DDT	12	13	—	19	13	12	18	24	28	10	11	24
	Dieldrin	—	14	—	40	28	21	22	11	12	—	—	—
1968	DDE	15	14	17	15	15	23	18	14	T	15	12	19
	TDE	15	12	15	15	19	29	26	25	T	20	T	18
	DDT	—	—	—	—	11	14	15	18	—	T	T	T
	Dieldrin	—	—	12	12	16	16	T	T	—	—	T	—

TABLE N-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Virginia*—Continued

Year	Compound	Residues in PPB (µg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 7.—DEEP WATER SHOALS—69 SAMPLES [1]—Continued													
1969	DDE	T	T	10	11	T	10	18	14	17	20	28	
	TDE	10	T	12	T	T	16	12	16	23	28	40	
	DDT	—	—	—	—	T	T	—	16	21	20	29	
	Dieldrin	—	Lost	—	—	T	11	13	T	—	T	14	
1970	DDE	41	19	10	27	10	12	T	40	20	25	12	17
	TDE	43	22	11	12	T	21	22	71	40	43	17	28
	DDT	60	—	T	—	—	17	T	10	14	25	T	12
	Dieldrin	T	—	14	—	—	12	16	--	--	—	—	—
1971	DDE					40				T		16	
	TDE					35				13		21	
	DDT					T				—		—	
	Dieldrin					31				T		17	
	[3] PCB's					1,000				—		560	
1972	DDE		15										
	TDE		15										
	DDT		—										
	Dieldrin		10										
	PCB's		[3] 760										
STATION 8.—NANSEMOND RIDGE—64 SAMPLES [1]													
1965	DDE							16	17	17	T	27	36
	TDE							59	43	29	14	37	49
	DDT							53	39	25	T	24	31
	Dieldrin							17	T	—	—	T	11
1966	DDE	28	14	16	30	30	36		34	13	10	11	11
	TDE	35	14	17	34	29	52		55	18	15	16	19
	DDT	15	—	—	13	13	29		29	—	10	16	10
	Dieldrin	—	—	—	17	14	22		14	—	—	—	—
1967	DDE	14	17	22	18	15	14	14	18	T	13	17	20
	TDE	17	16	20	18	17	20	24	35	16	24	24	25
	DDT	T	T	11	T	T	12	11	20	T	10	13	10
	Dieldrin	—	—	—	15	12	T	12	14	—	—	10	11
1968	DDE	12	15	14	12	32	24	23	14	—	T	16	16
	TDE	14	16	15	12	47	32	38	29	—	12	13	22
	DDT	—	T	T	—	45	27	23	15	—	—	T	T
	Dieldrin	—	11	—	10	21	15	T	-	--	—	—	—
1969	DDE	—	T	10	T	T	11	T	50	11	16		16
	TDE	T	T	11	T	T	23	T	37	14	28		26
	DDT	—	—	—	—	—	10	--	23	T	13		12
	Dieldrin	—	—	—	—	—	10	—	—	—	11		T

TABLE N-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Virginia*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 8.—NANSEMOND RIDGE—64 SAMPLES [1]—Continued													
1970	DDE	12	15	—				10	16	10	T		T
	TDE	18	40	13				27	23	14	12		14
	DDT	22	11	35				—	13	11	10		T
	Dieldrin	T	—	T				T	—	—	—		—
1971	DDE					22				11			16
	TDE					18				13			15
	DDT					T				—			—
	Dieldrin					15				T			20
	PCB's					[3] 1,000				—			[2] 440
1972	DDE		16										
	TDE		14										
	DDT		—										
	Dieldrin		—										
STATION 9.—HOSPITAL POINT—58 SAMPLES [1]													
1966	DDE			140	82	63	83	66	37	20	24	26	27
	TDE			120	73	60	130	96	63	36	53	42	40
	DDT			40	32	31	89	39	43	22	35	27	24
	Dieldrin			13	18	15	20	—	—	—	—	—	T
1967	DDE	42	52	26	34	33	37	29	11	20	39	43	54
	TDE	48	53	17	25	42	76	67	55	59	83	78	64
	DDT	31	29	T	—	20	58	36	31	62	63	100	37
	Dieldrin	T	16	—	—	11	16	—	—	10	15	16	19
1968	DDE	68	92	60	57	48	50	30	20	14	13	11	26
	TDE	79	67	48	49	48	93	67	62	30	24	17	21
	DDT	52	55	18	20	34	83	35	19	12	10	T	T
	Dieldrin	13	19	15	12	12	13	10	T	—	10	11	—
1969	DDE	12	17	31	22	15	15	15	28	33	32		29
	TDE	T	11	32	21	28	28	33	70	84	92		56
	DDT	—	—	11	19	11	27	15	41	46	71		36
	Dieldrin	—	T	10	10	T	T	10	T	19	10		T
1970	DDE	15	32	15				T	18	24	13	T	15
	TDE	21	54	35				22	37	57	30	13	23
	DDT	T	29	—				10	19	28	14	—	11
	Dieldrin	T	14	—				—	—	—	—	—	—
1971	DDE					40				27			27
	TDE					40				49			32
	DDT					20				—			—
	Dieldrin					T				12			24
	[3] PCB's					2,800				—			960

TABLE N-2.—*Distribution of organochlorine residues in* C. virginica *for each sampling station by date of collection—Virginia*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 9.—HOSPITAL POINT—58 SAMPLES [1]—Continued													
1972	DDE		34										
	TDE		32										
	DDT		—										
	Dieldrin		Lost										
	[3] PCB's		1,440										
STATION 10.—LYNNHAVEN BAY—62 SAMPLES [1]													
1965	DDE							26	18	13	14	13	31
	TDE							49	33	10	24	14	40
	DDT							17	12	T	—	T	T
	Dieldrin							10	—	—	—	—	—
1966	DDE	19	20	17	32	16	25	36	16	16	14	17	14
	TDE	25	29	20	39	20	41	59	21	24	22	27	26
	DDT	—	—	—	T	—	11	15	—	—	T	—	T
1967	DDE	16	22	19	29	15	24	34	17	21	20	20	18
	TDE	19	27	21	36	18	35	57	33	33	32	27	25
	DDT	T	T	—	T	T	20	22	17	16	17	11	10
	Dieldrin	—	—	—	16	—	—	—	—	—	—	—	—
1968	DDE	19	T	29	18	27	30	15	14	11	16	15	19
	TDE	22	—	43	18	28	45	T	21	13	20	12	20
	DDT	T	—	12	—	10	27	12	T	—	T	—	T
	Dieldrin	—	—	13	—	—	—	—	—	—	—	—	—
1969	DDE	18	16	12	11	16	12		27	14	11	20	18
	TDE	16	22	21	11	21	16		28	18	20	28	23
	DDT	—	T	T	—	T	—		17	T	10	—	—
	Dieldrin	—	—	—	—	—	—		—	—	—	10	—
1970	DDE	—	20	—	T	11	28			18		18	14
	TDE	—	26	—	—	—	29			20		12	23
	DDT	T	—	—	—	—	T			10		T	T

[1] Each sample represents 15 or more mature mollusks.
[2] Calculated as Aroclor 1242®.
[3] Calculated as Aroclor 1254®.

SECTION O.—WASHINGTON

The Pacific oyster, *Crassostrea gigas*, was used to monitor 19 estuarine sites at monthly intervals in the period October 1965 December 1968. All samples were analyzed at the Gulf Breeze Laboratory. The approximate station locations are shown in Fig. O-1. A summary of data on organochlorine residues in the monitored species, *C. gigas*, is presented in Table O-1, and the distribution of residues in this species for each sampling station by date of collection in Table O-2.

The monitoring program was terminated in Washington after 3 years because of the absence of detectable DDT residues in most samples. This was due to the absence of DDT pollution and not because of any lack of sensitivity on the part of the monitored species. Analyses of samples of the Pacific oyster in California waters had demonstrated its ability to store organochlorine residues at levels comparable to other molluscan species in the same estuary.

The overall incidence of DDT residues in Washington samples was only 11%. The maximum residue detected, 176 ppb, was the obvious result of a single pollution incident. Station 18 was the only one demonstrating a continuing, but low-level pollution problem. The fact that residues at this station were primarily DDT rather than one of its metabolites suggests a direct application of the pesticide to coastal waters. Analytical data are too few, even at Station 18, to indicate any trend in DDT pollution. The overall picture is that of an estuarine area of the United States that was remarkably free from DDT pollution in the period 1965-68.

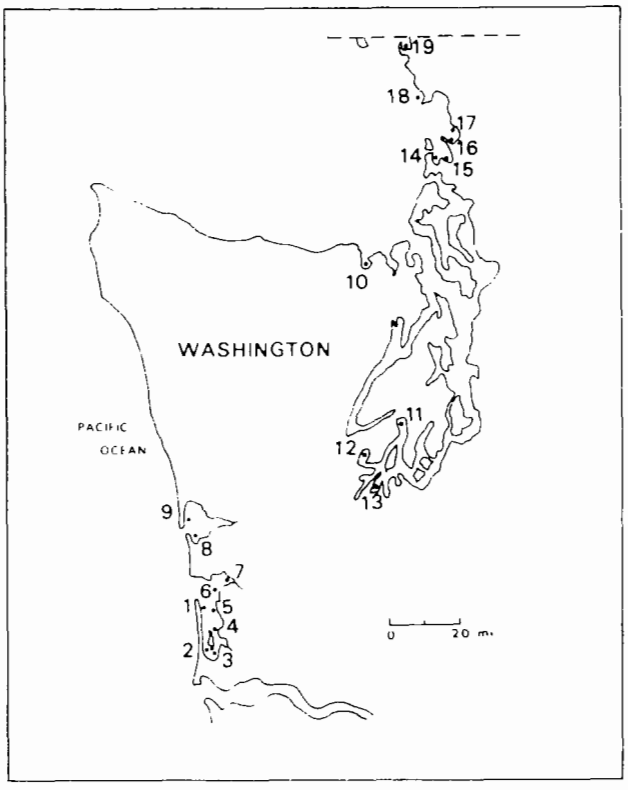


FIGURE O-1.—*Diagram of coastal Washington showing approximate location of monitoring stations*

1. Stackpole Harbor—Willapa Bay
2. Olson Slough—Willapa Bay
3. Bear River—Willapa Bay
4. Naselle River—Willapa Bay
5. Nemah River—Willapa Bay
6. Stony Point—Willapa Bay
7. South Bend—Willapa Bay
8. Beardslee Slough—Grays Harbor
9. Oyehut—Grays Harbor
10. Sequim Bay
11. North Bay Reserve—Puget Sound
12. Oakland Bay Reserve—Puget Sound
13. Mud Bay—Puget Sound
14. Padilla Bay—Padilla Bay
15. Swinomish—Padilla Bay
16. Scott Point—Samish Bay
17. Rock Point—Samish Bay
18. Lummi—Lummi Bay
19. Blaine—Drayton Harbor

TABLE O-1.—*Summary of data on organochlorine residues in the monitored species* (C. gigas), *1965-68—Washington*

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (µG/KG)	
				DDT	Dieldrin
1	Stackpole Harbor	1965-68	38	9 (25)	
2	Olson Slough	1966-68	30	7 (55)	
3	Bear River	1965-68	38	3 (17)	
4	Naselle River	1965-68	38	1 (11)	1 (120)
5	Nemah River	1965-68	39	4 (21)	
6	Stony Point	1965-68	39	10 (176)	
7	South Bend	1965-68	39	6 (23)	
8	Beardslee Slough	1965-68	37	2 (27)	
9	Oyehut	1966-68	36		
10	Sequim Bay	1966-68	31		
11	North Bay Reserve	1965-68	33		
12	Oakland Bay Reserve	1965-68	33		

TABLE O-1.—*Summary of data on organochlorine residues in the monitored species* (C. gigas), *1965-68—Washington*—Continued

Station Number	Location	Monitoring Period	Number of Samples [1]	Number of Positive Samples and Maximum Residue ( ) Detected in PPB (μg/kg)	
				DDT	Dieldrin
13	Mud Bay	1965-68	32		
14	Padilla Bay	1965-68	39	8 (17)	
15	Swinomish	1965-68	38	1 (T)	
16	Scott Point	1965-68	39	4 (10)	
17	Rock Point	1965-68	37		
18	Lummi	1965-68	38	23 (99)	
19	Blaine	1965-68	38		
	Occasional stations (2)	1966	3		
Total number of samples			695		
Percent of samples positive for indicated compound				11	<1

NOTE: T = >5 but <10 ppb.
[1] Each sample represents 15 or more mature mollusks.

TABLE O-2.—*Distribution of organochlorine residues in* C. gigas *for each sampling station by date of collection—Washington*
[Blank = no sample collected; — = no residue detected above 5 ppb; T = >5 but <10 ppb]

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 1.—STACKPOLE HARBOR—38 SAMPLES [1]													
1965	DDE										T	T	—
	TDE										T	—	—
	DDT										T	—	—
1966	DDE	—	—	T	T	T	13	11	—	T	—	—	—
	TDE	—	—	T	—	—	12	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	T	—	—	—		—	—	—
	TDE	—	—	—	—	T	—	—	—		—	—	—
	DDT	—	—	—	—	—	—	—	—		—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
STATION 2.—OLSON SLOUGH—30 SAMPLES [1]													
1966	DDE						—	20	T	—	—	—	—
	TDE						—	19	10	—	—	—	—
	DDT						—	16	—	—	—	—	—
1967	DDE	—	T	—	T	—	T	—	—		—	—	—
	TDE	—	11	—	T	—	12	—	—		—	—	—
	DDT	—	14	—	14	—	14	—	—		—	—	—

TABLE O-2.—*Distribution of organochlorine residues in* C. gigas *for each sampling station by date of collection—Washington*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 2.—OLSON SLOUGH—30 SAMPLES[1]—Continued													
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	T	—	—	—	—	—	—	—	—	—	—	—
	DDT	15	—	14	—	—	—	—	—	—	—	—	—
STATION 3.—BEAR RIVER—38 SAMPLES[1]													
1965	DDE										—	—	T
	TDE										—	—	—
	DDT										—	—	—
1966	DDE	—	—	—	—	—	T	—	—	—	—	—	—
	TDE	—	—	—	—	—	12	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	T	—	—		—	—	—
	TDE	—	—	—	—	—	T	—	—		—	—	—
	DDT	—	—	—	—	—	T	—	—		—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
STATION 4.—NASELLE RIVER—38 SAMPLES[1]													
1965	DDE										—	—	—
	TDE										—	—	—
	DDT										—	—	—
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—		—	—	—
	TDE	—	—	—	—	—	—	—	—		—	—	—
	DDT	—	—	—	—	—	—	—	—		—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	11	—	—	—	—	—	—	—	—	—	—	—
	Dieldrin	—	—	—	—	—	—	120	—	—	—	—	—
STATION 5.—NEMAH RIVER—39 SAMPLES[1]													
1965	DDE										—	T	—
	TDE										—	—	—
	DDT										—	—	—

TABLE O-2.—*Distribution of organochlorine residues in* C. gigas *for each sampling station by date of collection—Washington*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 5.—NEMAH RIVER—39 SAMPLES [1]—Continued													
1966	DDE	—	—	—	—	—	11	T	—	—	—	—	—
	TDE	—	—	—	—	—	10	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	T	—	—	—	—	—	—	—	—	—	—	—
STATION 6.—STONY POINT—39 SAMPLES [1]													
1965	DDE										T	T	—
	TDE										14	T	—
	DDT										T	—	—
1966	DDE	T	—	—	T	T	T	13	—	—	—	—	—
	TDE	T	—	—	T	—	11	11	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	26	—	—	—	—
	DDT	—	—	—	—	—	—	—	150	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	T	—	T	—	—	—	—	—	—	—	—	—
STATION 7.—SOUTH BEND—39 SAMPLES [1]													
1965	DDE										—	—	—
	TDE										—	—	—
	DDT										—	—	—
1966	DDE	T	—	T	T	T	—	10	—	—	—	—	—
	TDE	T	—	16	T	—	—	13	—	—	—	—	—
	DDT	—	—	—	T	—	—	—	—	—	—	—	—
1967	DDE	—	T	—	—	—	—	—	—	—	—	—	—
	TDE	—	T	—	—	—	—	—	—	—	—	—	—
	DDT	—	T	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—

TABLE O-2.—*Distribution of organochlorine residues in* C. gigas *for each sampling station by date of collection—Washington*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 8.—BEARDSLEE SLOUGH—37 SAMPLES [1]													
1965	DDE												—
	TDE												—
	DDT												—
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	T	—	T	—	—	—	—	—
	TDE	—	—	—	—	11	—	11	—	—	—	—	—
	DDT	—	—	—	—	11	—	10	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
STATION 9.—OYEHUT—36 SAMPLES [1]													
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
STATION 10.—SEQUIM BAY—31 SAMPLES [1]													
1966	DDE			—			—	—	—	—	—	—	—
	TDE			—			—	—	—	—	—	—	—
	DDT			—			—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	
	TDE	—	—	—	—	—	—	—	—	—	—	—	
	DDT	—	—	—	—	—	—	—	—	—	—	—	
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—

TABLE O-2.—*Distribution of organochlorine residues in* C. gigas *for each sampling station by date of collection—Washington*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 11.—NORTH BAY RESERVE—33 SAMPLES [1]													
1965	DDE												—
	TDE												—
	DDT												—
1966	DDE	—	—	—		—	—	—	—	—	—	—	—
	TDE	—	—	—		—	—	—	—	—	—	—	—
	DDT	—	—	—		—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—			
	TDE	—	—	—	—	—	—	—	—	—			
	DDT	—	—	—	—	—	—	—	—	—			
STATION 12.—OAKLAND BAY RESERVE—33 SAMPLES [1]													
1965	DDE												—
	TDE												—
	DDT												—
1966	DDE	—	—	—		—	—	—	—	—	—	—	—
	TDE	—	—	—		—	—	—	—	—	—	—	—
	DDT	—	—	—		—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—			
	TDE	—	—	—	—	—	—	—	—	—			
	DDT	—	—	—	—	—	—	—	—	—			
STATION 13.—MUD BAY—32 SAMPLES [1]													
1965	DDE												—
	TDE												—
	DDT												—
1966	DDE	—	—	—		—	—	—	—	—	—	—	—
	TDE	—	—	—		—	—	—	—	—	—	—	—
	DDT	—	—	—		—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—

TABLE O-2.—*Distribution of organochlorine residues in C. gigas for each sampling station by date of collection—Washington*—Continued

YEAR	COMPOUND	RESIDUES IN PPB (ug KG)											
		JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
STATION 13.—MUD BAY—32 SAMPLES [1]—Continued													
1968	DDE		—	—	—	—	—	—	—	—			
	TDE		—	—	—	—	—	—	—	—			
	DDT		—	—	—	—	—	—	—	—			
STATION 14.—PADILLA BAY—39 SAMPLES [1]													
1965	DDE										T	T	—
	TDE										—	—	—
	DDT										—	—	—
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	T	—	—	—	T	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	T	T	T	—
	TDE	—	—	—	—	—	—	—	—	—	—	T	—
	DDT	—	—	—	—	—	—	—	—	12	T	T	—
1968	DDE	T	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	T	—	—	—	—	—	—	—	—	—	—	—
STATION 15.—SWINOMISH—38 SAMPLES [1]													
1965	DDE										—	—	—
	TDE										—	—	—
	DDT										—	—	—
1966	DDE	—	—	—	—	—	—	—	—	—		—	—
	TDE	—	—	—	—	—	—	—	—	—		—	—
	DDT	—	—	—	—	—	—	—	—	—		—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	T	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
STATION 16.—SCOTT POINT—39 SAMPLES [1]													
1965	DDE										—	—	—
	TDE										—	—	—
	DDT										—	—	—

TABLE O-2.—*Distribution of organochlorine residues in* C. gigas *for each sampling station by date of collection—Washington*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 16.—SCOTT POINT—39 SAMPLES [1]—Continued													
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	T	T	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	T	T	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	T	—	—	—	—
STATION 17.—ROCK POINT—37 SAMPLES [1]													
1965	DDE										—	—	—
	TDE										—	—	—
	DDT										—	—	—
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—		—	
	TDE	—	—	—	—	—	—	—	—	—		—	
	DDT	—	—	—	—	—	—	—	—	—		—	
STATION 18.—LUMMI—38 SAMPLES [1]													
1965	DDE										—	—	—
	TDE										—	—	—
	DDT										—	—	—
1966	DDE	—	—	—	—	—	—	—	—	—	—	T	T
	TDE	—	—	—	—	—	—	—	—	—	—	15	—
	DDT	—	—	—	—	—	T	—	—	—	—	—	18
1967	DDE	T	—	T	T	T	11	T	T	—	T	—	T
	TDE	10	—	T	11	T	14	T	T	T	T	—	T
	DDT	25	19	29	44	42	74	34	14	15	21	21	19
1968	DDE	T	—	—	—	10	T	T	T	—	—	—	
	TDE	—	—	—	—	T	T	—	—	—	—	—	
	DDT	17	19	22	33	25	24	14	17	—	—	—	

TABLE O-2.—*Distribution of organochlorine residues in* C. gigas *for each sampling station by date of collection—Washington*—Continued

Year	Compound	Residues in PPB (μg/kg)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
STATION 19.—BLAINE—38 SAMPLES [1]													
1965	DDE										—	—	—
	TDE										—	—	—
	DDT										—	—	—
1966	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1967	DDE	—	—	—	—	—	—	—	—	—	—	—	—
	TDE	—	—	—	—	—	—	—	—	—	—	—	—
	DDT	—	—	—	—	—	—	—	—	—	—	—	—
1968	DDE	—	—	—	—	—	—	—	—	—	—	—	
	TDE	—	—	—	—	—	—	—	—	—	—	—	
	DDT	—	—	—	—	—	—	—	—	—	—	—	

[1] Each sample represents 15 or more mature mollusks.

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Accumulation and Movement of Mirex in Selected Estuaries of South Carolina, 1969-71 [1]

P. W. Borthwick[2], T. W. Duke[2], A. J. Wilson, Jr.[3], J. I. Lowe[2], J. M. Patrick, Jr.[2], and J. C. Oberheu[3]

ABSTRACT

In conjunction with a fire ant eradication program during which mirex was aerially applied to coastal areas near Charleston, S. C., field studies were conducted to monitor the movement and accumulation of mirex in the estuarine system.

Collections of background and periodic posttreatment samples of water, bottom sediments, shrimp, crabs, fish, and estuary-dependent birds and mammals were analyzed for mirex using electron-capture gas chromatography.

The data revealed that (1) mirex was translocated from treated lands and high marsh to estuarine biota—all animal classes sampled contained mirex; and (2) biological concentration of mirex occurred—especially in predators such as racoons and birds.

Mirex residue ranges for respective sample categories were: water (<10.01 ppb); sediment (0-0.07 ppm); crabs (0-0.60 ppm); fishes (0-0.82 ppm); shrimps (0-1.3 ppm); mammals (0-4.4 ppm); and birds (0-17.0 ppm). No mass mortalities were observed during the study.

Introduction

Mirex, a chlorinated hydrocarbon, is the insecticide component of a bait used in the Southeastern United States to control the imported fire ant (*Solenopsis saevissima richteri* Forel). This bait was developed after various pesticides and pesticide-bait formulations applied to control the ants proved to be toxic to nontarget organisms. Large-scale applications of dieldrin and heptachlor were especially destructive to fish and wildlife (*2*). Mirex was developed specifically to control fire ants and, until recently, was not considered to be toxic to nontarget organisms.

Independent experiments conducted under controlled conditions in the laboratory at Gulf Breeze, Fla., and at Bears Bluff, S. C., showed this chemical to be toxic to decapod crustaceans, including juvenile blue crabs and penaeid shrimp (*1, 5, 6*). Because of these and other results and concern of commercial fishermen that application of mirex to marsh areas could adversely affect fishery resources, application of mirex to the coastal environment was suspended and this cooperative study was undertaken.

The Gulf Breeze Laboratory (formerly a biological laboratory of the Bureau of Commercial Fisheries, Fish and Wildlife Service, U.S. Department of the Interior) entered an agreement with the U.S. Department of Agriculture in September 1969, to study the accumulation and movement of mirex in the estuarine environment near Charleston, S. C. The Bureau of Sport Fisheries and Wildlife of the Fish and Wildlife Service also agreed to participate in the study which terminated July 1, 1971.

The Gulf Breeze Laboratory was responsible for designing the study; collecting samples of bottom sediment, water, shrimp, crabs, and fish; and for analyzing all samples. The Bureau of Sport Fisheries and Wildlife was responsible for collecting birds and mammals.

The purposes of this investigation were (1) to observe the possible movement of mirex from treated areas near

[1] Contribution No. 156 from the Gulf Breeze Environmental Research Laboratroy, U.S. Environmental Protection Agency, Gulf Breeze, Fla. 32561, an Associate Laboratory of the National Environmental Research Center, Corvallis, Oreg.

[2] Gulf Breeze Environmental Research Laboratory, U.S. Environmental Protection Agency, Gulf Breeze, Fla. 32561.

[3] Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior, Atlanta, Ga. 30323.

Charleston, S. C., to the estuarine environment and (2) to determine levels of mirex in organisms, particularly crabs and shrimp, before, during, and after treatment of the area. The investigation began approximately 1 week before the first treatment was applied. Pretreatment samples were collected to establish "background" levels of mirex in the environment. The short time period between the start of the investigation and the application of mirex, however, precluded studies necessary to determine the ecological impact of this chemical on the study area.

Methods

STUDY AREA

The estuaries in which these studies were conducted border on a fire ant treatment area that extended 30 miles on either side of a line from Columbia to Charleston, S. C. The boundary of the treatment area ended approximately 12 miles from the coast (Fig. 1). Mirex, therefore, was not applied directly to the salt marsh, except in an experimental plot near Toogoodoo Creek.

The topography and biota of the estuarine environment are unique and often present special problems to environmental studies. Estuaries along this portion of the East Coast of the United States are protected by barrier islands and are supplied silt-laden fresh water by creeks and rivers on the mainland. These typical "Spartina" marshes support transient populations of crabs, shrimp, and fish that develop to maturity in the estuary, then return to the sea. In addition, resident populations of shellfish and some fin-fish inhabit the estuary throughout their lives. Many predatory birds and mammals depend upon the estuarine organisms for food.

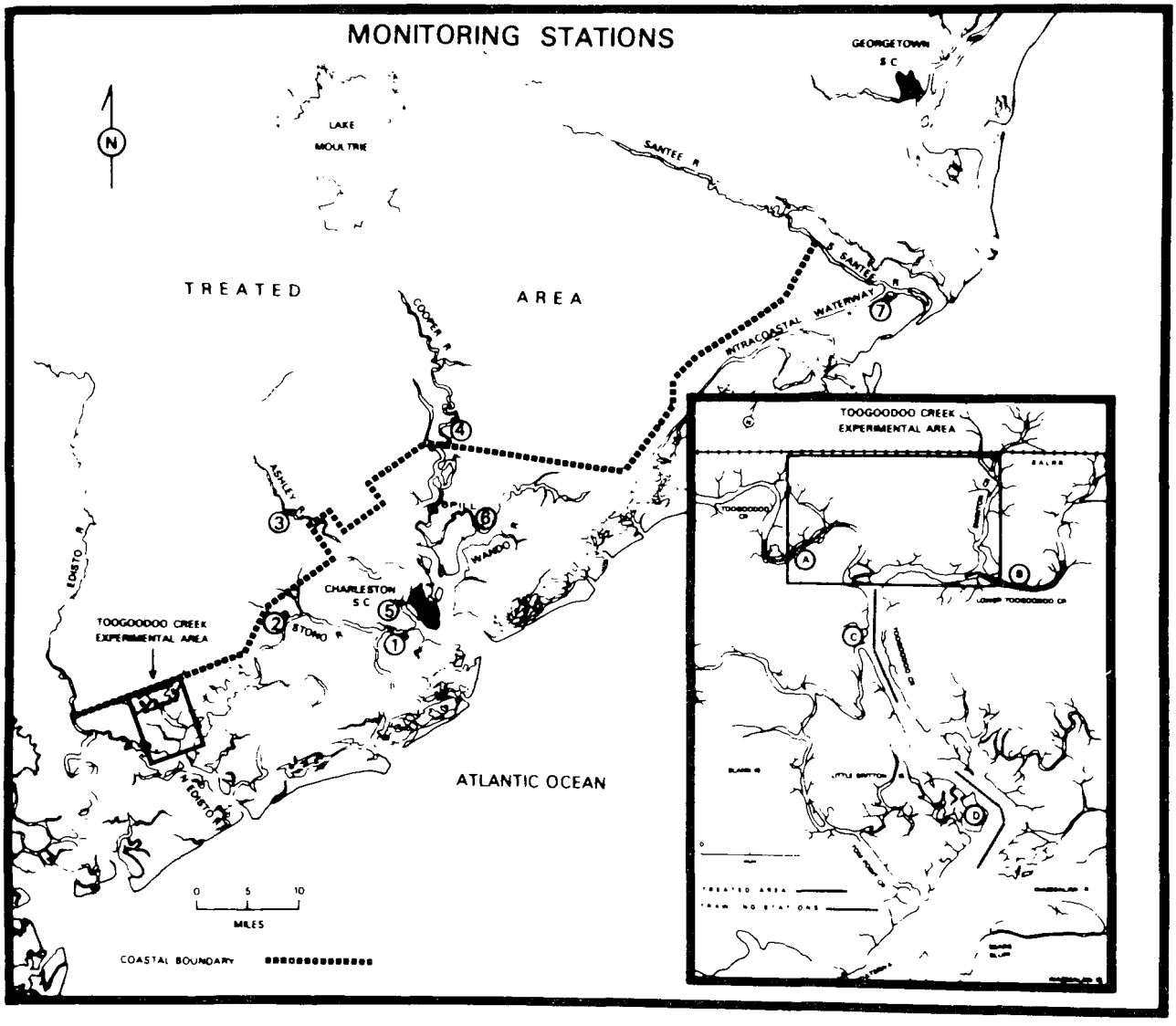


FIGURE 1.—*Sampling sites in selected estuaries of South Carolina, 1969-71*

Descriptions of sampling sites with the dates of mirex applications are given in Table 1.

Perodically, levels of mirex in water, sediment, and biota were monitored at (1) four stations near the main inland mirex-treated area and within and near a 2-square mile plot of salt marsh that was treated experimentally, (2) six stations located on the major rivers that drain the main inland mirex-treated areas, and (3) a station in a semi-enclosed tidal pond 4 miles from the main inland mirex-treated area; the banks of the pond (2.5 acres) were treated with mirex by hand spreader.

APPLICATION OF MIREX

The mirex 4X Bait formulation contained 84.7% corncob grits, 15.0% soybean oil, and 0.3% mirex. The bait was applied at a rate of 1.25 lb per acre or 1.7 g of technical mirex. This produced approximately 16 particles of bait per square foot.

TABLE 1.—*Description of sampling sites and dates of mirex application, South Carolina, 1969-71*

Map Location Number (Fig. 1)	Name and Location of Sampling Site	Remarks	Application Dates		
			1st	2d	3d
A	Toogoodoo Creek Lat. 32° 41′ N Long. 80° 18′ W	Within the main inland mirex-treated area on the upper reaches of the left branch of the Creek. Tidal marshlands predominate along the north bank; pine woodlands lie to the south. Several homes are in the area. Also treated experimentally by helicopter.	10/14-15/69	6/3-4/70	10/27-28/70
B	do.	Begins at the mouth of Swinton Creek and continues eastward on lower part of the Creek; partially within the mainland treated zone; marsh and woodland areas are on each bank. A few homes are along the south bank. Also treated experimentally by helicopter.	do.	do.	do.
C	do.	Extends southward from the fork of Toogoodoo Creek, just south of the experimental area treated by helicopter. Extensive farmlands are on the west bank, tidal marshlands on the east.	do.	do.	do.
D	do.	Begins 2 miles south of the helicopter-treated area and continues to the mouth of Toogoodoo Creek on the Intracoastal Waterway. Uninhabited low marsh areas on both sides of the creek are riddled with many small tidal creeks.	do.	do.	do.
1	Riverland Terrace Pond Lat. 32° 46′ N Long. 79° 59′ W	Seven miles from main inland treatment area, includes one of two adjoining 1-acre ponds in Riverland Terrace (a residential area). The east pond floods and drains with the tides into Wappoo Creek (Intracoastal Waterway), via two large culverts. The banks of the east pond were treated above the high tide mark by hand spreader.	12/3/69	7/24/70	12/1/70
2	Stono River (Intracoastal Waterway) at Log Bridge Creek Lat. 32° 45′ N Long. 80° 08′ W	Two miles from main inland treatment area. Several homes along the east side of the creek; the west side is bordered by tidal marshes.	10/23/69	6/18/70	—
3	Ashley River at Runnymeade Plantation Lat. 32° 53′ N Long. 80° 05′ W	Old plantations and rural homes are located on the east bank, and tidal marshes lie along the west side. This fresh water station is inside the main inland mirex-treated area.	10/22/69	6/11/70	—
4	Cooper River at U.S. Naval Ammunition Depot Lat. 32° 57′ N Long. 79° 56′ W	Bordered on the west bank by high wooded ground. On the east side, tidal marshes predominate. This fresh water station is inside the main inland mirex-treated area.	10/17/69	6/10/70	—
5	Ashley River at Oldtown Creek Lat. 32° 48′ N Long. 79° 58′ W	Six miles from the main inland treated area. The Citadel Military College is on the east bank, Charles Towne Landing on the west. Several industrial plants and homes, in addition to tidal marshes, are on this portion of the river.	10/22/69	6/10/70	—
6	Wando River at Beresford Creek Lat. 32° 53′ N Long. 79° 53′ W	Six miles from the main inland treated area. This uninhabited area is bordered by expansive marshlands and marsh islands.	10/17/69	6/8/70	—
7	South Santee River (Intracoastal Waterway) at Alligator Creek Lat. 32° 08′ N Long. 79° 19′ W	Six miles from the main inland treated area. This location is uninhabited and near the Cape Romain Migratory Bird Refuge.	9/18/69	5/20/70	—

Mirex 4X Bait was applied by fixed wing multi-engine aircraft (PV-2) to the inland treatment area, by helicopter to the Toogoodoo experimental plot, and by hand spreaders to the banks of a tidal pond. All applications were supervised by USDA.

The inland treatment area was divided into blocks that varied from 350,000 to 450,000 acres. An electronic guidance system (Decca Survey System) was set up in each block. This system consisted of three transmitting and receiving stations (one master station and two slave stations). The master and one of the slave stations produced an electronic tracking signal. Equipment mounted in the application aircraft received this signal which was fed through a computer into a dacometer. By use of the dacometer, the aircraft pilot could follow the signal from one point to another along the tracking path. Uniform application was made across each block by flying along a series of parallel signals. The master station and the second slave station produced a ranging signal that designated the points along the tracking path where the spraying was cut off. These signals, tracking and ranging, were converted to a numbering system and the system oriented to a map of the area being treated.

To allow for better control, helicopter applications of the mirex bait at the recommended rate were made to 800 acres of the 1,200-acre Toogoodoo Experimental Area. The Bell G-2-A helicopter used for spraying was equipped with two side-mounted hoppers (300 pounds capacity each) with electrically operated hopper gates, agitators, spinner-vane wheels, and spreaders for "positive control" at the "cut-off point." Flying at an airspeed of 40 miles per hour and an altitude of 80 feet produced a 75-foot wide swath; at 40 feet, a 60-foot wide swath. Helium-filled kytoons were used to mark each swath path. Bait distribution was even at both altitudes, but wind caused considerable aerial drift.

Approximately 2.5 acres surrounding the tidal pond were treated three times at 6-month intervals by hand-operated seed spreaders.

SAMPLING

Materials Sampled

Pesticides entering the estuarine environment become part of the biogeochemical cycles continually in operation within that environment. Therefore, the water, sediment, and various biota were sampled to determine routes of movement and reservoirs of the chemical in the study area. Common and scientific names of aquatic animals, birds, and mammals collected for mirex analysis are listed in Table 2. Crabs and shrimp were of special interest because of their sensitivity to mirex. Bottom and filter-feeding fish were also collected since they could accumulate mirex from food web organisms that were often difficult to obtain because of their transitory nature and movement to deeper water during the winter. In general, representatives of all species of aquatic organisms caught in the trawl were analyzed for mirex.

In selecting the birds and mammals for residue analysis, an effort was made to pick species that were (a) directly dependent on the estuarine environment for food; (b) most likely to live and feed in the area where they were collected; and (c) plentiful enough to allow periodic collections with a minimum of difficulty.

The raccoon was selected as the mammal for sampling since it is the most abundant carnivore in the salt marsh, where it preys heavily upon crustaceans and shellfish. It was more difficult to decide upon a bird species. Numerous gulls, shore birds, wading birds, and other water birds are found in the estuary, but most of them are migratory by nature, moving up and down the coast in search of food or as a result of weather changes. Few water birds are year-round residents. The clapper rail was finally selected as the most sedentary and widely distributed bird in the estuaries; when rails were not available at a particular sampling station, wading birds were collected. All animals collected during this study are listed in Table 2.

Collection of samples

Aquatic animals were collected with a 12-foot, ¾-inch bar mesh, otter trawl towed at 3 to 6 knots for 20 minutes at each station. Pond collections were made with a 15-foot, ¼-inch mesh haul seine. Occasionally, animals near the surface were taken with dip nets; fidder crabs and oysters were collected by hand. Raccoons were captured with wire live-traps; birds were hunted on foot or from a boat.

Several methods for collecting water (including carbon filtration) were considered, but because of the high silt content of the well-mixed water, water was collected just below the surface in a 1-gallon glass jug and sealed with a teflon-lined cap. A modified grab sampler was used to collect bottom sediment at each station. A sampler especially designed to collect the upper few centimeters of bottom sediment was used in specified instances.

Frequency of sampling

A pretreatment (background) and numerous posttreatment samples were taken at all stations. Quarterly collections of birds and mammals were scheduled around the first week in September, December, March, and June. Periodically, this schedule was altered by 2 or 3 weeks to select the week of highest tides for the best

TABLE 2.—*Common and scientific names of aquatic animals, birds, and mammals collected for mirex residue analysis*

CRABS	
Blue crab	*Callinectes sapidus* Rathbun
Common mud crab	*Panopeus herbstii* H. Milne Edwards
Mud crab	*Rithropanopeus harrisii* Gould
Portunid crab	*Callinectes ornatus* Ordway
Sand fiddler	*Uca pugilator* (Bosc)
SHRIMPS	
Brown shrimp	*Penaeus aztecus* Ives
Brown-spotted shrimp	*Penaeus duorarum* Burkenroad
Grass shrimp	*Palaemonetes pugio* Holthuis
River shrimp	*Macrobrachium ohione* (Smith)
White shrimp	*Penaeus setiferus* (Linnaeus)
FISHES	
American eel	*Anguilla rostrata* (Lesueur)
Atlantic croaker	*Micropogon undulatus* (Linnaeus)
Atlantic menhaden	*Brevoortia tyrannus* (Latrobe)
Atlantic silverside	*Menidia menidia* (Linnaeus)
Atlantic thread herring	*Opisthonema oglinum* (Lesueur)
Bay anchovy	*Anchoa mitchilli* (Valenciennes)
Blackcheek tonguefish	*Symphurus plagiusa* (Linnaeus)
Black drum	*Pogonias cromis* (Linnaeus)
Black sea bass	*Centropristis striata* (Linnaeus)
Bluefish	*Pomatomus saltatrix* (Linnaeus)
Fourspot flounder	*Paralichthys oblongus* (Mitchill)
Hogchoker	*Trinectes maculatus* (Bloch and Schneider)
Mummichog	*Fundulus heteroclitus* (Linnaeus)
Pinfish	*Lagodon rhomboides* (Linnaeus)
Sailfin molly	*Poecilia latipinna* (Lesueur)
Sea catfish	*Arius felis* (Linnaeus)
Searobin	*Prionotus* sp.
Sheepshead	*Archosargus probatocephalus* (Walbaum)
Silver perch	*Bairdiella chrysura* Lacépède
Snook	*Centropomus undecimalis* (Bloch)
Southern kingfish	*Menticirrhus americanus* (Linnaeus)
Spot	*Leiostomus xanthurus* Lacépède
Spotted hake	*Urophycis regius* (Walbaum)
Spotted seatrout	*Cynoscion nebulosus* (Cuvier and Valenciennes)
Star drum	*Stellifer lanceolatus* (Holbrook)
Striped killifish	*Fundulus majalis* (Walbaum)
Striped mullet	*Mugil cephalus* Linnaeus
Weakfish	*Cynoscion regalis* (Bloch and Schneider)
White catfish	*Ictalurus catus* (Linnaeus)
White mullet	*Mugil curema* Valenciennes
Winter flounder	*Pseudopleuronectes americanus* (Walbaum)
MISCELLANEOUS AQUATIC ANIMALS	
American oyster	*Crassostrea virginica* (Gmelin)
Brief squid	*Lolliguncula brevis* (Blainville)
Nudibranch	*Doris* sp.
Southern periwinkle	*Littorina irrorata* (Say)
BIRDS	
American bittern	*Botaurus lentiginosus* (Rackett)
American egret	*Casmerodius albus* (Linnaeus)
Anhinga	*Anhinga anhinga* (Linnaeus)
Belted kingfisher	*Megaceryle alcyon* (Linnaeus)
Clapper rail	*Rallus longirostris* Boddgert
Common snipe	*Capella gallinago* (Linnaeus)
Green heron	*Butorides virescens* (Linnaeus)
Least bittern	*Ixobrychus exilis* (Gmelin)
Little blue heron	*Florida caerulea* (Linnaeus)
Louisiana heron	*Hydranassa tricolor* (Müller)
Marsh hawk	*Circus cyaneus* (Linnaeus)
Pied-billed grebe	*Podilymbus podiceps* (Linnaeus)
Plover	*Charadrius* sp.
Snowy egret	*Leucophoyx thula* (Molina)
Sora rail	*Porzana carolina* (Linnaeus)
Virginia rail	*Rallus limicola* Vieillot
White ibis	*Eudocimus albus* (Linnaeus)
Willet	*Catoptrophorus semipalmatus* (Gmelin)
Yellow-crowned night heron	*Nyctanassa violacea* (Linnaeus)
MAMMALS	
Opossum	*Didelphis virginiana* (Linnaeus)
Raccoon	*Procyon lotor* (Linnaeus)

rail hunting. Samples were taken at the six river stations 24 hours and 3 months after each of two applications of mirex to the inland treatment area by fixed wing aircraft. Biweekly collections were made at the four stations in the Toogoodoo Creek Plot during the entire 18 months of the study; mirex was applied to the experimental plot by helicopter three times at 6-month intervals to high marsh only, i.e., marsh not normally covered by tidal waters. Samples were taken from the tidal pond 24 hours after each of three hand-spread treatments and at irregular intervals between applications.

ANALYTICAL PROCEDURES

Preparation of samples

Crabs, shrimp, and fish were prepared separately by pooling whole individuals, but birds and mammals were prepared individually. Muscle tissue from breast and upper wing in birds and thigh in raccoons and oil glands and eggs from birds were analyzed. All samples were ground and mixed thoroughly in a blender. A 30-g subsample was blended with a desiccant mix composed of 10% QUSO (a microfine precipitated silica) and 90% anhydrous sodium sulfate. This mixture was alternately frozen and blended until a free-flowing powder was obtained.

Sediment samples were spread on sheets of aluminum foil and dried at room temperature. The dry sediment was pulversized to a fine powder in a blender.

At this stage of preparation, the samples were wrapped in aluminum foil, packaged in plastic bags, and mailed to the Gulf Breeze Laboratory for extracting and pesticide residue analysis.

Water samples were refrigerated for up to 2 weeks until they were mailed to Gulf Breeze for analysis.

Analysis of samples

Tissues of shrimp, crabs, and fish mixed with the desiccant were extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated to approximately 10 ml and transferred in 3- to 4-ml portions to a 400-mm by 20-mm chromatographic column that contained 76 mm of unactivated Florisil. After each portion settled in the column, vacuum was applied to evaporate the solvent. This was repeated after each addition and after three 5-ml petroleum ether rinses of the extraction flask. The vacuum pump was disconnected after all solvent had evaporated, and the residue was eluted from the column with 70 ml of 9:1 mixture of acetonitrile and distilled water. The eluate was evaporated to dryness and the residue transferred to a Florisil column (7) with petroleum ether.

Sediment samples were dried at room temperature and extracted for 4 hours with 10% acetone in petroleum ether in a Soxhlet apparatus. Extracts were concentrated to approximately 10 ml and transferred to a Florisil column (7).

Water samples were not filtered before being extracted with petroleum ether. The extracts were dried with anhydrous sodium sulfate and reduced to an appropriate volume.

The extracts of all substrates were identified and measured by electron capture gas chromatography. Extract volumes were adjusted to obtain a sensitivity of 0.01 ppm (mg/kg) for tissue and sediment samples and 0.01 ppb (μg/liter) for water samples. Operating conditions of the two 152.4-cm by 3.2-mm glass columns used were:

Liquid phase:	2% OV-101	1:1 2% OV-101
Solid Support:	100 120 Gas Chrom Q	100/120 Gas Chrom Q
Temperatures:		
Oven:	188° C	180° C
Injector and detector	210° C	210° C
N_2 flow rate:	25 ml min	25 ml min

Laboratory tests indicated recovery rates for mirex were greater than 85%. Data in this report do not include a correction factor for percent recovery. All residues reported are on a wet-weight basis, except those of sediments, which are reported on a dry-weight basis. Thin layer chromatography, "p" values, and mass spectrometry were used to confirm the presence of mirex.

STATISTICAL ANALYSIS OF DATA

All statistical comparisons were made with the χ^2-test for independent samples (8), and differences were considered real at the 0.01 level of significance. The movement of mirex into the aquatic environment and its consequent accumulation in populations of estuarine animals were presumed to be greatest in areas where a significantly greater proportion of samples was positive for mirex (>0.01 ppm).

Average residues reported were computed by assuming that samples where mirex was not detected (<0.01 ppm) actually had no residue.

Bird and mammal residue data were not analyzed statistically; however, average mirex residues in muscle tissues are tabulated for herons and egrets (Table 10), clapper rails (Table 11), and raccoons (Table 13).

Results

PRETREATMENT SAMPLES

Mirex was not detected in any pretreatment samples of crabs, shrimp, fish, sediment, or water taken from the six monitoring stations, Toogoodoo stations, or the Riverland Terrace pond. Mirex residues were found in about one-third of the pretreatment samples of migratory birds (Table 9). Only one raccoon was collected before treatment, and this sample was free of mirex residues. Background residues in birds collected in the Charleston area are discussed in the section on "Significance of Data."

WATER AND SEDIMENT SAMPLES

Mirex was not detected in water samples during the study. No attempt was made, however, to concentrate water samples (such as carbon filtration of large volumes of water).

Sediment samples were negative, except in six instances (three samples from Riverland Terrace pond and three from the Ashley River within the treated zone). Accordingly, mirex residues in water and sediment are not reported in tabular form.

ACCUMULATION OF MIREX IN BIOTA

Biological concentration of mirex occurred in the estuarine food web as shown below:

Sample	Residue Range (ppm)	Percent of Posttreatment Samples with Mirex Residues
Water	< 0.01 ppb	0
Sediment	0*-0.07	3
Crabs	0-0.60	31
Fishes	0-0.82	15
Shrimps	0-1.3	10
Mammals	0-4.4	54
Birds	0-17.0	78

*0 = < 0.01 ppm

Differences in the amounts of mirex accumulated by the animals were probably caused by variables such as proximity to the treated area, duration of exposure to a

mirex-contaminated habitat, seasonal habits, avoidance ability, and position in the food web.

Additional variation may depend upon parameters such as method of application, amount and frequency of rainfall, surface runoff, variations in sea level, and degradation.

Levels of mirex found in the biota are listed in Tables 5 to 14.

Discussion

ANIMAL MORTALITIES

Procedures used in this study were neither capable of, nor intended to comprehensively detect mortality of aquatic organisms. The sampling areas were, however, inspected for dead or affected animals during each sampling period, and mass mortalities would probably have been detected visually or in trawl catches. No mass mortalities of organisms were observed during the study. Our laboratory experiments (5) suggest that mortalities in a population of marine crustaceans due to mirex would not all occur at the same time. Symptoms of mirex poisoning exhibited by shrimp and crabs prior to death are irritability, uncoordinated movement, loss of equilibrium, and paralysis. An affected crab may live several days or even weeks after the initial exposure to mirex. Animals in advanced stages of poisoning would be highly susceptible to predation by larger carnivores and could be swept out of estuaries by tidal action. Thus, affected animals could be removed from the system without leaving visible evidence of their condition. Further, any dead animals would generally enter the detritus pool soon after death.

TOOGOODOO CREEK EXPERIMENTAL AREA—*1,200-acre plot (Fig. 1) treated by helicopter on Oct. 14-15, 1969; June 3-4, 1970; and Oct. 27-28, 1970*

Movement of mirex from treated lands above the high-tide mark undoubtedly occurred after each of the three treatments, especially the first. The mechanisms of transporting mirex from treated land areas to the Toogoodoo Creek estuary are poorly understood. Surface runoff into the drainage system (especially after heavy rain) is one suspected cause.

All species sampled contained mirex. Residues first appeared in a shrimp sample 2 weeks after the first treatment. From then on, the relative frequency of mirex-positive samples and the "average" levels of mirex residues fluctuated greatly. Mirex was present, however, in at least one sample in 33 of 44 collections. Some of the statistically significant relationships occurring within these fluctuations are discussed in the following paragraphs.

Application effects

The relative number of samples that contained mirex appeared to increase during the first 10 weeks after each treatment and then to decrease in the 10- to 20-week period (Table 3). After the first and third treatments, the number of positive samples appeared to decrease even further in the third interval, 20 to 32 weeks.

These decreases for each individual treatment were not statistically significant; however, when data from all treatments were evaluated together, the relationship between time elapsed since spraying and the decreasing number of positive samples proved to be real.

Decreases in the percent occurrence of mirex residues from the first to the third treatment were significant. This could be due to: (1) mirex being translocated rapidly from the estuarine biota to reservoirs in fatty tissues of predacious birds and mammals, (2) transient estuarine animals (e.g., crabs, shrimp) translocating mirex during emigration from nursery areas of Toogoodoo Creek, (3) possible differences in the manner in which mirex bait was applied by the two helicopter pilots, or (4) degradation of mirex by physical, chemical, or biological processes.

Location effects

The relative number of samples positive for mirex also fluctuated with station location. Stations A and B were located within the treated area, and Stations C and D were downstream from the treated area (Fig. 1). Mirex levels at these stations gave some indication of the movement of mirex from treated land areas into untreated areas downstream.

As expected, more animals from the treated area contained mirex than did those from downstream stations (Table 4). This relationship was statistically significant after the first and third applications, but was only apparent after the second treatment. The frequencies of positive samples at stations located within the treated zone (A and B) were not significantly different. As shown in Table 4, there was an apparent decrease in frequency of positive samples with increased distance from the treated zone.

Species effects

The percent occurrence of mirex was higher in crab samples than in fish or shrimp samples. Although this difference was apparent at Stations A through D, it was statistically significant only at Station B. Overall, however, the higher residues in crabs were significant after the first two helicopter treatments, but was not statistically significant after the third treatment. The frequency of positive samples appeared unrelated to size of crabs or to species of fish.

TABLE 3.—*Percent occurrence of mirex in crab, shrimp, and fish samples by time of sample collection in respect to mirex application at Toogoodoo Creek Experimental Area*

Application	Percent Occurrence of Mirex by Sampling Time in Weeks Since Application			
	0-10	10-20	20-32	Total Weeks
First (Oct. 14-15, 1969)	37	36	18	30 (0-32 weeks)
Second (June 3-4, 1970)	22	9		16 (0-20 weeks)
Third (Oct. 27-28, 1970)	28	12	2	12 (0-32 weeks)
Overall Percent Occurrence	28	17	8	19

TABLE 4.—*Percent occurrence of mirex in aquatic animals by sampling site with respect to treated area*

Sampling Site	Location with Respect to Treated Area	Percent Occurrence Mirex in All Post-Treatment Samples of Crabs, Shrimp, and Fish
A	Inside treated area	29%
B	Inside treated area	24%
C	Just outside treated area	17%
D	2 miles downstream from treated area	8%
3	Upstream stations located inside main inland treated area	70%
4		70%
2	Downstream stations located outside main inland treated area	30%
5		0%
6		25%
7		0%

MONITORING STATIONS—*mirex was applied to inland treatment areas (Fig. 1) by fixed-wing aircraft during September-Ocotber 1969 and May-June 1970*

Trends in the data were similar to those observed in the Toogoodoo Creek Experimental Area. The greatest number of positive samples occurred shortly after treatment and diminished with time. Samples positive for mirex were significantly more frequent within the treated area, upstream Stations 3 and 4, than at sites located outside the treated area, downstream Stations 2, 5, 6, and 7 (Table 4).

Although significant differences in percent occurrence were noted between these two groups, individual stations showed no significant differences because too few samples were taken and too few positive samples occurred for χ^2-analysis. Also, for the individual monitoring stations, the relative number of samples containing mirex did not vary depending on the type of animal sampled.

A more frequent occurrence of mirex residues was apparent after the second application than after the first, but this increase was not significant.

RIVERLAND TERRACE POND—*a 2.5-acre zone around the pond treated by hand-operated seed spreader on Dec. 3, 1969; July 24, 1970; and Dec. 1, 1970*

Although mirex was applied three times to the banks of the pond, it was obviously not accumulated by sampled biota. During the study, only two crab samples contained mirex. In addition, three sediment samples were positive. The method of treatment (mirex applied by hand to a narrow bank around the pond above the high-tide mark on hard mud banks) might have caused the occurrence of the mirex in the sediment samples. "Crab samples" of sediment often consisted of as many as 20 to 25 "grabs" taken near the edge of the pond and one or more particles of bait could have been picked up with the sample.

Special attention was given Riverland Terrace Pond to observe any individual- or mass-mortalities in the pond biota. Migration of animals was controlled by means of retaining screens (¼-inch mesh) placed over two culverts that flood and drain the treated pond. Daily screen checks were made for a 3-week period to reveal any distressed, moribund, or dead animals. Crabs, shrimp, and fish observed in the pond or on the screens never appeared to be affected.

During several pond collections after treatment, seine-hauls revealed large populations of grass shrimp (mostly *Palaemonetes pugio)* and many of the females were gravid. On one occasion, grass shrimp were held for several weeks in an aquarium, where the shrimp remained healthy and their eggs seemed to develop and hatch normally. At no time was mirex detected in the grass shrimp population.

SIGNIFICANCE OF DATA

Surprisingly, mirex appeared in one-third of the birds in the pretreatment sample. Since the study area had not been previously treated with a large-scale application of mirex, the birds must have accumulated the residues from some other area. A large acreage around Savannah, Ga., had been treated with three successive applications of mirex in a pilot study of the feasibility of eradication. This area is the likely source of the mirex residues. Thus, migration is an important factor in interpreting the study data. Stewart's (9) report that northern clapper rails banded at Chincoteague, Va. migrated southward to winter in the coastal marshes of the South Atlantic States, including South Carolina, supports this view.

Measurable levels of mirex appeared at all stations, demonstrating that tidal flushing, biological transport, or some other mechanism can distribute the chemical throughout the estuary, regardless of precautions taken

to avoid treatment in the tidal zone. This finding is evidence that mirex can become widespread in animal food webs.

The occurrence and amount of mirex in birds and mammals varied considerably at all stations. This is to be expected since all of these animals are more or less migratory, and food sources of individuals vary. Similarly, Keith (*4*) found that levels of insecticide residues in fish-eating birds vary considerably within local populations of most species. Even so, average residues that appeared at the different stations correlated well with station location in respect to treated area, with the highest residues occurring at stations within a treated area and the lowest at stations farthest from the source of contamination.

Approximately 78% of the 179 birds collected after treatment began contained measurable residues of mirex, whereas residues were present in only 54% of the raccoons. The greater mobility of birds is doubtless the reason for this difference. In any case, occurrence of mirex residues as well as the quantity of residue in the animal appear directly related to drainage and distance from a treated area.

Residues in animals collected from the 2-square-mile treatment area on the Toogoodoo creek marshes (Stations A, B, and C) were not as great as those from animals collected within the inland treatment zone. This is not surprising because water as well as food-chain organisms in the Toogoodoo marshes were flushed twice daily by 4- to 6- foot tides.

Local and seasonal migrations of the sampled species would tend to mask any evidence of residue buildup during the course of the study. Even so, the absence of any individuals with greatly elevated residue levels indicates that average levels did not continue to rise beyond levels reached in the first few months following treatment. Raccoons were the most sedentary animals sampled. Data in Table 12 indicate that there was no gradual buildup of mirex residues in raccoons, although some seasonal variations are apparent.

The highest mirex residue (17.0 ppm) found in any animal analyzed occurred in a kingfisher. The highest level found in a raccoon was 4.40 ppm. All birds and mammals that contained residues in excess of 1.00 ppm are listed in Table 14. All animals on this list, except the kingfisher from Station D and two raccoons from Station 2, came from stations classified as having a "high" mirex exposure potential.

See Appendix for chemical name of mirex

Acknowledgment

Special thanks are due Dr. Nelson Cooley for editorial help, Dave Hansen for assistance with statistical analysis, Jerry Forester and Johnnie Knight for analysis of samples, and Madeleine Brown and Steve Foss for assistance in manuscript preparation—all of the Gulf Breeze Environmental Research Laboratory, U.S. Environmental Protection Agency.

We also thank the following persons who supervised the application of mirex or approved or conducted various aspects of the collection and preparation of samples: Julian Mikell, Plant Protection Division U.S. Department of Agriculture; Charles Bearden and Michael McKenzie, Division of Marine Resources, S.C. Wildlife Resources Department; Alston Badger, Bears Bluff Field Station, U.S. Environmental Protection Agency; Frank McKinney, Grice Marine Biological Laboratory, College of Charleston.

LITERATURE CITED

(*1*) *Bookhout, C. G., A. J. Wilson, Jr., T. W. Duke, and J. I. Lowe. 1972.* Effects of mirex on the larval development of two crabs. Water, Air, Soil Pollut. 1(2):165-180.

(*2*) *Carson, R. 1962.* Silent Spring. p. 161-169. Houghton Mifflin Co., Boston, Mass.

(*3*) *Frear, D. E. H. 1969.* Pesticide Index, 4th ed. 399 p. College Science Publishers, State College, Pa. 16801.

(*4*) *Keith, J. O. 1968.* Insecticide residues in fish-eating birds and their environment. Aves 5(1):28-41.

(*5*) *Lowe, J. I., P. R. Parrish, A. J. Wilson, Jr., P. D. Wilson, and T. W. Duke. 1971.* Effects of mirex on selected estuarine organisms. *In* Transactions of the 36th North Am. Wildl. Natl. Resour. Conf. p. 171-186.

(*6*) *McKenzie, M. D. 1970.* Fluctuations in abundance of the blue crab and factors affecting mortalities. S. C. Wildl. Resour. Dep., Marine Resour. Div., Tech. Rep. No. 1, 45 p., Charleston, S. C.

(*7*) *Mills, P. A., J. H. Onley, and R. A. Gaither. 1963.* Rapid method for chlorinated pesticide residues in nonfatty foods. J. Assoc. Off. Anal. Chem. 46(2):186-191.

(*8*) *Siegel, S. 1956.* Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Co., Inc., New York, N. Y.

(*9*) *Stewart, R. E. 1954.* Migratory movements of the northern clapper rail. Bird Banding 25(1):1-5.

TABLE 5.—*Mirex residues in shrimps by sampling site and sampling time, South Carolina, 1969-71*
[— = not detected]

Species	Interval From Mirex Application to Sampling	Number in Composite Sample	Size of Individuals in Composite Samples (inches)	Mirex Residue (ppm—whole body)
SAMPLING SITE A—TOOGOODOO CREEK				
White shrimp	Background	3	2.5	—
First application (Oct. 14-15, 1969)				
White shrimp	24 hrs	4	2.5	—
	2 wks	4	2.5	—
	4 wks	10	4-5	.014
Brown shrimp	30 wks	13	2.5-3.5	—
Brown-spotted shrimp	32 wks	10	3.5-4	—
Second application (June 3-4, 1970)				
Brown-spotted shrimp	24 hrs	12	3.5-5.5	.014
	2 wks	12	5-5.5	—
	4 wks	12	5.5-6	—
	6 wks	12	5-6	.024
	8 wks	12	3.5-5	—
White shrimp	10 wks	12	3.5-4.5	—
	12 wks	12	2.5-3.5	—
	14 wks	12	3-4.5	—
	16 wks	12	4-4.5	—
	18 wks	11	5.5-6	—
	20 wks	12	5-6	—
Third application (Oct. 27-28, 1970)				
White shrimp	24 hrs	12	5-6	—
	2 wks	4	5-6	—
Brown-spotted shrimp	20 wks	5	2.25-2.5	—
	24 wks	5	2.5-3.5	—
	28 wks	4	3.5-4.5	—
	30 wks	12	2.5-5	—
	32 wks	12	2-4	—
SAMPLING SITE B—TOOGOODOO CREEK				
White shrimp	Background	4	3	—
First application (Oct. 14-15, 1969)				
White shrimp	24 hrs	4	3	—
	2 wks	4	3-4	.040
	4 wks	10	4-5	.052
Brown-spotted shrimp	30 wks	2	3-5	—
	32 wks	3	4	—
Second application (June 3-4, 1970)				
Brown-spotted shrimp	24 hrs	12	5-6	—
	2 wks	12	5-5.5	—
	4 wks	2	4-6	—
	6 wks	5	4-7	—
	8 wks	12	4-5	—
White shrimp	10 wks	12	3-4	—
	12 wks	12	2-3	—
	14 wks	12	3.5-4.5	—
	16 wks	12	4-4.5	—
	18 wks	12	5-6	—
	20 wks	12	5	—
Third application (Oct. 27-28, 1970)				
White shrimp	24 hrs	12	5-6	—
	2 wks	3	4.5-6	—
Brown-spotted shrimp	18 wks	3	3-4.5	—
	22 wks	5	2-4	—
	24 wks	10	3-4	—
	26 wks	4	3-4.5	—
	28 wks	12	3-5.5	—
	30 wks	11	2.75-5	—
	32 wks	12	3-4.5	—

Species	Interval From Mirex Application to Sampling	Number in Composite Sample	Size of Individuals in Composite Samples (inches)	Mirex Residue (ppm—whole body)
SAMPLING SITE C—TOOGOODOO CREEK				
White shrimp	Background	3	5	—
First application (Oct. 14-15, 1969)				
White shrimp	24 hrs	6	4-5	—
	2 wks	4	3	—
	4 wks	10	4-5.5	.020
	6 wks	10	3.5-4	.014
Brown-spotted shrimp	30 wks	1	5.5	—
	32 wks	3	6-7	—
Second application (June 3-4, 1970)				
Brown-spotted shrimp	24 hrs.	9	4-5	.015
	2 wks	12	3-5	—
	4 wks	12	4-6	—
	6 wks	12	5-6	—
	8 wks	4	4	—
White shrimp	10 wks	1	4.5	—
	12 wks	12	2.5-4	—
	14 wks	12	4.5-5.5	—
	16 wks	12	4-4.5	—
	18 wks	12	5.5-6	—
	20 wks	10	5-6	—
Third application (Oct. 27-28, 1970)				
White shrimp	24 hrs	12	5-6	—
	2 wks	10	5-7	—
Brown-spotted shrimp	20 wks	4	3.5-4.5	—
Grass shrimp	24 wks	132	.75-1.25	—
Brown-spotted shrimp	26 wks	9	3.5-5.5	—
	28 wks	12	3.5-5	—
	30 wks	12	4-5	—
	32 wks	12	3.5-5	—
SAMPLING SITE D—TOOGOODOO CREEK				
White shrimp	Background	2	6	—
First application (Oct. 14-15, 1969)				
White shrimp	24 hrs	2	5	—
	2 wks	2	4.5	—
	4 wks	7	5-6	—
	6 wks	10	4-5	—
	8 wks	15	3.5-5	.027
Brown-spotted shrimp	24 wks	2	4.5-5	—
	30 wks	2	3-3.5	—
Second application (June 3-4, 1970)				
Brown-spotted shrimp	24 hrs	12	4-5	—
	2 wks	12	5-6	—
	4 wks	2	5	—
	6 wks	12	5-6	—
White shrimp	10 wks	4	3.5-5.5	—
Brown-spotted shrimp	12 wks	12	3-4.5	—
White shrimp	14 wks	12	4.5-5.5	—
	16 wks	12	4-5	—
	18 wks	12	5.5-6	—
	20 wks	10	5-6	—
Third application (Oct. 27-28, 1970)				
White shrimp	24 hrs	12	5-6	—
	2 wks	1	4.5	—
Brown-spotted shrimp	20 wks	2	5	—
	24 wks	6	3.5-4.5	—

TABLE 5.—*Mirex residues in shrimps by sampling site and sampling time, South Carolina, 1969-71*—Continued

[— = not detected]

Species	Interval From Mirex Application to Sampling	Number in Composite Sample	Size of Individuals in Composite Samples (inches)	Mirex Residue (ppm—whole body)
SAMPLING SITE D—TOOGOODOO CREEK—Continued				
Grass shrimp	24 wks	100	.75-1.25	—
	26 wks	12	2.5-4	—
	28 wks	12	3-4	—
	30 wks	1	4.5	—
	32 wks	6	3.5-5.5	—
STATION 1—RIVERLAND TERRACE POND (EAST)				
Grass shrimp	Background	178	.75-1	—
First application (Dec. 3, 1969)				
Grass shrimp	48 hrs	144	.75-1	—
	5 mos	77	1	—
Grass shrimp	Background	100	.75-1.25	—
Second application (July 24, 1970)				
White shrimp	72 hrs	5	2-3	—
Grass shrimp	3 mos	146	1-1.25	—
Brown-spotted shrimp	3 mos	5	2.25-3	—
Grass shrimp	4 mos.	176	.75-1.25	—
Third application (Dec. 1, 1970)				
Grass shrimp	48 hrs	159	.75-1.25	—
	2 wks	167	.75-1.25	—
	6 wks	193	.75-1.25	—
	3 mos	126	.75-1.25	—
STATION 2—STONO RIVER, LOG BRIDGE CREEK				
White shrimp	Background	13	Medium	—
Brown shrimp	Background	9	Small	—
First application (Oct. 23, 1969)				
White shrimp	24 hrs	4	3-4	—
Second application (June 18, 1970)				
Brown-spotted shrimp	24 hrs	12	4.5-5.5	—
White shrimp	3 mos	12	3.5-4.5	—
STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION				
River shrimp	Background	9	1.5-2.5	—
First application (Oct. 22, 1969)				
Second application (June 11, 1970)				
Brown-spotted shrimp	24 hrs	3	3-4	.11
White shrimp	3 mos	2	4.5	—
STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT				
First application (Oct. 17, 1969)				
River shrimp	24 hrs	1	3	1.3
White shrimp	24 hrs	5	2.5-3	.26
Second application (June 10, 1970)				
White shrimp	3 mos	10	3-4.5	—
STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK				
White shrimp	Background	6	4	—
First application (Oct. 22, 1969)				
White shrimp	24 hrs	4	3-4	—
Second application (June 10, 1970)				
Brown-spotted shrimp	24 hrs	3	2.5-3	—
White shrimp	3 mos	7	3-6	—
STATION 6—WANDO RIVER, BERESFORD CREEK				
White shrimp	Background	4	4.5-5.5	—
First application (Oct. 17, 1969)				
White shrimp	24 hrs	3	4-6	—
Second application (June 8, 1970)				
Brown-spotted shrimp	24 hrs	12	3.5-4.5	.015
White shrimp	3 mos	12	3.5-4	—
	8 mos	12	3.5-5	—
STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK				
White shrimp	Background	8	2.5-4	—
First application (Sept. 18, 1969)				
White shrimp	24 hrs	9	2-2.5	—
Second application (May 20, 1970)				
Brown-spotted shrimp	3 mos	12	3.5-4	—
White shrimp	8 mos	12	4.5-5	—

TABLE 6.—*Mirex residues in crabs by sampling site and sampling time, South Carolina, 1969-71*
[— = not detected]

Species	Interval from Mirex Application to Sampling	Number in Composite Sample	Size of Individuals in Composite Samples (inches)	Mirex Residue (ppm—whole body)
SAMPLING SITE A—TOOGOODOO CREEK				
Blue crab	Background	1	5	—
First application (Oct. 14-15, 1969)				
Blue crab	24 hrs	2	3	—
	4 wks	5	1-2.5	.12
	12 wks	2	4.5-5	.19
	14 wks	1	4.5	.12
	16 wks	1	2.5	.19
	22 wks	3	1-3	.040
	26 wks	4	3-4	.015
	28 wks	2	3-4	.026
	30 wks	4	3-6	.016
Second application (June 3-4, 1970)				
Blue crab	24 hrs	3	2-3	—
	4 wks	5	2.5-5	.19
	6 wks	3	3-6	—
	8 wks	5	2-5	.052
	10 wks	8	5-6	.053
	12 wks	12	1.5-2.5	—
	14 wks	12	2-2.5	—
	16 wks	6	2.5-5.5	—
	18 wks	1	5.5	.024
	20 wks	5	1-5	—
Third application (Oct. 27-28, 1970)				
Blue crab	24 hrs	3	6	—
	2 wks	3	1-5	—
	4 wks	3	2-5	—
	6 wks	11	1-2	.013
	8 wks	12	1-2.5	—
	10 wks	7	1-3	—
	14 wks	12	1-1.25	—
	16 wks	10	1-1.75	.022
	18 wks	6	1.5-2	—
	20 wks	8	1.25-1.5	—
	22 wks	8	1-3	—
	24 wks	5	3.5-5	.016
	26 wks	5	1.5-3	—
	28 wks	1	2.5	—
	30 wks	2	3.25-4.5	—
	32 wks	5	2-5.5	—
SAMPLING SITE B—TOOGOODOO CREEK				
Blue crab	Background	1	6	—
First application (Oct. 14-15, 1969)				
Blue crab	24 hrs	1	6	—
	4 wks	1	6	.24
	8 wks	1	3	.089
	14 wks	1	3	.20
	18 wks	2	4.5	—
	26 wks	2	3-4	—
	32 wks	3	4-6	.025
Second application (June 3-4, 1970)				
Blue crab	4 wks	6	2.5-5	.035
	8 wks	3	2-6	—
	10 wks	4	4-5	.017
	12 wks	12	1.25-2.25	—
	14 wks	6	3-5.5	—
	16 wks	1	5	—
	18 wks	1	5.5	—
Fiddler crab	21 wks	28	.33-.75	—
SAMPLING SITE B—TOOGOODOO CREEK—Continued				
Third application (Oct. 27-28, 1970)				
Blue crab	24 hrs	6	1-2.5	.049
Fiddler crab	24 hrs	30	.33-.75	—
Blue crab	4 wks	2	3-5	.012
	6 wks	11	1-2.5	.12
Fiddler crab	6 wks	29	.33-.75	—
Blue crab	8 wks	5	1-2	.041
	10 wks	4	1.5-5	.027
	12 wks	3	.75-3	.038
	14 wks	8	.75-2	—
	16 wks	9	2-5	.020
	18 wks	7	1.25-2	—
	20 wks	9	1-2	—
	22 wks	4	2-4	—
	24 wks	12	1.5-2.5	—
	26 wks	4	1-4.5	—
	28 wks	5	3-6	—
	32 wks	1	3.5	—
SAMPLING SITE C—TOOGOODOO CREEK				
Blue crab	Background	1	5	—
First application (Oct. 14-15, 1969)				
Blue crab	24 hrs	1	6	—
	2 wks	1	6	—
	4 wks	9	1-2	—
	6 wks	4	2-3	.015
	8 wks	1	6	.032
	12 wks	2	5.5	.090
	14 wks	1	2	.050
	18 wks	2	4.5	.056
	20 wks	2	4.5	.025
	22 wks	8	1-2	.038
	24 wks	2	1-2	—
	26 wks	1	3	—
	28 wks	2	4-5	—
	30 wks	1	4	—
Fiddler crab	32 wks	25	.5-1	—
Second application (June 3-4, 1970)				
Blue crab	24 hrs	1	4.5	.013
	2 wks	3	6-6.5	—
	6 wks	6	3-4.5	.027
	8 wks	4	4-5	—
	12 wks	4	2-4	—
	14 wks	1	5	—
	16 wks	2	4.5	—
	20 wks	2	6	—
Fiddler crab	21 wks	26	.5-.75	—
Third application (Oct. 27-28, 1970)				
Blue crab	24 hrs	3	3-5	—
Fiddler crab	24 hrs	27	.5-.75	—
Blue crab	2 wks	1	6	—
	4 wks	3	4-5	.010
	6 wks	4	4.5	—
Fiddler crab	6 wks	31	.5-1	—
Blue crab	8 wks	4	1.5-2.5	—
	14 wks	3	1-1.75	—
	16 wks	7	1-2.5	—
	20 wks	8	1-2	—
	22 wks	2	1.5-2.5	—
	24 wks	7	1.5-3	—
	26 wks	8	1-2.5	—
	28 wks	8	1.25-3.25	—
	30 wks	1	2	—

TABLE 6.—*Mirex residues in crabs by sampling site and sampling time, South Carolina, 1969-71*—Continued

[— = not detected]

Species	Interval from Mirex Application to Sampling	Number in Composite Sample	Size of Individuals in Composite Samples (inches)	Mirex Residue (ppm—whole body)
SAMPLING SITE D—TOOGOODOO CREEK				
Blue crab	Background	1	7	—
First application (Oct. 14-15, 1969)				
Blue crab	24 hrs	6	1-1.5	—
	2 wks	3	1-2	—
	4 wks	12	1-1.25	—
	6 wks	3	1.5-3	.065
	8 wks	12	1-1.75	.030
	12 wks	2	5.5	.051
	14 wks	2	5.5	—
	18 wks	3	1.5-5	—
	20 wks	3	3	—
	22 wks	2	3	—
	24 wks	6	2-3	—
	26 wks	1	4	—
	28 wks	2	4-5	—
	30 wks	1	5	—
	32 wks	2	3-6	—
Second application (June 3-4, 1970)				
Blue crab	24 hrs	5	3-6	—
	4 wks	4	3-4	.098
	6 wks	6	4-6	—
	10 wks	1	4.5	.015
	12 wks	12	1-2.5	—
	14 wks	2	4-5	—
	18 wks	2	5-6	—
	20 wks	3	2-5	—
Third application (Oct. 27-28, 1970)				
Blue crab	24 hrs.	8	1.5-2.5	—
	2 wks	4	2-5	—
	6 wks	5	1.5-4.5	—
	8 wks	12	1-2	—
	10 wks	4	1.5-4.5	—
	12 wks	5	1-5	—
	14 wks	3	1-2	—
	16 wks	12	1-2	—
	20 wks	10	1.5-2.5	—
Mud crab	24 wks	12	.5-1.25	—
Blue crab	26 wks	2	2.5-3.5	—
	28 wks	12	1.75-3.5	—
	30 wks	2	2-3	—
	32 wks	1	2.5	—
STATION 1—RIVERLAND TERRACE POND (EAST)				
Blue crab	Background	3	1-2.5	—
First application (Dec. 3, 1969)				
Blue crab	5 mos	8	.5-1.25	—
Second application (July 24, 1970)				
Blue crab	72 hrs	15	.5-2	.024
	3 mos	13	.75-1.5	—
Third application (Dec. 1, 1970)				
Blue crab	48 hrs	12	.5-2	—
Fiddler crab	48 hrs	4	.5-.75	—
Blue crab	2 wks	3	1-3	.026
	3 mos	12	1.5-4.5	—
STATION 2—STONO RIVER, LOG BRIDGE CREEK				
Blue crab	Background	7	1.5-3	—
First application (Oct. 23, 1969)				
STATION 2—STONO RIVER—Continued				
Second application (June 18, 1970)				
Blue crab	24 hrs	8	2.5-6	.010
	3 mos	2	5-5.5	.031
	8 mos	12	1.25-4	—
STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION				
Blue crab	Background	2	1.5	—
First application (Oct. 22, 1969)				
Blue crab	24 hrs	3	2-3	.60
Second application (June 11, 1970)				
Blue crab	24 hrs	3	5	.27
STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT				
Blue crab	Background	1	4	—
First application (Oct. 17, 1969)				
Second application (June 10, 1970)				
Blue crab	24 hrs	4	6-8	.042
	3 mos	1	4.5	—
Mud crab	8 mos	31	.25-.5	—
STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK				
Blue crab	Background	1	5.5	—
First application (Oct. 22, 1969)				
Blue crab	24 hrs	9	1	—
	3 mos	2	1-2.5	—
Second application (June 10, 1970)				
Blue crab	24 hrs	5	1.5-2.5	—
	3 mos	4	3-5	—
	8 mos	8	1-4	—
STATION 6—WANDO RIVER, BERESFORD CREEK				
Blue crab	Background	2	1.5-3	—
First application (Oct. 17, 1969)				
Blue crab	3 mos	2	5	—
Second application (June 8, 1970)				
Blue crab	24 hrs	2	2-5	.025
	3 mos	8	5-6	—
	8 mos	12	1.25-3.25	—
STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK				
Blue crab	Background	1	5.5	—
First application (Sept. 18, 1969)				
Blue crab	24 hrs	1	4.5	—
	3 mos	2	1.5-3	—
Second application (May 20, 1970)				
Blue crab	24 hrs	2	2-3	—
	3 mos	4	4-5	—
	8 mos	12	1-4	.012

TABLE 7.—*Mirex residues in fishes by sampling site and sampling time, South Carolina, 1969-71*
[— = not detected]

Species	Interval from Mirex Application to Sampling	Number in Composite Sample	Size of Individuals in Composite Samples (inches)	Mirex Residue (ppm—whole body)
SAMPLING SITE A—TOOGOODOO CREEK				
Blackcheek tonguefish	Background	2	3	—
Southern kingfish	Background	1	7	—
First application (Oct. 14-15, 1969)				
Silver perch	24 hrs	2	3.5	—
	2 wks	2	3.5	—
	4 wks	2	4.5	.073
	8 wks	11	3.5-4.5	.028
Atlantic menhaden	16 wks	10	3.5-4.5	—
	18 wks	10	4-5	—
Spot	30 wks	13	3-4	.015
Second application (June 3-4, 1970)				
Spot	24 hrs	12	3-4	—
	2 wks	7	3.5-5	—
	4 wks	12	4.5-5.5	—
	6 wks	5	3.5-4	—
	8 wks	5	3-4	—
	12 wks	6	2-3	.060
	14 wks	12	4-5	.043
	16 wks	6	3-5	—
Third application (Oct. 27-28, 1970)				
Weakfish	24 hrs	3	4.5-7	—
Silver perch	2 wks	3	4.5-5.5	.017
Striped mullet	6 wks	1	7	—
	10 wks	7	7-9	—
Silver perch	20 wks	2	4.5	—
Atlantic menhaden	20 wks	2	4.25	—
Bay anchovy	22 wks	12	2-3	—
Silver perch	26 wks	4	5.5-7	—
Spot	28 wks	12	1.5-3	—
	30 wks	12	2.25-3.25	—
	32 wks	12	2-3	—
SAMPLING SITE B—TOOGOODOO CREEK				
Silver perch	Background	1	3.5	—
First application (Oct. 14-15, 1969)				
Silver perch	24 hrs	2	3.5	—
	2 wks	1	4.5	—
	8 wks	1	4	—
Spotted seatrout	8 wks	1	7	.039
Atlantic menhaden	14 wks	12	2-4	—
	16 wks	10	3.5-4.5	—
	18 wks	4	4-5	—
Spot	28 wks	3	2-2.5	—
Bluefish	30 wks	1	7	—
Spot	32 wks	2	3-4	—
Second application (June 3-4, 1970)				
Spot	24 hrs	12	3.5-4.5	—
	2 wks	6	3.5-5.5	—
	4 wks	12	3-4.5	—
	6 wks	12	3-4	—
	8 wks	1	3.5	—
Striped mullet	8 wks	2	4-5	—
Silver perch	10 wks	6	4.5-6	.027
Spot	12 wks	12	3.5-4	.016
Silver perch	14 wks	7	4-5	—
Spot	16 wks	4	3-4.5	—
Silver perch	18 wks	4	6-6.5	—
	20 wks	1	5	—
Third application (Oct. 27-28, 1970)				
Silver perch	24 hrs	8	4-5	.034
Spot	2 wks	1	6	—
Striped mullet	6 wks	3	5.5-6.5	.018
Spot	20 wks	4	6.5-7.5	—
Fourspot flounder	22 wks	3	2.5	—
	24 wks	2	3-4	—
Spot	28 wks	12	1.5-2.75	—
	30 wks	5	3-4.5	—
	32 wks	11	2.5-4.25	—
SAMPLING SITE C—TOOGOODOO CREEK				
Blackcheek tonguefish	Background	3	4	—
First application (Oct. 14-15, 1969)				
Hogchoker	24 hrs	1	4	—
Silver perch	2 wks	2	4	—
Blackcheek tonguefish	4 wks	7	4-5	—
Silver perch	6 wks	5	3-4	.017
Atlantic menhaden	14 wks	12	3.5-4.5	—
	20 wks	4	4	—
Mixed fish	22 wks	2	2-4	—
	24 wks	3	2.5-4	—
Searobin	26 wks	1	7	—
Spotted hake	26 wks	1	4	—
Spot	30 wks	3	2-3	—
	32 wks	6	4-6	—
Second application (June 3-4, 1970)				
Spot	24 hrs	10	3.5-4.5	—
	2 wks	2	4-6	—
	4 wks	2	4.5	—
	6 wks	12	3-4	—
Weakfish	8 wks	3	4-5	—
Spot	10 wks	5	3.5-4.5	—
Silver perch	12 wks	12	2.5-3.5	—
	14 wks	12	3-4	—
	16 wks	3	4	—
Spot	18 wks	3	5-6.5	—
Third application (Oct. 27-28, 1970)				
Silver perch	24 hrs	1	4.5	—
	2 wks	2	4	.028
Bay anchovy	26 wks	12	2-3	—
Spot	28 wks	12	2.25-3.5	—
	30 wks	5	3-4.5	—
	32 wks	12	2.5-4.5	—
SAMPLING SITE D—TOOGOODOO CREEK				
Blackcheek tonguefish	Background	2	3	—
First application (Oct. 14-15, 1972)				
Blackcheek tonguefish	24 hrs	2	3.5	—
	2 wks	2	4	—
	4 wks	3	3-4	—
Silver perch	6 wks	10	3.5-4	—
	8 wks	12	3-4	—
Atlantic menhaden	12 wks	2	4	—
	14 wks	10	3-5	—
	16 wks	10	3.5-4.5	—
	18 wks	3	3.5-4	—
Mixed fish	20 wks	2	3-4.5	—
Silver perch	24 wks	1	7	—
Spotted hake	24 wks	2	4	—
Weakfish	26 wks	1	10.5	—
Spotted hake	26 wks	1	8.5	—
Mixed fish	26 wks	3	2-4	—
Spotted hake	28 wks	3	6	—
Bluefish	30 wks	2	3-6	—
Spot	32 wks	7	3-6	—

TABLE 7.—*Mirex residues in fishes by sampling site and sampling time, South Carolina, 1969-71*—Continued

[— = not detected]

Species	Interval from Mirex Application to Sampling	Number in Composite Sample	Size of Individuals in Composite Samples (inches)	Mirex Residue (ppm—whole body)
SAMPLING SITE D—TOOGOODOO CREEK—Continued				
Second application (June 3-4, 1970)				
Spot	24 hrs	12	4.5-5.5	—
	2 wks	8	3-5	—
	4 wks	2	4	—
	10 wks	2	3.5-5.5	—
Silver perch	12 wks	8	2.5-3.5	.046
	14 wks	7	4	—
	16 wks	2	3.5-5	—
Blackcheek tonguefish	18 wks	4	4-5	—
Third application (Oct. 27-28, 1970)				
Silver perch	2 wks	10	4-4.5	—
Winter flounder	8 wks	1	10.5	—
Spotted hake	18 wks	2	3-4.5	—
Spot	20 wks	1	4.5	—
Spotted hake	24 wks	7	3.5-6	—
Silver perch	26 wks	2	3-5	—
Spot	28 wks	12	2-3	—
	30 wks	2	3-3.5	—
	32 wks	12	3-4.5	—
STATION 1—RIVERLAND TERRACE POND (EAST)				
Mummichog	Background	13	1-2	—
White mullet	Background	2	4-5	—
Atlantic silverside	Background	30	1.5-2.5	—
First application (Dec. 3, 1969)				
Atlantic silverside	48 hrs	55	1.5-2	—
Mummichog	12 days	13	1-2.5	—
Atlantic silverside	12 days	49	1.5-2	—
White mullet	5 mos	23	1.25-1.5	—
Second application (July 24, 1970)				
Silver perch	72 hrs	12	2-2.5	—
Sailfin molly	3 mos	17	.5-1	—
Atlantic silverside	4 mos.	27	2-3	—
Third application (Dec. 1, 1970)				
Sailfin molly	48 hrs	18	1-1.5	—
White mullet	2 wks	25	.75-1	—
Mummichog	2 wks	20	1.25-2.25	—
Atlantic silverside	2 wks	11	1.75-2	—
	6 wks	25	1.5-2.5	—
White mullet	6 wks	12	2.5-3.75	—
	3 mos	42	1	—
STATION 2—STONO RIVER, LOG BRIDGE CREEK				
Blackcheek tonguefish	Background	4	3	—
Black drum	Background	1	5	—
Spotted seatrout	Background	1	7	—
First application (Oct. 23, 1969)				
Silver perch	24 hrs	2	3-4	—
Second application (June 18, 1970)				
Spot	24 hrs	12	2.5-3.5	—
Silver perch	3 mos	12	4-5	.054
Spot	8 mos	21	1.5-2.5	—
STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION				
Atlantic croaker	Background	1	5	—
Hog choker	Background	3	3	—
First application (Oct. 22, 1969)				
Hog choker	24 hrs	4	1-2.5	—
	2 mos	8	2-3	.053
White catfish	2 mos	6	2-3	.35
	3 mos	1	12.5	.086
Striped mullet	3 mos	1	8	—
Second application (June 11, 1970)				
Spot	24 hrs	8	3-5	.82
Silver perch	3 mos	3	4	.20
Hog choker	3 mos	8	2-2.5	.096
White catfish	8 mos	7	3.5-7	.19
STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT				
Spot	Background	3	2	—
First application (Oct. 17, 1969)				
Silver perch	24 hrs	2	3.5	.21
Mixed fish	2 mos	2	4-6	—
White catfish	3 mos	1	13	.036
Second application (June 10, 1970)				
Spot	24 hrs	9	2-3.5	.12
Hogchoker	24 hrs	6	1.5-2	—
Silver perch	3 mos	5	5-5.5	.14
White catfish	8 mos	1	5.5	.045
Spot	8 mos	2	4.5-5.5	—
Bay anchovy	8 mos	14	1.5-2.25	—
STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK				
Spot	Background	3	3.5	—
Winter flounder	Background	1	8	—
First application (Oct. 22, 1969)				
Blackcheek tonguefish	24 hrs	3	3	—
Atlantic menhaden	3 mos	12	2-4	—
Second application (June 10, 1970)				
Spot	24 hrs	8	2-3	—
Silver perch	3 mos	9	4-4.5	—
Atlantic menhaden	8 mos	5	4.5	—
Striped mullet	8 mos	2	5.5	—
Star drum	8 mos	43	1-1.75	—

TABLE 7.—*Mirex residues in fishes by sampling site and sampling time, South Carolina, 1969-71*—Continued
[— = not detected]

Species	Interval from Mirex Application to Sampling	Number in Composite Sample	Size of Individuals in Composite Samples (inches)	Mirex Residue (ppm—whole body)
STATION 6—WANDO RIVER, BERESFORD CREEK				
Silver perch	Background	2	4.5	—
First application (Oct. 17, 1969)				
Pinfish	24 hrs	1	7	—
Second application (June 8, 1970)				
Spot	24 hrs	12	2.5-3.5	.016
	3 mos	12	3.5-5	—
Silver perch	8 mos	22	1.5-2	—
STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK				
Atlantic croaker	Background	2	4	—
Hog choker	Background	4	2-2.5	—
Spot	Background	3	2-3	—
First application (Sept. 18, 1969)				
Spot	24 hrs	3	2-3	—
Sheepshead	3 mos	1	8.5	—
Second application (May 20, 1970)				
Spot	24 hrs	2	6	—
Silver perch	24 hrs	1	5	—
	3 mos	6	3.5-6.5	—
Spot	8 mos	12	3.75-4.5	.011

TABLE 8.—*Mirex residues in miscellaneous organisms by sampling site and sampling time, South Carolina, 1969-71*
[— = not detected]

Species	Interval from Mirex Application to Sampling	Number in Composite Sample	Size of Individuals in Composite Samples (inches)	Mirex Residue (ppm) [1]
SAMPLING SITE A—TOOGOODOO CREEK				
Second application (June 3-4, 1970)				
American oyster	12 wks	12	2-4	—
SAMPLING SITE B—TOOGOODOO CREEK				
First application (Oct. 14-15, 1969)				
Brief squid	32 wks	10	4-6	—
Second application (June 3-4, 1970)				
American oyster	12 wks	12	2-4	—
Third application (Oct. 27-28, 1970)				
American oyster	6 wks	28	2-4	—
	10 wks	18	2-4	—
	12 wks	10	2-4	—
SAMPLING SITE C—TOOGOODOO CREEK				
First application (Oct. 14-15, 1969)				
American oyster	32 wks	25	2-4	—
Southern periwinkle	32 wks	40	.5-.75	—
Phytoplankton (dry weight)	32 wks			—
Second application (June 3-4, 1970)				
American oyster	12 wks	12	2-4	—
Third application (Oct. 27-28, 1970)				
American oyster	6 wks	22	2-4	—
	10 wks	13	2-4	—
	12 wks	12	2-4	—
Nudibranch	16 wks	18	.5-1	—
SAMPLING SITE D—TOOGOODOO CREEK				
Second application (June 3-4, 1970)				
American oyster	12 wks	12	2-4	—
STATION 1—RIVERLAND TERRACE POND (EAST)				
First application (Dec. 3, 1969)				
Egg masses from 186 gravid grass shrimp	5 mos			—
Third application (Dec. 1, 1970)				
Dead white mullet	2 wks	1	5	—
STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK				
Second application (June 10, 1970)				
Dead striped mullet	24 hrs	1	11.5	—
STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK				
First application (Oct. 18, 1969)				
Pied-billed grebe (breast muscle) (drowned in net)	3 mos			—

[1] Residues are whole-body basis unless otherwise indicated.

TABLE 9.—*Mirex residues in birds by sampling site and sampling time, South Carolina, 1969-71*
[— = not detected]

Quarterly Collection	Species	Mirex Residue (ppm) [1]
SAMPLING SITES A-B-C—TOOGOODOO CREEK		
December 1969	Green Heron	.99
	Kingfisher	1.30
	Clapper Rail	.29
	do.	1.40
	do.	.05
	do.	.10
	do.	.15
	do.	1.90
	do.	.29
	Oil glands from 5 Clapper Rails	.52
March 1970	Clapper Rail	—
	do.	.06
	do.	.05
	do.	.08
	do.	.10
	do.	.06
	Oil glands from 6 Clapper Rails	.29
May 1970	Green Heron	.09
	do.	.17
	do.	—
	do.	.07
	Yellow Crowned Night Heron	.11
	Little Blue Heron	—
	Clapper Rail	.11
September 1970	Clapper Rail	—
	do.	.17
	do.	—
	do.	.02
	do.	—
	do.	—
	do.	.02
	do.	.05
	do.	.63
	do.	.02
	do.	.19
	do.	.04
	do.	—
December 1970	Clapper Rail	.15
	do.	—
	10 Rails (Muscle)	.07
	10 Rails (Fat)	1.20
	Willet	—
	Kingfisher	.15
February 1971	Clapper Rail	.04
	do.	.13
	do.	.10
	do.	.03
	do.	.19
	do.	.04
	do.	.03
	do.	.07
	do.	.05
	do.	.75
	do.	—
	Oil glands from 6 Clapper Rails	.58
	Louisiana Heron	.11
May 1971	Clapper Rail	.01
	Louisiana Heron	.94
	do.	.14
	Little Blue Heron	.01
	do.	.22
	American Egret	5.40
	do.	.05
SAMPLING SITE D—TOOGOODOO CREEK		
December 1969	Attempts to collect birds unsuccessful	
March 1970	Clapper Rail	—
	do.	.11
May 1970	Plover	—
	Clapper Rail	—
	do.	.61
September 1970	Kingfisher	—
	do.	1.50
	do.	.04
	Clapper Rail	—
	do.	—
	do.	—
December 1970	Clapper Rail	.06
	do.	.03
	do.	.03
February 1971	Clapper Rail	.06
	Kingfisher	—
May 1971	Snowy Egret	.17
	Green Heron	.04
	Louisiana Heron	—
	do.	.03
STATION 2—STONO RIVER, LOG BRIDGE CREEK		
September 1969	American Egret	—
	Little Blue Heron	.12
	Clapper Rail	.19
December 1969	Clapper Rail	.55
	do.	.90
March 1970	Clapper Rail	—
	do.	.18
	do.	.11
	Sora Rail	—
May 1970	Clapper Rail	.08
	do.	.05
September 1970	Clapper Rail	.10
	do.	.21
	do.	.16
December 1970	Clapper Rail	.11
	do.	.06
February 1971	Clapper Rail	.09
	do.	.13
May 1971	Clapper Rail	.09
	Little Blue Heron	.70
STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION		
September 1969	Green Heron	.25
	do.	.15
	do.	.13
December 1969	Snowy Egret	.69
	American Bittern	1.80
March 1970	Snipe	.11
	do.	1.10
May 1970	Anhinga (Juvenile)	—
	Anhinga	1.70
	Kingfisher	17.00
	(internal organs)	8.30
September 1970	Pied-Billed Grebe	.28
	Anhinga	.35
December 1970	Snipe	.05
	do.	.34
	do.	[illegible]
	do.	[illegible]

TABLE 9.—*Mirex residues in birds by sampling site and sampling time, South Carolina, 1969-71*—Continued
[— = not detected]

Quarterly Collection	Species	Mirex Residue (ppm) [1]
STATION 3—UPPER ASHLEY RIVER—Continued		
February 1971	Snowy Egret	.60
	Marsh Hawk	1.50
May 1971	Least Bittern	2.20
	Louisiana Heron	1.10
STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT		
September 1969	Louisiana Heron	—
	do.	—
December 1969	Attempts to collect birds unsuccessful	
March 1970	Snipe	.14
	do.	.89
May 1970	Grebe	—
September 1970	Louisiana Heron	.38
	Green Heron	1.00
December 1970	Clapper Rail	.60
	do.	.94
February 1971	Clapper Rail	.19
	American Bittern	.04
	Grebe	1.10
May 1971	Least Bittern	2.80
	Clapper Rail	.18
STATION 5—LOWER ASHLEY RIVER, *OLD TOWN CREEK*		
September 1969	Louisiana Heron	—
	American Egret	—
December 1969	Clapper Rail	.11
	do.	.35
March 1970	Clapper Rail	.09
	do.	.07
	Sora Rail	.09
	Virginia Rail	.06
May 1970	Clapper Rail	—
	do.	—
	Eggs	.08
September 1970	Clapper Rail	—
	do.	.04
December 1970	Clapper Rail	.07
	do.	.06
February 1971	Clapper Rail	.09
	Sora Rail	.01

[1] Residues are breast and upper wing muscle unless otherwise indicated.

Collection Quarterly	Species	Mirex Residue Mirex
STATION 5—LOWER ASHLEY RIVER—Continued		
May 1971	Clapper Rail	.10
	do.	—
	do.	.04
	Eggs	.05
STATION 6—WANDO RIVER, BERESFORD CREEK		
September 1969	Snowy Egret	.89
	American Egret	—
December 1969	Attempts to collect birds unsuccessful	
March 1970	Clapper Rail	.05
	Snipe	—
May 1970	Clapper Rail	.47
	White Ibis	.29
September 1970	Kingfisher	—
	do.	—
	Sora Rail	—
	Clapper Rail	.04
	do.	—
December 1970	Clapper Rail	.82
	do.	.25
February 1971	Clapper Rail	.71
	do.	.07
May 1971	Green Heron	.14
	Little Blue Heron	.17
STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK		
September 1969	Snowy Egret	—
	Little Blue Heron	—
December 1969	Clapper Rail	—
	do.	—
March 1970	Grebe	—
May 1970	Snowy Egret	—
	do.	.20
September 1970	Kingfisher	—
	do.	.06
December 1970	Snowy Egret	.51
	American Egret	.45
February 1971	Snowy Egret	.13
	Louisiana Heron	.17
May 1971	Clapper Rail	.10
	Snowy Egret	.11

TABLE 10.—*Average mirex residues in muscle tissue of herons and egrets at each station, South Carolina, 1969-71*

Station	Average Residues in ppm and Number of Birds ( )							
	Sept. 1969	Dec. 1969	Mar. 1970	May 1970	Sept. 1970	Dec. 1970	Feb. 1971	May 1971
A-B-C		.99 (1)		.07 (6)			.11 (1)	1.13 (6)
D								.06 (4)
2	.06 (2)							.70 (1)
3	.18 (3)	.69 (1)					.60 (1)	1.10 (1)
4	.00 (2)				.69 (2)			
5	.00 (2)							
6	.45 (2)							.16 (2)
7	.00 (2)			.10 (2)		.48 (2)	.15 (2)	.11 (1)
Overall average	.12	.84		.08	.69	.48	.25	.61
Total birds	(13)	(2)		(8)	(2)	(2)	(4)	(15)

TABLE 11.—*Average mirex residues in muscle tissue of clapper rails at each station, South Carolina, 1969-71*

Station	Average Residues in ppm and Number of Birds ( )							
	Sept. 1969	Dec. 1969	Mar. 1970	May 1970	Sept. 1970	Dec. 1970	Feb. 1971	May 1971
A-B-C		.60 (7)	.07 (6)	.11 (1)	.09 (13)	.07 (12)	.14 (11)	.01 (1)
D			.06 (2)	.31 (2)	.00 (3)	.04 (3)	.06 (1)	
2	.19 (1)	.73 (2)	.10 (3)	.07 (2)	.16 (3)	.08 (2)	.11 (2)	.09 (1)
3								
4						.77 (2)	.19 (1)	.18 (1)
5		.23 (2)	.08 (2)	.00 (2)	.02 (2)	.06 (2)	.09 (1)	.05 (3)
6			.05 (1)	.47 (1)	.02 (2)	.54 (2)	.39 (2)	
7	.00 (2)							.10 (1)
Overall average	.19	.47	.07	.16	.07	.23	.15	.07
Total birds	(1)	(13)	(14)	(8)	(23)	(23)	(18)	(7)

TABLE 12.—*Mirex residues in mammals by sampling site and sampling time, South Carolina, 1969-71*
[— = not detected]

Quarterly Collection	Species	Mirex Residue (ppm) [1]
SAMPLING SITE A—TOOGOODOO CREEK		
December 1969	Raccoon	.14
	Fat	.97
March 1970	Raccoon	.05
May 1970	Raccoon	1.30
	Raccoon	.20
September 1970	Raccoon	.02
	Raccoon	.26
December 1970	Raccoon	.02
	Raccoon	—
February 1971	Raccoon	—
	Raccoon	.16
May 1971	Raccoon	.88
	Raccoon	—
SAMPLING SITE B—TOOGOODOO CREEK		
December 1969	Raccoon	—
	Fat	—
	Raccoon	—
	Fat	.16
March 1970	Raccoon	.07
	Raccoon	.43
May 1970	Raccoon	.03
	Raccoon	.66
	Raccoon	—
	Raccoon	.12
September 1970	Raccoon	.21
	Raccoon	—
December 1970	Raccoon	—
	Raccoon	—
February 1971	Raccoon	.04
	Raccoon	.12
May 1971	Raccoon	.16
	Raccoon	.04
SAMPLING SITE C—TOOGOODOO CREEK		
December 1960	Raccoon	—
	Raccoon	—
March 1970	Attempts to collect raccoons unsuccessful	
May 1970	Attempts to collect raccoons unsuccessful	
September 1970	Raccoon	.15
	Raccoon	.24
December 1970	Raccoon	.02
	Raccoon	.08
February 1971	Raccoon	.04
	Raccoon	.09
May 1971	Raccoon	.09
	Raccoon	.02

TABLE 12.—*Mirex residues in mammals by sampling site and sampling time, South Carolina, 1969-71*—Continued
[— = not detected]

Quarterly Collection	Species	Mirex Residue (ppm) [1]
SAMPLING SITE D—TOOGOODOO CREEK		
December 1969	Raccoon	—
	Raccoon	—
	Fat	—
March 1970	Attempts to collect raccoons unsuccessful	
May 1970	Raccoon	.01
	Raccoon	.04
September 1970	Raccoon	.01
	Raccoon	—
December 1970	Raccoon	—
	Raccoon	—
February 1971	Raccoon	—
	Raccoon	.04
May 1971	Raccoon	—
	Raccoon	—
STATION 2—STONO RIVER, LOG BRIDGE CREEK		
December 1969	Raccoon	—
	Fat	—
	Raccoon	—
	Fat	—
March 1970	Raccoon	—
May 1970	Raccoon	1.40
	Raccoon	.07
September 1970	Raccoon	.07
	Raccoon	1.90
December 1970	Raccoon	—
	Raccoon	—
February 1971	Raccoon	.20
	Raccoon	.04
May 1971	Raccoon	—
STATION 3—UPPER ASHLEY RIVER, RUNNYMEADE PLANTATION		
December 1969	Attempts to collect raccoons unsuccessful	
March 1970	Attempts to collect raccoons unsuccessful	
May 1970	Raccoon	—
September 1970	Raccoon	.56
	Raccoon	.12
	Raccoon	.88
December 1970	Raccoon	.10
	Raccoon	—
February 1971	Raccoon	.19
May 1971	Raccoon	1.90
	Raccoon	.09
	Opossum	3.30
STATION 4—COOPER RIVER, U.S. NAVAL AMMUNITION DEPOT		
December 1969	Raccoon	.80
	Raccoon	.39
March 1970	Raccoon	.60
	Raccoon	4.40
May 1970	Raccoon	.28
September 1970	Raccoon	.90
	Raccoon	1.30
December 1970	Attempts to collect raccoons unsuccessful	
February 1971	Attempts to collect raccoons unsuccessful	
May 1971	Attempts to collect raccoons unsuccessful	
STATION 5—LOWER ASHLEY RIVER, OLD TOWN CREEK		
December 1969	Raccoon	—
	Raccoon	—
March 1970	Raccoon	—
	Raccoon	—
May 1970	Raccoon	—
	Raccoon	—
September 1970	Raccoon	.06
	Raccoon	—
December 1970	Raccoon	.03
	Raccoon	.02
February 1971	Raccoon	—
	Raccoon	—
May 1971	Raccoon	—
	Raccoon	.05
STATION 6—WANDO RIVER, BERESFORD CREEK		
December 1969	Raccoon	.04
	Raccoon	—
March 1970	Raccoon	—
	Raccoon	—
May 1970	Raccoon	—
	Raccoon	.16
September 1970	Raccoon	—
December 1970	Raccoon	.02
	Raccoon	.02
February 1971	Raccoon	.04
	Raccoon	—
May 1971	Raccoon	—
	Opossum	.80
	Opossum	2.20
STATION 7—SOUTH SANTEE RIVER, ALLIGATOR CREEK		
September 1969	Raccoon	—
December 1969	Raccoon	—
	Raccoon	—
March 1970	Raccoon	—
	Raccoon	—
May 1970	Attempts to collect raccoons unsuccessful	
September 1970	Attempts to collect raccoons unsuccessful	
December 1970	Raccoon	—
	Raccoon	—
February 1971	Raccoon	.06
	Raccoon	—
May 1971	Raccoon	.03

[1] Residues for thigh muscles unless otherwise indicated.

TABLE 13.—*Average mirex residues in muscle tissue of raccoons at each station, South Carolina, 1969-71*

Station	Average Residues in ppm and Number of Raccoons ( )							
	Sept. 1969	Dec. 1969	Mar. 1970	May 1970	Sept. 1970	Dec. 1970	Feb. 1971	May 1971
A		.14 (1)	.05 (1)	.75 (2)	.14 (2)	.01 (2)	.08 (2)	.44 (2)
B		.00 (2)	.25 (2)	.20 (4)	.11 (2)	.00 (2)	.08 (2)	.10 (2)
C		.00 (2)			.20 (2)	.05 (2)	.07 (2)	.06 (2)
D		.00 (2)		.03 (2)	.01 (2)	.00 (2)	.02 (2)	.00 (2)
2		.00 (2)	.00 (1)	.74 (2)	.99 (2)	.00 (2)	.12 (2)	.00 (2)
3				.00 (1)	.52 (3)	.05 (2)	.19 (1)	1.00 (2)
4		.60 (2)	2.50 (2)	.28 (1)	1.10 (2)			
5		.00 (2)	.00 (2)	.00 (2)	.03 (2)	.03 (2)	.00 (2)	.03 (2)
6		.02 (2)	.00 (2)	.08 (2)	.00 (1)	.02 (2)	.02 (2)	.20 (1)
7	.00 (1)	.00 (2)	.00 (2)			.00 (2)	.03 (2)	.03 (1)
Overall average	.00	.08	.46	.27	.37	.02	.06	.22
Total raccoons	(1)	(17)	(12)	(16)	(18)	(18)	(17)	(15)

TABLE 14.—*Summary of birds and mammals containing mirex residues in excess of 1.0 ppm, South Carolina, 1969-71*

Station	Animal Species	Mirex Residues in ppm	Month of Sample Collection
	BIRDS		
A-B-C	Kingfisher	1.30	December 1969
A-B-C	Clapper Rail	1.40	December 1969
A-B-C	Clapper Rail	1.90	December 1969
A-B-C	American Egret	5.40	May 1971
D	Kingfisher	1.50	September 1970
3	American Bittern	1.80	December 1969
3	Snipe	1.10	March 1970
3	Anhinga	1.70	May 1970
3	Kingfisher	17.00	May 1970
3	Marsh Hawk	1.50	February 1971
3	Least Bittern	2.20	May 1971
3	Louisiana Heron	1.10	May 1971
4	Louisiana Heron	1.00	September 1970
4	Grebe	1.10	February 1971
4	Least Bittern	2.80	May 1971
	MAMMALS		
A	Raccoon	1.30	May 1970
2	Raccoon	1.40	May 1970
2	Raccoon	1.90	September 1970
3	Raccoon	1.90	May 1971
3	Opossum	3.30	May 1971
4	Raccoon	4.40	March 1970
4	Raccoon	1.30	September 1970
6	Opossum	2.20	May 1971

Relation Between Simple Dynamic Pool and Surplus Production Models for Yield from a Fishery[1]

A. L. JENSEN

Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory,[2] Sabine Island, Gulf Breeze, Fla. 32561, USA

JENSEN, A. L. 1973. Relation between simple dynamic pool and surplus production models for yield from a fishery. J. Fish. Res. Board Can. 30: 998–1002.

Dynamic pool models without self-regenerating properties are continuous age models, and surplus production models are continuous time models. Self-regenerating dynamic pool models are continuous age-discrete generation models and, also, discrete time-discrete age models. In a steady state specification of the regulatory function and direct estimation of biomass results in the surplus production model. Estimation of biomass by specifying the functions with respect to age for size of a cohort and individual weight and application of the coefficient of fishing mortality result in the dynamic pool model. A third approach, not applied in fisheries, is to specify the regulatory function and functions with respect to age of cohort size and individual growth in weight. In a steady state all methods for calculating yield give the same results if the functions specified are realistic. Specification of the functions requires that many assumptions be made. The dynamic pool model may be more accurate than the surplus production model because the regulatory function may be more difficult to determine than the functions with respect to age of cohort size and growth in individual weight.

JENSEN, A. L. 1973. Relation between simple dynamic pool and surplus production models for yield from a fishery. J. Fish. Res. Board Can. 30: 998–1002.

Des modèles pools dynamiques dépourvus d'autorégénération sont des modèles à âge continu, alors que les modèles surplus-production sont des modèles à temps continu. Les modèles pools dynamiques à autorégénération sont des modèles à âge continu-génération discontinue; ils sont également des modèles à temps discontinu-âge discontinu. Dans un état d'équilibre, la spécification de la fonction régulatrice et l'estimation directe de la biomasse aboutissent au modèle surplus-production. Par ailleurs, l'estimation de la biomasse par spécification des fonctions qui ont trait à l'âge correspondant à la taille d'une cohorte et au poids individuel d'une part, et l'application du coefficient de mortalité due à la pêche d'autre part, aboutissent au modèle pool dynamique. Une troisième méthode, non appliquée aux pêches, consiste à spécifier la fonction régulatrice et les fonctions qui ont trait à l'âge correspondant à la taille d'une cohorte et à la croissance pondérale individuelle. Dans un état d'équilibre, toutes les méthodes de calcul de rendement donnent les mêmes résultats, pourvu que les fonctions spécifiées soient réalistes. La spécification des fonctions requiert l'élaboration de plusieurs hypothèses. Le modèle pool dynamique peut être plus précis que le modèle surplus-production, parce que la fonction régulatrice est parfois plus difficile à établir que les fonctions qui ont trait à l'âge correspondant à la taille de la cohorte et à la croissance pondérale individuelle.

Received January 9, 1973

MATHEMATICAL models applied to fish populations have been grouped into two basic categories: dynamic pool models such as those of Baranov (1918), Thompson and Bell (1934), Ricker (1944), and Beverton and Holt (1957); and surplus production models such as those of Hjort et al. (1933), Graham (1935), and Schaefer (1954, 1957). These two types of models have been compared by Schaefer and Beverton (1963) and by Silliman (1971).

These models differ in a fundamental way that does not appear to have been explicitly discussed in the literature. In dynamic pool models without self-regenerating properties the variables are continuous functions of age, whereas in surplus production models the variables are continuous functions of time. In this note the dynamic pool model without self-regeneration properties and the surplus production model will be reviewed with attention drawn to their time and age properties, and then the models will be compared.

[1]Contribution No. 157, Gulf Breeze Laboratory.
[2]Associate Laboratory of the National Environmental Research Center, Corvallis, Oreg., USA.

Printed in Canada (J2790)

Mathematical symbols used are:

$B(t)$ = population size in biomass at time t;
B_∞ = environmental carrying capacity in terms of biomass;
B = annual biomass accumulation;
$f(B(t))$ = regulatory function that gives instantaneous rate of change in biomass;
F = fishing mortality coefficient;
F_1 = coefficient of effect of fishing on population density;
F_2 = coefficient of effect of fishing on average individual weight;
g = coefficient of growth in weight per individual averaged over the entire population;
K = coefficient of individual fish growth in weight or length;
k = coefficient of population growth in number of individuals and weight;
$N(x,t)$ = number of individuals of age x at time t;
$N(x)$ = number of individuals of a cohort alive at age x;
N_∞ = environmental carrying capacity in terms of the number of individuals;
$N(t)$ = total number of individuals in population at time t;
$P(t)$ = population size at time t in biomass or numbers;
R = recruitment;
r = coefficient of growth in number of individuals;
t = time measured in years;
v = greatest age attainable by an individual fish;
$W(x,t)$ = average weight of an individual of age x at time t;
$W(x)$ = average weight of an individual of age x;
W_∞ = maximum attainable weight of an individual fish;
$\overline{W}(t)$ = average weight of all individuals in the population at time t;
x = age measured in years;
x_0 = theoretical age at which length equals zero;
Y = yield in weight;
Y_E = equilibrium yield in weight;
Z = total instantaneous mortality coefficient.

In the above list, symbols of the form $B(t)$ indicate that B is a function of t and should not be confused with multiplication.

Review of typical dynamic pool model — The dynamic pool model for change in yield with respect to age is given by

$$\frac{dY}{dx} = F\ N(x)\ W(x)\ dx \qquad (1)$$

where F is a constant and $N(x)$ and $W(x)$ are unspecified functions of age. Change in the number of individuals in a cohort with respect to age is given by

$$\frac{dN}{dx} = -Z\ N(x) \qquad (2)$$

which after integration becomes

$$N(x) = R\ e^{-Zx}. \qquad (3)$$

A number of different models have been applied to describe weight as a function of age; Richards (1959) has shown that these models are related. Von Bertalanffy's equation

$$W(x) = W_\infty\ (1 - e^{-K(x-x_0)})^3 \qquad (4)$$

has frequently been applied for this purpose and appears to adequately describe the growth of many species of fish. Substitution of equations (3) and (4) into equation (1) gives the simple Beverton and Holt yield equation

$$\frac{dY}{dx} = F\ R\ e^{-Zx}\ W_\infty\ (1 - e^{-K(x-x_0)})^3. \qquad (5)$$

This model has recently been discussed in considerable detail by Gulland (1969). In equations (3), (4), and (5) population density, weight, and yield are all clearly functions of age. Under steady state conditions it has been shown that yield per generation equals annual yield from an entire population (Thompson and Bell 1934; Beverton and Holt 1957). In this situation annual yield can be calculated using equation (5), but it is constant with respect to time. In practice, to compensate for variation in recruitment, equation (5) is usually divided by recruitment to obtain an equation for yield per recruit (Gulland 1972). In most fisheries variation in recruitment is important and has led to investigation of stock–recruitment relations (Ricker 1958).

Self-regeneration properties of dynamic pool models, such as those proposed by Ricker (1954) and Beverton and Holt (1957), produce a continuous age-discrete generation model. These self-regenerating dynamic pool models usually have been applied to obtain yield per generation (Ricker 1958; Larkin and Ricker 1964; Tautz et al. 1969; Waller et al. 1971). A simple bookkeeping procedure can easily be established to obtain yield per year from a population consisting of several age-groups (Walters 1969). Continuous age models such as the Von Bertalanffy growth equation may be applied to calculate the constants in self-regenerating dynamic pool models, but these continuous age-discrete generation models are also discrete time-discrete age models as is clearly revealed by the nature of Walters' (1969) computer program. Self-regenerating dynamic pool models may offer the best approach for obtaining realistic models. However, these models are complex and are difficult to compare with the simpler models; their inclusion in this study would result in an undesirable degree of complexity.

Review of typical surplus production model — The general form of this model for an exploited population under average environmental conditions was formulated by Schaefer (1954) as

$$\frac{dP}{dt} = P(t)\ f[P(t)] - F\ P(t). \qquad (6)$$

If $P(t)$ is taken as biomass, then

$$P(t) = B(t) = N(t)\,\bar{W}(t) \tag{7}$$

where $N(t)$ is the total number of individuals of all ages at any point in time

$$N(t) = \int_0^v N(x,t)\ dx \tag{8}$$

and $\bar{W}(t)$ the average weight of an individual at time t

$$W(t) = \frac{\int_0^v N(x,t)\ W(x,t)\ dx}{\int_0^v N(x,t)\ dx} \tag{9}$$

It can be shown (Jensen 1972), by applying the equation

$$\frac{dB}{dt} = \bar{W}(t)\,\frac{dN}{dt} + N(t)\,\frac{d\bar{W}}{dt} \tag{10}$$

which is obtained by differentiating equation (7), that, if change in biomass with respect to time is given by the linear surplus production model

$$\frac{dB}{dt} = k\ B(t)\left(\frac{B_\infty - B(t)}{B_\infty}\right) - F\ B(t) \tag{11}$$

then suitable equations for $\frac{dN}{dt}$ and $d\bar{W}/dt$ are given by

$$\frac{dN}{dt} = rN(t) - \frac{rN(t)\,^2\bar{W}(t)}{B_\infty} - F_1N(t) \tag{12}$$

$$\frac{d\bar{W}}{dt} = g\bar{W}(t) - \frac{gW(t)\,^2N(t)}{B_\infty} - F_2\bar{W}(t) \tag{13}$$

where $k = r + g$. Yield with respect to time is given by the equation

$$\frac{dY}{dt} = F\ N(t)\ \bar{W}(t). \tag{14}$$

Substitution of equations (12) and (13) into equation (14) gives

$$\frac{dY}{dt} = F\int_{\Delta t}\left[rN(t) - \frac{rN(t)^2\,\bar{W}(t)}{B_\infty} - F_1N(t)\right]dt \cdot \int_{\Delta t}\left[g\bar{W}(t) - \frac{g\bar{W}(t)^2\,N(t)}{B_\infty} - F_2\bar{W}(t)\right]dt. \tag{15}$$

Clearly, equations (12), (13), and (15) give population size, average individual weight, and yield as functions of time.

Comparison of simple dynamic pool and surplus production models under steady state conditions — Virtually no exploited fish population is in a steady state but, as a first approximation in modeling a fish population, a steady state is usually assumed (Gulland 1972). In the steady state the difference between dynamic pool and surplus production models results from specifying different functions. In a steady state, where $dB/dt = 0$, $dN/dt = 0$, and $d\bar{W}/dt = 0$, annual yield from a fishery is given by

$$Y_E = F\,B = B\,f(B) \tag{16}$$

which is obtained from equations (6) and (7). Biomass accumulation of a cohort during its lifetime in a steady state is given by

$$\int_0^v N(x)\ W(x)\ dx \tag{17}$$

where $N(x)$ and $W(x)$ are unspecified functions of age. Biomass accumulation from an entire population during 1 year of life under steady state conditions is given by

$$\int_0^1 N(t)\ W(t)\ dt \tag{18}$$

but $N(t)$ and $\bar{W}(t)$ are constant with respect to time. Substituting equations (8) and (9) into expression (18) gives

$$\int^1\int^v N(x)\ dx\left(\frac{\int_0^v N(x)\ W(x)\ dx}{\int_0^v N(x)\ dx}\right)dt \tag{19}$$

and, after cancellation of similar terms and integration with respect to time from 0 to 1, expression (19) becomes the same as expression (17). Under steady state conditions the biomass accumulation of a cohort during its lifetime equals the biomass accumulation of an entire population during 1 year.

Substitution of expression (17) into equation (16) for annual biomass accumulation gives

$$Y_E = F\int_0^v N(x)\ W(x)\ dx = f(B) \cdot \int_0^v N(x)\ W(x)\ dx. \tag{20}$$

From equations (16) and (20) it is clear that yield in a steady state can be calculated by four different approaches:

(1) Specify the form of no functions and apply the equation

$$Y_E = F\,B. \tag{21}$$

This method was applied by Ricker (1945, 1958), who separated the life of the fish into segments and applied equation (21) to each segment and then summed over the segments to obtain yield from the entire population.

(2) Specify the form of the regulatory function, $f(B)$, and apply the equation

$$Y_E = f(B)\, B. \tag{22}$$

This is the surplus production approach. Examples of the application of these models to specific fisheries are given by Ricker (1958), Schaefer (1954), and Gulland (1972). Ricker reviews the assumptions of this approach.

(3) Specify the form of $W(x)$ and $N(x)$ and apply the equation

$$Y_E = F \int_0^{\infty} N(x)\, W(x)\, dx. \tag{23}$$

This is the dynamic pool approach. Examples of the application of these models are given by Gulland (1972), Beverton and Holt (1957), and Gulland (1969). Ricker (1958) and Beverton and Holt (1957) review the assumptions of this approach.

(4) Specify the form of $f(B)$, $N(x)$, and $W(x)$ and apply the equation

$$Y_E = f(B) \int_0^{\infty} W(x)\, N(x)\, dx. \tag{24}$$

Equation (24) has not been applied. Application of equation (24) is equivalent to applying both the dynamic pool and surplus production models to the same fish population. Gulland (1972) has applied both the linear surplus production model and the Beverton and Holt yield equation to the eastern Pacific yellowfin tuna fishery, but he did not combine the two models into a single equation. Equation (24) combines the surplus production concept of the environmental carrying capacity with the age-specific events of the dynamic pool model. Application of equation (24) requires many of the assumptions of both equations (22) and (23). Equation (24) may not be of practical value, but it is of interest for it shows that in a steady state the constant for fishing mortality, F, of the dynamic pool model equals the regulatory function, $f(B)$, of the surplus production model.

All four expressions for yield are equal and theoretically give the same result. However, in fitting specific models to data different results may be obtained with the four yield equations if the functions selected for $f(B)$, $N(x)$, and $W(x)$ are not realistic. For example, if biomass is calculated by functions $N(x)$ and $W(x)$ that poorly approximate mortality and growth, the annual biomass accumulation estimated directly from the weight of the catch may not equal the annual biomass accumulation calculated by expression (17). Therefore, yield calculated by the surplus production approach (equation 22) may not equal yield calculated by the dynamic pool approach (equation 23).

The dynamic pool yield equation may be more accurate than the surplus production yield equation because the form of the functions $N(x)$ and $W(x)$ can be determined more easily than the form of the function $f(B)$. To obtain data on the form of $W(x)$ and $N(x)$ in a steady state, either observations can be made throughout the lifetime of a cohort or observations can be made on the entire population at a single point in time. To obtain data on the form of $f(B)$, estimates of yield and biomass are required for different steady states. The dynamic pool model must be used if the parameters it contains, such as age of entry into the fishery, are of interest. Gulland (1972) has discussed in considerable detail the practical aspects of applying both the dynamic pool and surplus production models.

Schaefer and Beverton (1963, equation 1) give a general equation that describes the dynamics with respect to time of an exploited fish stock. The terms in this equation for changes with respect to time were confused with the terms for changes with respect to age that are contained in the expressions for biomass in both the dynamic pool and the surplus production models. This led to the incorrect conclusion that the dynamic pool model describes a population in terms of recruitment, growth, and natural and fishing mortality, whereas the surplus production model combines these effects into a common function of the mean population size. Equation (23) shows that yield calculated by the dynamic pool model is the product of the fishing mortality coefficient and annual biomass accumulation. Annual biomass accumulation is estimated analytically using functions $N(x)$ and $W(x)$. In the dynamic pool model the terms for recruitment, growth, and natural and fishing mortality are contained in the expression for annual biomass accumulation. Equation (22) shows that yield calculated by the surplus production model is the product of the regulatory function and annual biomass accumulation. In practice annual biomass accumulation is estimated directly from the biomass of the catch without aid of the functions $W(x)$ and $N(x)$. In the surplus production model the terms for recruitment, growth, and natural and fishing mortality are all combined in the expression for annual biomass accumulation. The regulatory function is a function of biomass; therefore, the regulatory function is also a function of recruitment,

growth, and natural and fishing mortality. In addition, however, the regulatory function contains terms that have no counterpart in the dynamic pool model. These terms are the coefficient for growth in number of individuals with respect to time, r; the coefficient of growth of average individual weight, g; and the environmental carrying capacity in terms of biomass, B_∞. The regulatory function is a complex function.

In summary, the surplus production model is a function of time and the simple dynamic pool model is a function of age. In the steady state the differences between the dynamic pool and surplus production models result from specifying different functions: (1) In the dynamic pool model analytical functions are applied to determine biomass. In surplus production models biomass is estimated directly from the weight of the catch; (2) In dynamic pool models the proportion of the annual biomass accumulation that is captured by fishing is estimated directly by the coefficient of fishing mortality. In surplus production models the proportion of the annual biomass accumulation that is captured by fishing is equated to the regulatory function. The regulatory function gives the rate of growth of biomass per unit of biomass that would occur in the absence of fishing. If fishing were stopped, biomass would increase to the environmental carrying capacity, and the rate of biomass increase at the moment fishing stopped would equal the coefficient of fishing mortality. The dynamic pool and surplus production models are not based on mutually exclusive theories, but when specific functions are defined the results calculated by these two methods may not agree. The dynamic pool and surplus production approaches are only two of four possible approaches for calculating yield under steady state conditions. Some of the four approaches may be more accurate than others.

Acknowledgments—I thank R. P. Silliman for valuable suggestions that he made on a draft of this note.

BARANOV, F. I. 1918. On the question of the biological basis of fisheries [in Russian]. Nauch.-Issled. Ikhtiol. Inst. Izv. 1: 81–128.

BEVERTON, R. J. H., AND S. J. HOLT. 1957. On the dynamics of exploited fish populations. H.M.S.O., London. 533 p.

GRAHAM, M. 1935. Modern theory of exploiting a fishery, and application to North Sea trawling. J. Cons. Cons. Perma. Int. Explor. Mer 10: 264–274.

GULLAND, J. A. 1969. Manual of methods for fish stock assessment. Part 1. Fish population analysis. FAO Man. Fish. Sci. No. 4, Rome. 154 p.

1972. Population dynamics of world fisheries. University of Washington Press, Seattle, Wash. 336 p.

HJORT, J., G. JAHN, AND P. OTTESTAD. 1933. The optimum catch. Hvalradets Skr. 7: 92–127.

JENSEN, A. L. 1972. Population biomass, number of individuals, average individual weight, and the linear surplus-production model. J. Fish. Res. Board Can. 29: 1651–1655.

LARKIN, P. A., AND W. E. RICKER. 1964. Further information on sustained yields from fluctuating environments. J. Fish. Res. Board Can. 20: 647–678.

RICHARDS, F. J. 1959. A flexible growth function for empirical use. J. Exp. Bot. 10: 290–300.

RICKER, W. E. 1944. Further notes on fishing mortality and effort. Copeia 1944: 23–44.

1945. A method of estimating minimum size limits for obtaining maximum yield. Copeia 1945: 84–94.

1954. Stock and recruitment. J. Fish. Res. Board Can. 11: 559–623.

1958. Maximum sustained yields from fluctuating environments and mixed stocks. J. Fish. Res. Board Can. 15: 991–1006.

SCHAEFER, M. B. 1954. Some aspects of the dynamics of populations important to the management of commercial marine fisheries. Bull. Inter-Amer. Trop. Tuna Comm. 1: 27–56.

1957. A study of the dynamics of the fishery for yellowfin tuna in the eastern tropical Pacific Ocean. Bull. Inter-Amer. Trop. Tuna Comm. 2: 247–268.

SCHAEFER, M. B., AND R. J. H. BEVERTON. 1963. Fishery dynamics — their analysis and interpretation, p. 464-483. *In* M. N. Hill [ed.] The sea. John Wiley & Sons, New York, N.Y.

SILLIMAN, R. P. 1971. Advantages and limitations of "simple" fishery models in light of laboratory experiments. J. Fish. Res. Board Can. 28: 1211–1214.

TAUTZ, A., P. A. LARKIN, AND W. E. RICKER. 1969. Some effects of simulated long-term environmental fluctuations on maximum sustained yield. J. Fish. Res. Board Can. 26: 2715–2726.

THOMPSON, W. F., AND F. H. BELL. 1934. Biological statistics of the Pacific halibut fishery. (2) Effects of changes in intensity upon total yield and yield per unit of gear. Rep. Int. Fish. Comm., No. 8. Seattle, Wash. 49 p.

WALLER, W. T., M. L. DAHLBERG, R. E. SPARKS, AND J. CAIRNS, JR. 1971. A computer simulation of the effects of superimposed mortality due to pollutants on populations of fathead minnows (*Pimephales promelas*). J. Fish. Res. Board Can. 28: 1107–1112.

WALTERS, C. J. 1969. A generalized computer simulation model for fish population studies. Trans. Amer. Fish. Soc. 98: 505–512.

Reprinted from THE JOURNAL OF PROTOZOOLOGY.
J. PROTOZOOL., 20(3), 443–445 (1973).
Made in United States of America

The Polychlorinated Biphenyls, Aroclors® 1248 and 1260: Effect on and Accumulation by *Tetrahymena pyriformis**

NELSON R. COOLEY, JAMES M. KELTNER, JR. and JERROLD FORESTER†

Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561; Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon

SYNOPSIS. Effects of 2 polychlorinated biphenyls, Aroclor 1248 and 1260, on axenic *Tetrahymena pyriformis* strain W were investigated and compared with published data on Aroclor 1254. Aroclors 1248 and 1260 at 1 mg/liter in the presence of 0.1% (v/v) polyethylene glycol 200 reduced significantly ($P < 0.005$) growth rates and 96-hr populations of *T. pyriformis* grown at 26 C. Both toxicants were ~ 0.001 as toxic as Aroclor 1254. Ciliates were exposed for 7 days to concentrated Aroclors 1248 40×, 1254 60×, and 1260 79× over initial concentrations in the media. Accumulation of Aroclors increased with increased chlorination. It is suggested that if levels in the environment reached those used in these studies, the chief ecologic effect of Aroclor 1254 would be reduction of availability of the ciliates as food and as nutrient regenerators, but with Aroclors 1248 and 1260, this effect would be secondary to accumulation of the toxicants by the ciliates. Accumulation of polychlorinated biphenyls by ciliates would permit the toxicants to enter aquatic food chains. Thus the compounds could exert toxic effects at higher trophic levels.

Index Key Words: *Tetrahymena pyriformis* strain W; axenic cultivation; Aroclor 1248; Aroclor 1254; Aroclor 1260; toxic effect on, and accumulation by ciliates.

THE Aroclors are mixtures of polychlorinated biphenyls (PCBs) or terphenyls. The PCBs are related structurally to DDT and some are toxic to and accumulated by animals and man (17). Aroclor 1254, reported from water, sediment, and biota of Escambia Bay, Florida (5), in laboratory experiments is toxic to and accumulated by the ciliate, *Tetrahymena pyriformis,* and by shrimp, fiddler crabs, oysters, and fishes (4, 5, 9, 10, 13, 14). The ubiquity and significance of these and related PCBs in the environment have been reviewed (6, 5, 17).

Individual Aroclors are identified by 4-digit numbers, the first 2 indicating the type of molecule and the last 2, the weight percentage of chlorine in the molecule. For example, Aroclor 1248 is a polychlorinated biphenyl that contains 48% chlorine. The Aroclors have been used in lubricants; as plasticizers in paints, plastics, and chlorinated rubbers; as heat-exchange fluids in industrial heating systems; and as dielectric compounds in large electrical transformers and capacitors (17). Recently, use of these compounds has been restricted to their dielectric application (17).

We report here the effects of Aroclors 1248 and 1260 on growth of populations of *T. pyriformis* and the accumulation of these compounds by this ciliate. The data are compared with our previous observations on response of *T. pyriformis* to Aroclor 1254 (4).

MATERIALS AND METHODS

Tetrahymena pyriformis strain W was grown in optically matched culture tubes containing 10 ml of proteose peptone [2% (w/v)] medium, supplemented with 0.1% (w/v) dehydrated yeast extract and 0.5% (w/v) glucose, at 26 C. Culture tubes were slanted at 60° to enhance aeration.

Stock solutions of toxicants in polyethylene glycol 200 were prepared, and chemical residue analyses in media and cells were performed by methods already described (4), with one exception. Previously, petroleum ether was used in extraction before gas-chromatographic analysis. In this study, a mixture of equal parts of ethyl and petroleum ethers was used.

Aroclors were tested in the presence of 0.1% (v/v) polyethylene glycol 200 for effect on *Tetrahymena* growth and for accumulation by the ciliates. This concentration of polyethylene glycol had no toxic effect on the ciliates (4) and we had no reason to suspect that effects of polyethylene glycol and Aroclors were additive. Aroclor 1248 was tested at 0.01, 0.1, and 1 mg/liter. Aroclor 1260 was tested for effect on population growth at 0.001, 0.01, 0.1, 1, and 10 mg/liter and for accumulation at 0.001, 0.01, 0.1, and 1 mg/liter. Each concentration was tested for effect on population growth in 6 replicate 96-hr experiments and for accumulation in 3 replicate 7-day experiments. Initial concentrations of each Aroclor in the media were confirmed by electron-capture gas chromatography, and were within 17% of the desired concentrations.

Growth was measured as absorbance at 540 nm. Observations were made at 0, 4, 8, 16, 24, 36, 48, 60, 72, 84, and 96 hr and the data graphed. Exponential growth rate of each population was estimated as the quantity b of the least squares estimate of the line $y = a + bx$ of the exponential part of the growth curve. The calculated regression lines for this segment of the growth curves closely fitted the experimental data—for Aroclor 1248, $r \geqslant 0.89$; for Aroclor 1260, $r \geqslant 0.87$. In addition, populations were compared at 96 hr, when densities of control populations were maximal. Values for all treatments in 6 replicates were subjected to analysis of variance with 2-way classification and individual treatments were compared by Scheffé's test (16). Differences were considered significant at $P < 0.05$.

RESULTS

Aroclor 1248

Effect on population growth.—Exponential growth rate of the ciliate (Table 1) was reduced significantly by Aroclor 1248 [variance ratio, $F_{6,35} = 6.77$; $P < 0.005$], growth rates of populations exposed to 1 mg/liter of the compound being 18.9%

® Registered Trademark, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, Mo. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

* Contribution No. 158, Gulf Breeze Environmental Research Laboratory.

† We thank Dr. John O. Corliss for the cultures of *T. pyriformis* strain W from which our stock was derived, the Monsanto Company for the samples of Aroclors 1248 and 1260 used in this investigation, and David J. Hansen and Dr. Alvin L. Jensen for valuable advice on the statistical analysis of our data.

TABLE 1. *Comparison of the effect of Aroclors 1248 and 1260 on growth of* T. pyriformis *strain W at 26 C.*

Toxicant	Concentration (mg/liter)	Mean growth rate* (*b*)	Difference (%)	Mean population density at 96 hr* (absorbance)	Difference (%)
AROCLOR 1248	0	0.0164		0.7732	
	0.01	0.0168	+ 2.2	0.7883	+ 1.9
	0.1	0.0161	− 1.8	0.7915	+ 2.4
	1.0	0.0133	−18.9†	0.6992	− 9.6‡
AROCLOR 1260	*Series 1*				
	0	0.0168		0.8472	
	0.001	0.0176	+ 4.8	0.8487	+ 1.8
	0.01	0.0179	+ 6.5	0.8533	+ 7.2
	0.1	0.0171	+ 1.8	0.8317	−18.3
	1.0	0.0126	−25.0§	0.6577	−22.4‖
	Series 2				
	0	0.0194		1.0175	
	1.0	0.0157	−19.1**	0.8788	−13.6††
	10.0	0.0113	−41.1**	0.7075	−30.5††

* Means of 6 replicate experiments.
† $F_{(3,\ 15)} = 6.77$, $P < 0.005$; ‡ $F_{(3,\ 15)} = 9.35$, $P < 0.005$; § $F_{(4,\ 20)} = 5.85$, $P < 0.005$; ‖ $F_{(4,\ 20)} = 6.06$, $P < 0.005$; ** $F_{(2,\ 10)} = 485.00$, $P < 0.005$; †† $F_{(2,\ 10)} = 217.90$, $P < 0.005$.

less than those of control populations. There was no statistically significant difference between growth rates of control and experimental populations exposed to lower concentrations or among growth rates of experimental populations exposed to lower concentrations.

Population density at 96 hr (Table 1) was reduced (9.6%) significantly by 1 mg/liter [$F_{(3,\ 15)} = 9.35$; $P < 0.005$], but not by lower concentrations of the toxicant.

Accumulation.—Tetrahymena pyriformis accumulated Aroclor 1248 from media containing 0.01, 0.1, or 1 mg/liter during 7-days exposure. Uptake of the toxicant from the medium was linear with increasing concentration. When initial concentration in the medium was plotted against mean concentration in the cells, the data agreed closely ($r = 0.998$) with the calculated regression line $\overline{Y}_m = 0.9322X - 1.4746$. Concentration factors ranged from 14.8 to 40.6. Ciliates exposed to 0.01 mg/liter absorbed 15.1-25.5% ($\bar{x} = 21.6\%$) of the toxicant in the medium, those exposed to 0.1 mg/liter absorbed 13.4-19.2% ($\bar{x} = 15.7\%$), and those exposed to 1 mg/liter absorbed 14.9-19.2% ($\bar{x} = 16.8\%$).

We observed no indication of change in isomeric composition of Aroclor 1248 as was reported for Aroclor 1254 in tissues of fish (9) and shrimp (14).

Aroclor 1260

Effect on population growth.—Exponential growth rate of the ciliate (Table 1) was reduced significantly by Aroclor 1260 [$F_{(4,\ 20)} = 5.85$, $P < 0.005$, in the 1st series; $F_{(2,\ 10)} = 485.00$, $P < 0.005$, in a 2nd series], growth rates of organisms exposed to the highest concentrations being 19.1-25.0% less at 1 mg/liter and 41.1% less at 10 mg/liter than those of control populations. There was no statistically significant difference between growth rates of control populations and populations exposed to lower concentrations of the toxicant.

At 96 hr, population density was reduced significantly [$F_{(4,\ 20)} = 6.06$, $P < 0.005$, in the 1st; and $F_{(2,\ 10)} = 217.9$, $P < 0.005$ in the 2nd experimental series]. The reduction ranged 13.6-22.4% in ciliate populations exposed to 1 mg/liter and 30.5% in those exposed to 10 mg/liter. No significant reductions in population density were observed in the presence of a lower concentration of the toxicant.

Accumulation.—Tetrahymena pyriformis accumulated Aroclor 1260 from medium that contained 0.001, 0.01, 0.1, or 1 mg/liter during 7-days exposure. Uptake of the toxicant from the medium was linear with increasing concentration. When initial concentration in the medium was plotted against mean concentration in the cells, the data agreed closely ($r = 0.917$) with the calculated regression line $\overline{Y}_m = 0.06967X - 0.2191$. Concentration factors ranged 21-79. Ciliates exposed to 0.001 mg/liter absorbed amounts that ranged from detected-but-unquantifiable to 61.7% ($\bar{x} = 38.2\%$) of the toxicant in the medium; those exposed to 0.01 mg/liter, 16.4-49.3% ($\bar{x} = 36.8\%$), those exposed to 0.1 mg/liter, 15.1-68.1% ($\bar{x} = 41.7\%$), and those exposed to 1 mg/liter, 26.4-81.0% ($\bar{x} = 53.3\%$).

We observed no indication of change in isomeric composition of Aroclor 1260 as was reported for Aroclor 1254 in tissues of fish (9) and shrimp (14).

Comparison of accumulation of Aroclor 1248 and Aroclor 1260 by *T. pyriformis* by the *t*-test (differences considered significant at $P \leqslant 0.05$) (16) revealed that (a) when concentration of each compound in the medium increased, concentrations of Aroclor 1248 in the cells increased at the same rate as did Aroclor 1260 ($P > 0.05$) and (b) the mean amounts of Aroclor 1248 and Aroclor 1260 accumulated were not significantly different ($P > 0.05$).

DISCUSSION

Protozoa, algae, and bacteria form the broad base of aquatic food chains. Ciliates are among the most numerous organisms of the estuarine benthos (1, 7) and may be more important as nutrient regenerators, particularly of nitrogen and phosphorus, than are bacteria (11, 12). Also, some ciliates, including *T. pyriformis,* can concentrate certain persistent pesticides and PCBs (4, 8), and thus help translocate them up the trophic pyramid. It has been shown that PCBs build up in food chains (6). It is possible, therefore, that PCBs acting on or through these ciliates could be toxic at higher trophic levels, either

through disruption of nutrient cycles or through translocation and bioaccumulation in the food chain (2, 3, 17).

Tests of Aroclor 1254 included concentrations found in natural waters and sediments (5). Testing similar concentrations of Aroclor 1248 and 1260 permitted comparison of effects and accumulation of the 3 compounds. All were toxic to and accumulated by *T. pyriformis,* although in different degree. Aroclor 1254 was the most toxic, as judged by significant reduction of both growth rate and 96-hr population density at 0.001 mg/liter concentration (4), whereas Aroclors 1248 and 1254 did not reduce significantly either population growth or 96-hr population density at concentrations below 1 mg/liter. Concentration of these PCBs by *T. pyriformis* increased with increasing chlorination of the toxicants: Aroclor 1248, 40 ×; Aroclor 1254, 60 ×; and Aroclor 1260, 79 ×. This trend appears to be in agreement with the suggestion that PCBs with fewer chlorines tend to be metabolized or excreted faster than those more chlorinated, so that the latter compounds tend to increase in food chains (10, 17).

At the concentrations tested, the chief effect of Aroclor 1254 on natural populations of *T. pyriformis* and ciliates that respond similarly might be reduction of population density. This would reduce their availability as food organisms and nutrient regenerators. Conversely, with Aroclors 1248 and 1260, reduction of population density is secondary in importance to accumulation of the toxicants by the ciliates. The ability of these ciliates to concentrate them would enable these PCBs to enter aquatic food chains, thereby permitting the toxicants to pass to, and possibly exert their effect at higher trophic levels.

REFERENCES

1. Borror, A. C. 1963. Morphology and ecology of the benthic ciliated protozoa of Alligator Harbor, Florida. *Arch. Protistenk.* **106**, 465-534.

2. Burdick, G. E., Harris, E. J., Dean, H. J., Walker, T. M., Skea, J. & Colby, D. 1964. The accumulation of DDT in lake trout and the effect on reproduction. *Trans. Am. Fish. Soc.* **93**, 127-36.

3. Butler, P. A. 1969. Monitoring pesticide pollution. *BioScience* **19**, 889-91.

4. Cooley, N. R., Keltner, J. M., Jr. & Forester, J. 1972. Mirex and Aroclor® 1254: Effect on and accumulation by *Tetrahymena pyriformis* strain W. *J. Protozool.* **19**, 636-8.

5. Duke, T. W., Lowe, J. I. & Wilson, A. J., Jr. 1970. A polychlorinated biphenyl (Aroclor® 1254), in water, sediment, and biota of Escambia Bay, Florida. *Bull. Environ. Contam. Toxicol.* **5**, 171-80.

6. Dustman, E. H., Stickel, L. F., Blus, L. J., Reichel, W. L. & Wiemeyer, S. N. 1971. The occurrence and significance of polychlorinated biphenyls in the environment. *Trans. 36th N. Am. Wildl. Nat. Resour. Conf.* 118-33.

7. Fenchel, T. 1967. The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. *Ophelia* **4**, 121-37.

8. Gregory, W. W., Jr., Reed, J. K. & Priester, L. E., Jr. 1969. Accumulation of parathion and DDT by some algae and protozoa. *J. Protozool.* **16**, 69-71.

9. Hansen, D. J., Parrish, P. R., Lowe, J. I., Wilson, A. J., Jr. & Wilson, P. D. 1971. Chronic toxicity, uptake, and retention of Aroclor® 1254 in two estuarine fishes. *Bull. Environ. Contam. Toxicol.* **6**, 113-9.

10. Jensen, S., Johnels, A. G., Olsson, M. & Otterlind, G. 1969. DDT and PCB in marine animals from Swedish waters. *Nature* (London) **224**, 247-50.

11. Johannes, R. E. 1965. Influence of marine protozoa on nutrient regeneration. *Limnol. Oceanogr.* **10**, 434-42.

12. ——— 1968. Nutrient regeneration in lakes and oceans, *in* Droop, M. R. & Ferguson Wood, E. J., eds., *Advances in Microbiology of the Sea*, Academic Press, New York **1**, 203-13.

13. Nimmo, D. R., Wilson, P. D., Blackman, R. R. & Wilson, A. J., Jr. 1971. Polychlorinated biphenyl absorbed from sediments by fiddler crabs and pink shrimp. *Nature* **231**, 50-2.

14. ———, Blackman, R. R., Wilson, A. J., Jr. & Forester, J. 1971. Toxicity and distribution of Aroclor® 1254 in pink shrimp *Penaeus duorarum*. *Mar. Biol.* (Berlin) **11**, 191-7.

15. Peakall, D. B. & Lincer, J. L. 1970. Polychlorinated biphenyls: another long-life widespread chemical in the environment. *BioScience* **20**, 958-64.

16. Snedecor, G. W. & Cochran, W. G. 1971. *Statistical Methods.* 6th ed. Iowa State Univ. Press, Ames.

17. U. S. Interdepartmental Task Force on PCBs. 1972. *Polychlorinated Biphenyls and the Environment.* Final Report. COM-72-10419. 192 pp. (National Technical Information Service, U. S. Dept. of Commerce, Springfield, Va. 22151.)

Differential Responses of Marine Phytoplankton to Herbicides: Oxygen Evolution

by TERRENCE A. HOLLISTER
Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory, Sabine Island
Gulf Breeze, Fla. 32561
and
GERALD E. WALSH
Associate Laboratory of the National Environmental Research Center
Corvallis, Ore.

INTRODUCTION

Marine unicellular algae vary in their responses to a variety of toxicants, including chlorinated hydrocarbon insecticides (UKELES 1962, MENZEL et al. 1970), organophosphate insecticides (DERBY and RUBER 1970), and fungicides (UKELES 1962). Little is known, however, about toxicities of herbicides to marine unicellular algae. Responses of four algal species to 30 herbicidal formulations have been reported (WALSH 1972) and the urea and triazine herbicides were the most toxic. Four urea herbicides also caused depression of the carbohydrate contents of six species of algae, and effect was directly proportional to salinity of the growth medium (WALSH and GROW 1971).

The work reported here was done to learn if marine unicellular algae differ in their responses to herbicides. We tested 18 species against the substituted ureas, neburon and diuron, and the triazines, atrazine and ametryne.

MATERIALS AND METHODS

The algae were obtained from the culture collections of the Woods Hole Oceanographic Institution, Scripps Institution of Oceanography, and Indiana University. All were maintained and tested in a growth medium composed of artificial seawater [2] supplemented with trace elements and vitamins. The supplements, per liter of medium, were: 30 mg Na_2EDTA, 14 mg $FeCl_2 \cdot 6H_2O$, 34 mg H_3BO_3, 4 mg $MnCl_2 \cdot 4H_2O$, 2 mg $ZnSO_4 \cdot 7H_2O$, 6 mg K_3PO_4, 100 mg $NaNO_3$, 40 mg $Na_2SiO_3 \cdot 9H_2O$, 5µg $CuSO_4$, 12 µg $CoCl_2$, 50 µg thiamine hydrochloride, 1 µg vitamin B_{12}, and 0.01 µg biotin. Salinity was 30 parts per thousand and the pH ranged between 7.9 and 8.1. The medium was sterilized by autoclaving for 15 minutes at 121°C.

Five ml of stock algae were inoculated into 100 ml of growth medium and incubated at 20°C under 6,000 lux illumination from fluorescent tubes with alternating 12-hour periods of light and

[1] Contribution No. 159 from the Gulf Breeze Laboratory.

[2] From Rila Products, Teaneck, New Jersey. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

Bulletin of Environmental Contamination & Toxicology,
Vol. 9, No. 5 © 1973 by Springer-Verlag New York Inc.

darkness for three days. Then, the cultures were centrifuged gently and resuspended in growth medium to an optical density of 0.100 at 525 mμ on a Fisher electrophotometer. The algal cultures were not axenic.

Effects of herbicides were measured as inhibition of oxygen evolution. Both ureas and triazines inhibit photosynthesis and move quickly into the cells (ZWEIG 1969) and the concentrations required for inhibition of both growth and photosynthesis are the same (WALSH 1972).

Concentrations of herbicides in the suspending media ranged from zero to those which inhibited evolution of oxygen by approximately 25, 50, and 75%. Concentrations were calculated as parts per billion (ppb) of the technical preparation. From each cell suspension, 5.0 ml were placed in reaction vessels of a Gilson photosynthesis model differential respirometer. After equilibrating at 20°C for 30 minutes, oxygen evolution was measured for 60 minutes with a CO_2 buffer in the well of the reaction vessel (UMBREIT et al. 1964). Duplicate flasks were analyzed and each test was performed three times.

All data were subjected to statistical analysis. Mean percentage inhibition after 60 minutes was calculated and EC50 values (effective concentrations at which evolution of oxygen was 50% that of untreated cell suspensions) were calculated by the least squares method. Concentrations were converted to logarithms and responses to probits and the standard error obtained for each series of tests.

RESULTS AND DISCUSSION

The EC50 values for the four herbicides and 18 algal species tested are shown in Table 1 and summarized by family in Table 2. Atrazine was the least toxic; ametryne, neburon, and diuron were approximately equal in toxicity. Species of the family Bacillariophyceae were generally the least sensitive, requiring as much as 5.8 times more ametryne to reduce oxygen evolution by 50% than did species of the other families.

Wide variations occured in response to the toxicants among the individual species of the families Chlorophyceae, Bacillariophyceae, and Chrysophyceae. A measure of the range of responses among species was calculated by derivation of the ratio of the highest EC50 to the lowest EC50. The ratio, here called the "Difference Factor", is given for each family and herbicide in Table 2. Difference Factors were greatest in the Bacillariophyceae, being as high as 11.9 for neburon-treated algae. In that case, the EC50 for Cyclotella nana was 11 ppb, whereas for Nitzschia (Indiana strain 684) it was 131 ppb.

TABLE 1. EC50 (ppb) of neburon, diuron, atrazine, and ametryne on oxygen evolution by marine unicellular algae. Standard errors (SE) were derived by unweighted probit analysis.

Family	Species	Neburon		Diuron		Atrazine		Ametryne	
		EC50	SE	EC50	SE	EC50	SE	EC50	SE
Chlorophyceae									
	Chlamydomonas sp.	37	5	37	3	60	8	41	5
	Dunaliella tertiolecta	10	3	10	3	159	18	40	6
	Platymonas sp.	12	5	17	3	102	8	24	4
	Chlorella sp.	22	3	19	2	143	8	32	3
	Neochloris sp.	39	6	28	5	82	7	36	7
	Chlorococcum sp.	20	3	20	4	80	7	10	3
Bacillariophyceae									
	Thalassiosira fluviatilis	108	9	95	10	110	19	58	7
	Navicula inserta	124	11	93	12	460	15	97	9
	Amphora exigua	82	5	31	4	300	21	26	4
	Achnanthes brevipes	23	4	24	1	93	11	19	1
	Stauroneis amphoroides	17	3	31	2	348	67	65	11
	Cyclotella nana	11	4	39	7	84	19	55	8
	Nitzschia closterium	120	13	50	6	287	68	62	6
	Nitzschia (Ind. 684)	131	9	169	17	434	84	135	11
Chrysophyceae									
	Monochrysis lutheri	12	4	18	3	77	23	14	4
	Isochrysis galbana	20	5	10	3	100	17	10	4
	Phaeodactylum tricornutum	40	7	10	3	100	19	10	5
Rhodophyceae									
	Porphyridium cruentum	24	4	24	3	79	9	35	4

TABLE 2. Average EC50 values (ppb) for four herbicides and four families of marine unicellular algae. The Difference Factor (DF) is the ratio of the highest to the lowest EC50 among the algal species.

Family	Number of Species Tested	Neburon		Diuron		Atrazine		Ametryne	
		EC50	DF	EC50	DF	EC50	DF	EC50	DF
Chlorophyceae	6	23	3.9	22	3.7	104	2.6	31	4.1
Bacillariophyceae	8	77	11.9	67	7.0	265	5.5	65	7.1
Chrysophyceae	3	24	3.3	13	1.8	92	1.3	11	1.4
Rhodophyceae	1	24	-	24	-	79	-	35	-

These data show that when bioassay analyses are conducted for effects of herbicides on marine unicellular algae, two factors are particularly important: (1) the response in relation to familial taxonomic position, and (2) the wide range of responses by individual species within a given family. It is necessary, therefore, to use several species from each of several families in algal bioassay studies to obtain realistic data concerning effects of herbicides on algae.

ACKNOWLEDGEMENT

We thank E. I. DuPont de Nemours and Co. for providing the urea herbicides, and Geigy Agricultural Chemicals for providing the triazine herbicides used in these experiments.

REFERENCES

DERBY, S. B. (SLEEPER) and E. RUBER. Bull. Environ. Contam. Toxicol. 5:553-558, (1970).

MENZEL, D. W., J. ANDERSON, and A. RANDTKE. Science 167: 1724-1726, (1970).

UKELES, R. Appl. Microbiol. 10:532-537, (1962).

UMBREIT, W. W., R. H. BURRIS, and J. F. STAUFFER. Manometric Techniques, 4th ed., Burgess Pub. Co., Minneapolis, Minn., 305 pp. (1964).

WALSH, G. E. Hyacinth Control J. 10:45-48, (1972).

WALSH, G. E. and T. E. GROW. Weed Sci. 19:568-570, (1971).

ZWEIG, G. Residue Rev. 25:69-79, (1969).

CONTRIBUTION NO. 163

Comp. Biochem. Physiol., 1974, *Vol.* 49**B**, *pp.* 375 *to* 379. *Pergamon Press. Printed in Great Britain*

SOME ASPECTS OF MYOSIN ADENOSINE TRIPHOSPHATASE OF PINK SHRIMP (*PENAEUS DUORARUM*)*

W. P. SCHOOR

U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561, U.S.A. (Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon)

(*Received* 22 *October* 1973)

Abstract—1. Myosin ATP'ases of shrimp and rabbit muscle behave similarily except at temperatures above 30°C where the activity in shrimp started to decline rapidly.

2. There is a correlation between the effects of temperature on myosin ATP'ase and behavior of shrimp in nature; this compares well with similar observations in crayfish (Maruyama, 1958).

3. Data obtained emphasize that under certain conditions caution must be used in determining initial enzymatic activities.

INTRODUCTION

PURIFICATION of myosin adenosine triphosphatase (ATP'ase, E.C. 3.6.1.3) is usually carried out by modification of the method of Szent-Györgyi (1951) in combination with one of a variety of buffers. The characteristics of proteins in solution are such, however, that changes in ions, as well as changes in pH and temperature, can affect their conformation (Flory, 1956). If the proteins possess catalytic function, altered conformation may cause changes in their specific activity. In the research reported here, some aspects of shrimp myosin ATP'ase in solution were investigated, using the method of Szent-Györgyi for purification and a minimal amount of histidine buffer.

MATERIALS AND METHODS

Pink shrimp (*Pénaeus duorarum*) from the Gulf of Mexico near Tampa, Florida, were held in large tanks with flowing sea water and fed fish muscle for 2 weeks prior to use. Sea water (February–May) averaged 10°C and 23‰ salinity. Heads of shrimp were removed and tails diced after removal of exoskeleton, nerve cord and digestive tract. Ten g of diced muscle were homogenized in a blender for 30 sec with 50 ml of 0·5 M KCl that contained 0·002 M histidine at pH 7·0 (KCl–histidine buffer), and centrifuged in a Beckman Model L 3-50 ultracentrifuge at 70,000 *g* (max.) for 45 min, using a SW-25.2 rotor. The supernate, usually 35–40 ml, was decanted and diluted 1 : 19 with 0·5 mM histidine at pH 7·0. The resulting flocculate was allowed to settle for 30 min before centrifugation at 17,000 *g* (max.) for 10 min, using a SW-50.1 rotor. The supernate was decanted and sufficient

* Contribution No. 163, Gulf Breeze Environmental Research Laboratory.

KCl–histidine buffer added to bring the volume to 25 ml. The resulting solution was adjusted to 0·5 M in KCl by the addition of powdered KCl. This procedure was repeated twice more. The final enzyme solution was centrifuged at 100,000 *g* (max.) for 1 hr to remove aggregates and diluted to the desired enzyme concentration with KCl–histidine buffer.

The specific activity of the myosin ATP'ase is expressed here as the number of micro-equivalents of inorganic phosphate (P_i) liberated per gram of protein per second (μ-equiv. P_i/g per sec). The ATP'ase activity was determined by the pH-stat technique (Kay & Brahms, 1963) with a Radiometer pH Meter 26, Titrator II, Autoburette ABU 12, Titigraph SBR-2-c, and G-222-B and K 401 electrodes. The reaction mixture was kept at constant temperature in a jacketed vessel connected to a Lauda K-2/R Circulator. The reaction volume was 5 ml and no adjustment was made for the small error in concentration caused by dilution with base. The order of addition of reagents was inconsequential, but it was convenient to add the enzyme last. Unless otherwise indicated, only initial activities are given. Reagent blanks were determined in the absence of myosin. The Biuret method with bovine serum albumin as the standard was used for protein assay.

RESULTS

The enzymatic activities for shrimp myosin ranged from 3 to 5 μ-equiv. P_i/g per sec and were linear up to 2 mg of protein per assay, after which the activity declined.

Table 1 shows the change in the specific activity as a function of pH and time. Specific activity between pH 6·5 and 8·0 increased with increasing pH and remained constant for at least 4 min. After 1 min at pH 8·5 and 9·0, the activity of the ATP'ase decreased. True initial activity at those pH values may have been underestimated because of rapid inactivation of the enzyme.

TABLE 1—EFFECT OF pH ON SPECIFIC ACTIVITY OF MYOSIN ATP'ase OF SHRIMP (*Penaeus duorarum*)*

	Specific activity (min)			
pH	1	2	3	4
5·5	0	0	0	0
6·5	0·70	0·70	0·70	0·70
7·0	0·92	0·92	0·92	0·92
7·5	1·58	1·58	1·58	1·58
8·0	3·32	3·32	3·32	3·32
8·5	5·22	1·38	0·94	0·34
9·0	3·00	1·38	0·94	0·34

* Assay conditions: 1·44 mg protein/assay, 0·5 M KCl, 0·1 M $CaCl_2$, 2 mM ATP, 25°C. Values represent the average of three determinations; error was ± 10 per cent maximum.

Table 2 shows the same type of enzymatic response as a function of temperature. There was no change in the specific activity of myosin ATP'ase during the 3-min reaction interval between 20 and 25°C. The specific activities rose during the first minute up to 30°C but declined at 35°C, at which temperature the true initial

TABLE 2—EFFECT OF TEMPERATURE ON THE SPECIFIC ACTIVITY OF MYOSIN ATP'ase OF SHRIMP (*Penaeus duorarum*)*

Temperature (°C)	Specific activity (min)	
	1	3
20	2·29	2·29
25	2·92	2·92
30	3·48	1·53
35	3·36	0·10

* Assay conditions: 1·44 mg protein/assay, 0·5 M KCl, 0·1 M $CaCl_2$, 2 mM ATP, pH 8·0. Values represent the average of three determinations; error was ± 10 per cent maximum.

activity may have been underestimated because of rapid inactivation of the enzyme.

Table 3 shows the effects of storage at 0°C and $CaCl_2$ on the shrimp ATP'ase system. Activation by the addition of $CaCl_2$ continued as the specific activity decreased with increasing length of storage at 0°C. Concentrations of $MgCl_2$ above 10^{-4} M completely inhibited the enzymatic activity.

TABLE 3—EFFECTS OF STORAGE AT 0°C AND $CaCl_2$ ON THE SPECIFIC ACTIVITY OF MYOSIN ATP'ase OF SHRIMP (*Penaeus duorarum*)*

Storage time (hr)	Specific activity ($CaCl_2$, moles/l.)				
	0	0·04	0·08	0·10	0·12
1	0·41	2·93	4·28	4·36	3·00
24	<0·10	2·11	2·28	1·79	0·87
48	<0·10	0·71	1·00	0·94	0·15

* Assay conditions: 1·50 mg protein/assay, 0·5 M KCl, 2 mM ATP, pH 8·0, 25°C. Values represent the average of three determinations; error was ± 10 per cent maximum.

DISCUSSION

A reduction in specific activity at concentrations above 2 mg protein per assay suggests that protein aggregation is taking place in the manner described for rabbit myosin (Lowey & Holtzer, 1959; Johnson & Rowe, 1961) and for cod myosin (Mackie, 1965). Other physical properties of the structural proteins of cod and carp, and rabbit myosin also appear to be closely related (Hamoir, 1955). The $CaCl_2$ activation effect still remained, even though the isolated enzyme system was unstable when stored at 0°C.

In these studies, shrimp myosin ATP'ase behaved similarly to rabbit myosin ATP'ase (Schoor & Mandelkern, unpublished results) under identical assay conditions. The range of $CaCl_2$ activation and complete loss of activity in the presence

of 10^{-4} M $MgCl_2$ was identical in both enzyme systems. However, differences in the maximum activities did exist, specific activities ranging from 8 to 10 μ-equiv. P_i/g per sec in rabbit myosin and 3–5 μ-equiv. P_i/g per sec in shrimp myosin.

Initial activities of the rabbit myosin remained linear for at least 5 min at temperatures up to 40°C; that of shrimp myosin decreased rapidly at 35°C. "Total" muscle ATP'ases show approximately the same activity in fishes, frog, mouse, bird and turtle at 30°C, but wide differences occur at 0°C (Steinbach, 1949; Davidson & Richards, 1954).

The cold-blooded animals showed higher activities at the lower temperatures than did warm-blooded ones. Although these data agree well with my findings, it must be remembered that "total" ATP'ase activity includes actomyosin ATP'ase, which was not measured in this study. Field studies have shown that pink shrimp tolerated water temperatures of 30–31°C, appeared hyperactive around 32°C and at 35°C had a low survival rate (Heald, personal communication). The same is true for the protozoeal stage of the pink shrimp (Thorhaug *et al.*, 1971). The actomyosin ATP'ase of crayfish (*Cambarus clarkii*) had an optimal activity at pH 7·0 at 30–35°C, the activity quickly declining at 37°C (Maruyama, 1958). This temperature is somewhat higher than the maximum found for the pink shrimp myosin ATP'ase, but parallels the higher temperatures to which the crayfish is exposed in its shallow water habitat.

Even so, until the temperature dependence of other shrimp enzyme systems has been established, it is speculative to suggest that inactivation of myosin ATP'ase is the reason for the reduced survival rate of shrimp at temperatures above 32°C. Nevertheless, observed differences in the effects of temperature on enzyme systems, having similar functions, seem to be a good example of adaptability of such systems in warm- and cold-blooded animal species.

REFERENCES

DAVIDSON J. A. & RICHARDS A. G. (1954) Muscle apyrase activity as a function of temperature in the cockroach, crayfish and minnow. *Archs Biochem. Biophys.* **48,** 484–486.

FLORY P. J. (1956) Role of crystallization in polymers and proteins. *Science, Wash.* **124,** 53–60.

HAMOIR G. (1955) Fish proteins. *Adv. Protein Chem.* **10,** 227–288.

JOHNSON P. & ROWE A. J. (1961) The spontaneous transformation reactions of myosin. *Biochim. biophys. Acta* **53,** 343–360.

KAY C. M. & BRAHMS J. (1963) The influence of ethylene glycol on the enzymatic adenosine triphosphatase activity and molecular conformation of fibrous muscle proteins. *J. biol. Chem.* **238,** 2945–2949.

LOWEY S. & HOLTZER A. (1959) The aggregation of myosin. *J. Am. Chem. Soc.* **81,** 1378–1383.

MACKIE I. M. (1965) The effect of adenosine triphosphate, inorganic pyrophosphate and inorganic tripolyphosphate on the stability of cod myosin. *Biochim. biophys. Acta* **115,** 160–172.

STEINBACH H. B. (1949) Temperature coefficients in muscle apyrase systems. *J. cell. comp. Physiol.* **33,** 123–131.

SZENT-GYÖRGYI A. (1951) *Chemistry of Muscular Contraction,* 2nd End., p. 146. Academic Press, New York.

THORAUG A., DEVANY T. & MURPHY B. (1971) Refining shrimp culture methods: the effect of temperature on early stages of commercial pink shrimp. Proc. 23rd Ann. Session, Gulf and Carib. Fish Inst., pp. 125–132.

Key Word Index—Pink shrimp (*Penaeus duorarum*); myosin ATP'ase; rabbit; temperature effects; purification; comparison.

COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY

Volume 49 *Number* 2*A* 1 *October* 1974

CONTRIBUTION NO. 164

AROCLOR® 1254: EFFECT ON COMPOSITION OF DEVELOPING ESTUARINE ANIMAL COMMUNITIES IN THE LABORATORY*

David J. Hansen

U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida, U.S.A. 32561
(Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon)

ABSTRACT

Aroclor® 1254, a polychlorinated biphenyl (PCB), affected the composition of communities of estuarine animals that developed from planktonic larvae in salt water that flowed through 10 control aquaria and 10 aquaria contaminated with 0.1, 1 or 10 μg/l of this PCB. Communities that developed in control aquaria and aquaria that received 0.1 μg/l of PCB in water for four months were dominated (>75%) by arthropods, primarily the amphipod *Corophium volutator*. In aquaria receiving 1 and 10 μg/l, the number of arthropods decreased and the number of chordates, primarily the tunicate *Mogula manhattensis*, increased: over 75% of the animals in 10 μg/l aquaria were tunicates. Numbers of phyla, species, and individuals (particularly amphipods, bryozoans, crabs, and mollusks) were decreased in this PCB, but there was no apparent effect on the abundance of annelids, brachiopods, coelenterates, echinoderms or nemerteans. The Shannon-Weaver index of species diversity was not altered by Aroclor 1254.

INTRODUCTION

Polychlorinated biphenyls (PCBs) have been manufactured for various uses (Broadhurst 1972) for over 40 years, but their occurrence in aquatic ecosystems was not confirmed until 1966 (Anonymous 1966). Since then, PCBs have been detected in estuarine organisms from 6 of 15 of the coastal United States (Butler 1973). One PCB, Aroclor 1254, was discovered in the water, sediment and biota of Escambia Bay, Florida (Duke *et al.* 1970).

Chronic and acute toxicity experiments conducted at the Gulf Breeze Laboratory have established that Aroclor 1254 is toxic to some estuarine organisms. A concentration of 100 μg/l of Aroclor was acutely toxic (48 to 96 hours) to pink shrimp, *Penaeus duorarum*, and oysters, *Crassostrea virginica*, but not to pinfish, *Lagodon rhomboides* (Duke *et al.* 1970). Chronic toxicity was up to 100 times

* Contribution No. 164, Gulf Breeze Environmental Research Laboratory. ®Registered trademark, Monsanto Company, St. Louis, Missouri, U.S.A. Mention of commercial products or trade names does not constitute endorsement by the Environmental Protective Agency.

greater than acute toxicity. In exposures lasting more than two weeks, 1 μg/l of Aroclor 1254 killed pink shrimp (Nimmo *et al.* 1971a), whereas 5 μg/l killed pinfish and spot, *Leiostomus xanthurus* (Hansen *et al.* 1971) and significantly reduced oyster growth rate (Parrish *et al.* 1972), but it was not lethal to blue crabs, *Callinectes sapidus* (Duke *et al.* 1970). Aroclor 1254 is, therefore, toxic to certain estuarine species exposed separately. However, its effect on communities of estuarine animals is not known. This study reports experiments that determine the effect of this chemical on development of estuarine animal communities in the laboratory.

MATERIALS AND METHODS

I investigated the effect of Aroclor 1254 on development of estuarine communities by comparing the number, species, and diversity of animals that grew from planktonic larvae in apparatuses continuously contaminated with 0.1, 1 or 10 μg/l of PCB for four months, 18 May to 25 September 1970, with animals from an identical apparatus that was not contaminated.

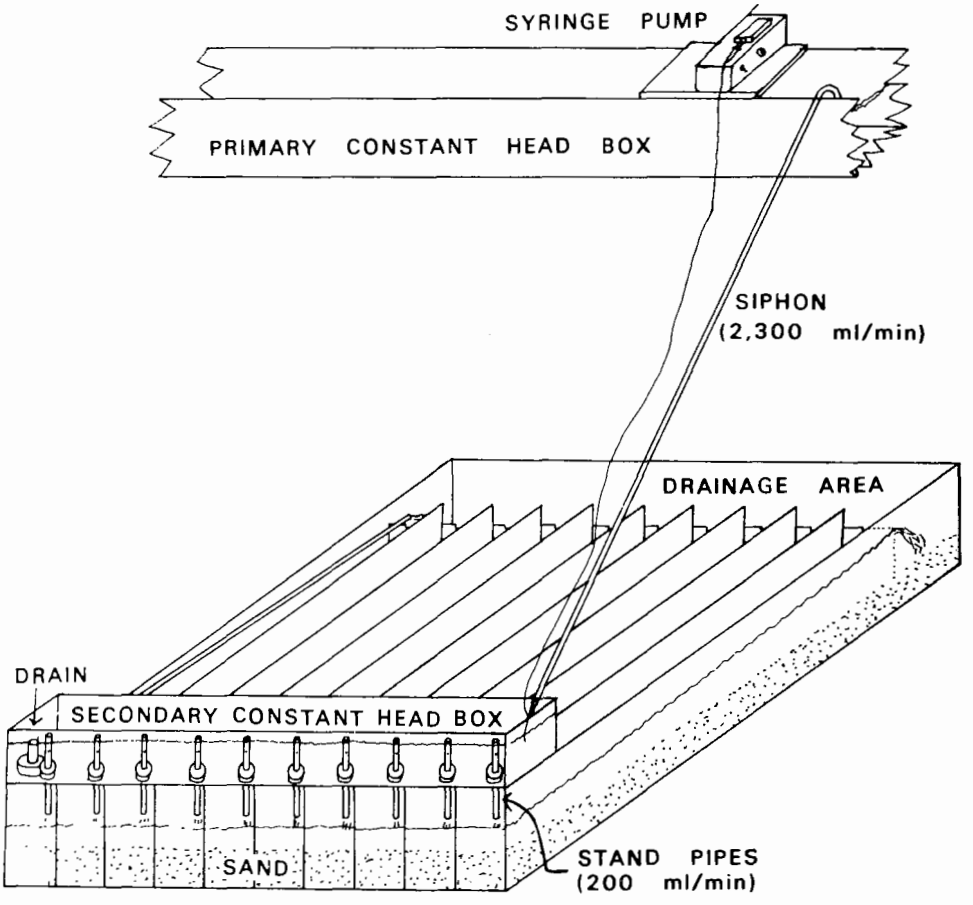


FIG. 1.—Apparatus used to test the effect of Aroclor 1254 on composition of estuarine animal communities.

The apparatus used in this investigation is illustrated in Fig. 1 (only one of four identical apparatuses is shown). Sea water with its natural component of plankton was pumped from the estuary adjacent to the laboratory into the primary constant head box. Salinity of the water ranged from 10 to 34‰ (average, 29.7‰) and temperature ranged from 22 to 33 C (average, 28.5 C). In contaminated apparatuses Aroclor 1254 was added to water after it was siphoned, at the rate of 2,300 ml/min, from the primary into the secondary constant head box; the control apparatus received the same flow of water. Water then flowed from the secondary constant head box to each of 10 adjacent aquaria-10 replicates for each treatment. (Treatment includes control and contaminated apparatuses.) Flow rate through each aquarium was maintained at 200 ml/min each by adjusting the height of a 2.7 mm diameter hole in each of the 10 standpipes in the secondary constant head box. Each aquarium was 44 cm long, 9 cm wide and 14 cm high. Water depth in each aquarium was maintained at 8 cm. PCB-free sand was placed in each aquarium to a depth of 6 cm. Planktonic larvae colonized this sand and the walls of the aquaria. The siphon and constant head boxes were cleaned weekly so that all aquaria received planktonic larvae from a common source-the incoming water. Water leaving each aquarium flowed through a V-shaped opening and into a common drain for the apparatus.

Aroclor 1254, dissolved in polyethylene glycol 200, was metered by a syringe pump into the water as it entered the secondary constant head box of each experimental apparatus. The same amount of polyethylene glycol (2 ml/day, 0.68 mg/l) was metered into the control apparatus. Solvent-induced effect was not expected because: (1) polyethylene glycol 200 did not affect development of two species of crabs at this concentration (Epifanio 1971); (2) concentrations up to 1% (v/v) were not lethal to grass shrimp, *Palaemonetes pugio*, or sheepshead minnows, *Cyprinodon variegatus*, in 96 hours in static tests (Hansen unpublished data); and (3) the toxicity of 5 μg/l of Aroclor 1254 to brown shrimp, *Penaeus aztecus*, and pinfish was not increased by increasing the concentration of solvent up to 100 times (0.1 to 10.0 mg/l) (Hansen unpublished data).

Concentrations of Aroclor in test water and sediment were determined by gas chromatography. Methods of analysis for water are described by Nimmo *et al.* (1971a) and for sediment by Nimmo *et al.* (1971b), except that an OV-101 column was used. Recovery rates were above 70%, but data in this report do not include a correction factor for recovery. Water from the secondary constant head box of each apparatus was analyzed twice a month (Table 1). Concentrations throughout this box were uniform; water from each standpipe, analyzed once during the 10 μg/l exposure, averaged 7.9 μg/l (range 6.8–8.7 μg/l). Sediment cores from 4 of 10 aquaria from each apparatus were analyzed at the end of the exposure (Table 1).

At the end of the four-month exposure, animals were scraped from the side of the aquaria and the contents of the aquaria siphoned into a 1 mm mesh sieve. During the last three months of the experiment, animals that left or were lost from the aquaria or were cleaned from the secondary constant head box and the drainage area were also collected in a 1 mm mesh sieve. Animals

TABLE 1

Range and average concentration of Aroclor® 1254 in water and sediment from experiment and control apparatuses. Water was analyzed twice monthly and sediment was analyzed at the end of the four month experimenta. Limit of quantification was 0.1 μg/l in water and 0.015 mg/kg (dry weight) in sediment. Correction for recovery (>70%) is not included.

Concentration in water, μg/l			Concentration in sediment, μg/g	
	Measured		Measured	
Nominal	Average	Range	Average	Range
Control	None		None	
0.1	<0.1	<0.1 –0.1	0.1	0.05–0.18
1.0	0.6	0.48–0.72	0.36	0.25–0.42
10.0	6.7	5.2 –7.8	2.0	1.5 –2.5

TABLE 2

Animals collected from control aquaria and from aquaria contaminated for four months with Aroclor® 1254. Ten aquaria were used for each treatment. Number of animals and number of aquaria from which they were collected are listed

Taxon	Control		Aroclor 1254						Total animals
			0.1 µg/l		1 µg/l		10 µg/l		
	Animals	Aquaria	Animals	Aquaria	Animals	Aquaria	Animals	Aquaria	
Annelida									
Armandia agilis	8	5	11	4	3	2	0	0	22
Capitella capitata	34	10	30	8	34	9	23	8	121
Cirratulus sp.	1	1	0	0	1	1	0	0	2
Dasybranchus sp.	0	0	0	0	1	1	0	0	1
Eupomatus dianthus	6	5	8	6	11	6	9	6	34
E. protulicola	8	4	13	9	5	4	17	9	43
Heteromastus filiformis	0	0	0	0	2	1	0	0	2
Laeonereis culveri	1	1	0	0	0	0	0	0	1
Lumbrinereis parvapedata	1	1	0	0	0	0	0	0	1
Marphysa sanguinea	1	1	0	0	0	0	0	0	1
Mediomastus californiensis	0	0	0	0	0	0	1	1	1
Nearthes succinea	8	6	11	7	12	6	9	7	40
Notomastus hemipodus	0	0	0	0	1	1	0	0	1
Podarke, near *guanica*	1	1	1	1	0	0	0	0	2
Polydora websteri	28	10	35	10	51	8	22	4	136
Polydora sp.	2	2	1	1	1	1	0	0	4
Prinospio sp.	1	1	2	1	0	0	0	0	3
Spiophanes bombyx	1	1	0	0	0	0	0	0	1
Streblospio benedicti	6	4	7	4	3	3	0	0	16
Capitellidae sp.	0	0	0	0	0	0	3	1	3
Spionidae sp.	6	1	0	0	0	0	0	0	6
Unidentified sp. #1	2	2	0	0	2	1	1	1	5
Unidentified sp. #2	1	1	0	0	1	1	0	0	2

TABLE 2—Continued

Taxon	Control		Aroclor 1254						Total animals
			0.1 µg/l		1 µg/l		10 µg/l		
	Animals	Aquaria	Animals	Aquaria	Animals	Aquaria	Animals	Aquaria	
Arthropoda									
Balanus sp.	6	4	2	2	9	6	2	1	19
Caprella sp.	0	0	11	1	3	3	0	0	14
Corophium volutator	1338	10	1693	10	736	10	3	3	3770
Neopanope texana	2	2	0	0	0	0	0	0	2
Upogebia affinis	0	0	0	0	0	0	1	1	1
Decapod larvae, unident. sp.	0	0	0	0	4	3	0	0	4
Pycnogonidae, unident. sp.	0	0	3	1	28	6	1	1	32
Brachiopoda									
Glottidia pyramidata	1	1	0	0	0	0	0	0	1
Chordata									
Bostrichobranchus pilularis	100	1	2	2	3	1	29	5	134
Branchiostoma caribaeum	2	1	1	1	0	0	0	0	3
Molgula manhattensis	91	7	141	10	434	9	499	10	1164
Coelenterata									
Leptomedusae*, unident. sp.	5	5	4	4	8	8	7	7	24
Zoantharia, unident. sp.	0	0	0	0	5	3	0	0	5
Echinodermata									
Hemipholis elongata	4	2	17	4	15	6	0	0	1
?*Amphioplus* sp.	1	1	0	0	0	0	0	0	36
Ectoprocta									
*Membranipora tenuis**	6	6	6	6	4	4	0	0	16
Mollusca									
Abra aequalis	15	8	11	6	5	5	0	0	31
Amygdalum papyria	10	6	4	3	0	0	6	6	20
Anachis translirata	2	2	0	0	0	0	0	0	2
Andara transversa	6	4	4	4	3	3	6	5	19
Barnea costata	5	4	1	1	1	1	1	1	8
Bittium varium	16	7	4	4	7	4	1	1	28

TABLE 2—Continued

Taxon	Control		Aroclor 1254						Total animals
			0.1 µg/l		1 µg/l		10 µg/l		
	Animals	Aquaria	Animals	Aquaria	Animals	Aquaria	Animals	Aquaria	
Caecum pulchellum	0	0	2	2	0	0	0	0	2
Crassostrea virginica	4	3	5	4	4	3	4	4	17
Crepidula fornicata	0	0	0	0	0	0	3	3	3
Epitonium humphreysi	0	0	0	0	1	1	0	0	1
Laevicardium mortoni	9	5	3	3	2	2	4	3	18
Lyonsia hyalina	2	1	1	1	1	1	0	0	4
Mactra fragilis	0	0	1	1	1	1	0	0	2
Mitrella lunata	2	2	0	0	1	1	0	0	3
Modiolus americanus	1	1	0	0	1	1	1	1	3
M. demissus	1	1	1	1	2	1	0	0	4
Mulinia lateralis	5	3	0	0	1	1	0	0	6
Musculus lateralis	7	4	3	2	2	1	3	2	15
Nassarius albus	1	1	0	0	2	2	0	0	3
N. vibex	0	0	0	0	1	1	0	0	1
Ostrea equestris	1	1	0	0	1	1	0	0	2
Retusa canaliculata	1	1	0	0	0	0	0	0	1
Rissoina catesbyana	1	1	0	0	0	0	0	0	1
Tagelus divisus	0	0	1	1	1	1	0	0	2
Tellina alternata	9	7	3	3	7	7	1	1	20
Eolidacea, unident. sp.	1	1	0	0	0	0	0	0	1
Gastropoda, unident. sp.	2	1	0	0	0	0	0	0	2
Nemertea									
Oerstedia dorsalis	2	2	0	0	0	0	0	0	2
Unidentified sp.	1	1	0	0	0	0	0	0	1

* Colonies: Counted as one animal.

retained by the sieve were placed in fingerbowls of seawater, relaxed with $HgCl_2$, preserved in 50% isopropanol and identified.

To determined effect of Aroclor 1254 in each treatment, an index of species diversity as well as the number and percent occurrence of various species in each treatment (contaminated and control aquaria) were compared. A species diversity index provides a numerical means of assessing community structure that is independent of sample size, expresses the relative importance of each species and is dimensionless. Modifications of the Shannon-Weaver (1963 formula, $H' = -\sum_{i=1}^{s} p_i \log p_i$, where p_i is the proportion of the i^{th} species in the collection and $s =$ the number of species, have been used in freshwater (Wilhm and Dorris 1968) and saltwater (Bechtel and Copeland 1970) to assess effects of pollution on natural communities. In unpolluted areas, many species of animals are abundant and diversity is high, but pollution can decrease diversity by making a few species very abundant and all others rare. In this study, the Shannon-Weaver formula ($\log_2$) was used to determine the usefulness of the species diversity index in assessing the effect of Aroclor 1254 on community structure in laboratory experiments.

Pooled data from each Aroclor concentration and control were compared statistically using the χ^2 test for independent samples. Data from each of the 10 aquaria receiving one treatment were compared with data from 10 aquaria receiving a different treatment using the Mann-Whitney "U" test (Siegal 1956). Differences were considered real at $\alpha = 0.01$.

RESULTS

A large number and variety of animals were found in all aquaria (Tables 2 & 3). Of the 67 species from nine phyla, 27 were mollusks (15 pelecypods and 12 gastropods), 23 annelids, 6 arthropods, 3 chordates, 2 coelenterates, 2 enchinoderms, 2 nemerteans, 1 brachiopod and 1 bryozoan. Anthropods were most abundant (3,842) of the 5,897 animals followed by chordates (1,302), annelids (448), mollusks (219) and animals from other phyla (86). The two most abundant animals were the amphipod, *Corophium volutator* (3,770), and the tunicate, *Molgula manhattensis* (1,164).

Species composition and abundance of individual species varied among the 40 aquaria. The number of species in each aquarium ranged from 5 to 23 (average 13). Annelids and mollusks were present in all aquaria. Animals from other phyla were present in from 1 to 36 aquaria (Table 3). Seventeen species were found in all treatments and 26 were in only one.

Aroclor 1254 prevented animals of certain phyla from colonizing (Table 3). Although nine phyla were found, the number of phyla represented in any aquarium ranged from three to seven. The number of phyla in the control, 0.1 and 1 μg/l aquaria averaged 5.7, 5.4 and 5.7, respectively. Fewer phyla, average 4.1, were in aquaria contaminated with 10 μg/l of Aroclor because fewer aquaria contained arthropods, and none contained bryozoans.

The total number of species found in each apparatus ranged from 25 to 52 but the percentage of species in each phylum was similar in all four treatments (Table 4). In each apparatus, more species of mollusks were found than species from any other phylum. The relative numbers of molluscan species were similar for all treatments (40–44 percent). The number of annelid species was only slightly less (29–35 percent). Although fewer arthropods and chordates were found, the relative numbers of each were similar in all treatments.

TABLE 3

Number of control and experimental aquaria that contained animals, by phylum. Ten aquaria were used for each control and contaminated apparatus. Experimental aquaria were contaminated continuously with Aroclor 1245 for four months.

Phylum	Control aquaria	Aroclor 1254 aquaria		
		0.1 µg/l	1.0 µg/l	10.0µg/l
Annelida	10	10	10	10
Arthropoda	10	10	10	4
Branchiopoda	1	0	0	0
Chordata	7	10	9	10
Coelenterata	5	4	9	7
Echinodermata	3	4	6	0
Ectoprocta	6	6	4	0
Mollusca	10	10	10	10
Nemertea	3	0	0	0
Total phyla in apparatus	9	7	7	6
Average number of phyla per aquarium	5.7	5.4	5.7	4.1

TABLE 4

Number of species, by phylum, that developed from planktonic larvae in control apparatus and in apparatuses contaminated continuously for four months with 0.1, 1 or 10 µg/l of Aroclor 1254. Each apparatus consisted of 10 aquaria.

Taxon	Control		Aroclor 1254					
			0.1 µg/l		1 µg/l		10 µg/l	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Annelida	18	34.6	10	29.4	14	32.6	8	32.0
Arthropoda	3	5.8	4	11.8	4	9.3	4	16.0
Chordata	3	5.8	3	8.8	2	4.6	2	8.0
Mollusca	21	40.4	14	41.2	19	44.2	10	40.0
Other phyla	7	13.4	3	8.8	4	9.3	1	4.0
Total	52	100.0	34	100.0	43	100.0	25	100.0

TABLE 5

Number of species, by phylum, that developed from planktonic larvae in 10 control aquaria and 10 aquaria in each apparatus contaminated continuously for four months with 0.1, 1 or 10 µg/l of Aroclor 1254.

Taxon	Control		Aroclor 1254					
			0.1 µg/l		1.0 µg/l		10.0 µg/l	
	Average number per aquarium	Range	Average number per aquarium	Range	Average number per aquarium	Range	Average number per aquarium	Range
Annelida	5.7	2–10	5.1	3–7	4.5	2–6	3.9	3–5
Arthropoda	1.6	1–2	1.4	1–4	2.8	2–4	0.6	0–3
Chordata	0.9	0–3	1.3	1–2	1.0	0–2	1.5	1–2
Mollusca	6.3	2–10	3.6	1–7	3.9	1–6	2.7	1–5
Other phyla	1.8	0–3	1.4	0–3	2.1	1–4	0.7	0–1
Total	16.3	7–23	12.8	9–18	14.3	7–18	9.4	5–15

The number of species in each aquarium of an apparatus was altered by Aroclor 1254 (Tables 2 & 5). The total number of species and the number of species from each phylum in the ten control, 0.1 and 1 μg/l contaminated aquaria were similar. However, there were significantly fewer species and the species composition differed in the ten aquaria contaminated by 10 μg/l of the PCB. The greatest shifts in species composition were found in arthropods, bryozoans, and mollusks. Although there were significant reductions in the number of molluscan species in the 10 μg/l aquaria, there was no difference in the gastropod—pelecypod ratio.

The total number of animals in each aquarium did not differ significantly among the four treatments; whereas the number and percentage occurrence of species was markedly different (Tables 2 & 6). Arthropods (primarily the tube-dwelling amphipod, *Corophium volutator*) were the dominant animals in the control (76 percent) and 0.1 μg/l PCB (84 percent) aquaria. In these aquaria, chordates (primarily *Molgula manhattensis*) were secondarily abundant. Arthropods were also abundant (55 percent) in aquaria that received 1 μg/l but a significant decrease in their abundance and an increase in abundance (31 percent) of chordates occurred. Dominance was different in aquaria contaminated by 10 μg/l; 80 percent of the animals were chordates. This difference from communities dominated by arthropods in control aquaria and in aquaria contaminated by 0.1 μg/l of Aroclor 1254 to communities dominated by chordates in aquaria receiving the highest concentration of this PCB was the most striking PCB-induced effect in this experiment.

The abundance of animals of other phyla, although less striking, was also altered by PCB. There were more mollusks in the control aquaria than in treated aquaria, but the percentage of their occurrence was not different in the PCB environments. Colonies of the encrusting bryozoan, *Membranipora tenuis*, were not counted, and therefore their numbers are not adequately represented in Table 6. However, their exclusion from the ten aquaria contaminated with 10 μg/l was significant. Abundance of polychaetes was not altered by any of the three concentrations of PCB.

The Shannon-Weaver (1963) index of species diversity calculated for each aquarium did not differ among the control and three contaminated apparatuses (Table 7). Species diversity is a function of two components, richness (number of species) (Table 5) and equitability or relative number of each species (Table 7) (Lloyd and Ghelardi 1964). In my study, species diversity is not correlated ($r = 0.094$) with richness of species but is correlated ($r = 0.882$) with relative abundance of each species: $J' =$ calculated diversity $\div$ maximum diversity (Pielou 1966). (Maximum diversity is defined as species diversity where all species are equally abundant.) Equitability did not differ between treatments because communities in this study were usually dominated by one species and were not rich in species. Therefore, the effect of Aroclor was not on species diversity but on species composition.

TABLE 6

Average number per aquarium and average percent frequency per aquarium of animals, by phylum (range in parentheses), that developed from planktonic larvae in 10 control aquaria and 10 aquaria that for four months received 0.1, 1 or 10 μg/l of Aroclor 1254.

			Aroclor 1254					
	Control		0.1 μg/l		1 μg/l		10 μg/l	
Phylum	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Annelida	11.6(3–23)	6.5(1.8–21.4)	11.9(4–21)	5.8(1.3–42.8)	12.8(7–22)	9.0(3.9–38.5)	8.5(5–16)	12.9(5.3–66.7)
Arthropoda	134.6(6–406)	75.8(12.7–94.0)	170.9(14–528)	83.6(28.6–96.2)	78.0(8–199)	54.9(20.5–86.9)	0.7(0–4)	1.1(0–2.8)
Chordata	19.3(0–112)	10.9(0–64.7)	14.4(1–52)	7.0(0.6–19.4)	43.7(0–130)	30.8(0–59.1)	52.8(3–160)	80.4(25.0–88.6)
Mollusca	10.1(3–18)	5.7(0.7–28.6)	4.4(1–10)	2.2(0.8–12.2)	4.4(1–9)	3.1(1.3–33.3)	3.0(1–6)	4.6(1.9–9.0)
Other phyla	2.0(0–4)	1.1(0–7.1)	2.7(0–11)	1.4(0–6.5)	3.2(1–7)	2.2(0.9–7.7)	0.7(0–1)	1.0(0–3.6)
Total	177.6(14–432)	100.0	204.3(49–589)	100.0	142.1(24–239)	100.0	65.7(12–186)	100.0

TABLE 7

Shannon-Weaver index of species diversity and index of species richness in the ten control aquaria and ten aquaria contaminated with 0.1, 1 or 10 μg/l or Aroclor 1254.

	Control			Aroclor 1254								
	Mean	Range	Std. error	Mean	0.1 μg/l Range	Std. error	Mean	1.0 μg/l Range	Std. error	Mean	10.0 μg/l Range	Std. error
Species diversity	1.80	0.57–2.83	0.24	1.42	0.36–3.17	0.26	2.07	0.94–3.26	0.20	1.62	1.03–2.19	0.13
Equitability (J)	0.47	0.14–0.89	0.07	0.38	0.10–0.81	0.07	0.55	0.25–0.86	0.06	0.53	0.26–0.94	0.06

TABLE 8

Species and total number of animals collected from the effluents of 10 control aquaria and 10 aquaria contaminated for four months with 0.1, 1 or 10 μg/l Aroclor 1254.

Taxon	Control	0.1 μg/l	1 μg/l	10 μg/l
Annelida				
Eupomatus dianthus	1	1	0	0
E. protulicola	0	1	1	0
Neanthes succinea	1	2	2	0
Total	2	4	3	0
Arthropoda				
Balanus sp.	10	2	2	1
Caprella sp.	0	8	4	0
Clibanaris tricolor	0	1	0	0
Corophium volutator	7	15	32	1
Eurypanopeus depresus	5	2	5	0
Neopanope texana	10	6	3	0
Pagurus longicarpus	1	0	0	0
Pinnixa chaetopterana	1	0	0	0
Upogebia affinis	1	1	0	0
Decapod zoea, unident. sp.	0	0	1	0
Portunidae, unident. sp.	2	1	0	0
Pycnogonidae, unident. sp.	0	0	3	0
Total	37	36	50	2
Chordata				
Bostrichobranchus pilularis	3	0	0	0
Branchiostoma caribaeum	1	0	1	0
Molgula manhattensis	72	103	304	35
Total	76	103	305	35
Coelenterata				
Leptomedusae*, unident. sp.	1	1	1	1
Echinodermata				
Hemipholis elongata	1	0	3	0
Ectoprocta				
*Membranipora tenuis**	1	1	1	0
Mollusca				
Anadara ovalis	1	0	0	0
A. transversa	12	0	4	1
Bittium alternata	0	1	0	0
B. varium	3	0	1	0
Crassostrea virginica	2	0	1	1
Doridella obscura	12	1	2	1
Laevicardium mortoni	1	0	0	0
Mitrella lunata	2	0	2	2
Musculus lateralis	1	0	1	0
Nassarius albus	2	0	0	0
Tagelus divisus	1	2	0	0
Eolidacea, unident. sp.	1	1	1	3
Total	38	5	12	8
Totals: Animals	156	150	375	47
Species	27	18	20	10

* Colonies: Counted as one animal.

Conclusions based on abundance and diversity of animals collected at the end of the four-month exposure were corroborated by the abundance and diversity of animals that migrated from and were washed from each apparatus during the exposure (Table 8). The total number of animals and species from aquaria with 10 μg/l was markedly lower than those from the other aquaria. Arthropods were abundant in collections from the effluents of control aquaria and aquaria with 0.1 and 1 μg/l but rare from the aquaria with 10 μg/l. The effect of Aroclor on crabs collected from the aquaria could not be assessed at the end of the exposure because only two were found in all aquaria. However, presence of exoskeletons in seven of 10 control. six of 10 0.1 μg/l and eight of 10 1 μg/l aquaria and absence of exoskeletons in the 10 μg/l aquaria strongly suggest that crabs were sensitive to the highest concentration of Aroclor 1254. This sensitivity was substantiated by collections of crabs from the effluents of the aquaria. *Eurypanopeus. Neopanope, Pinnixa* and portunids were abundant in effluents of control, 0.1 and 1 μg/l aquaria but absent from the effluent of the 10 μg/l aquaria. The bryozoan, *M. tenuis*, was absent in the effluent from the 10 μg/l aquaria, but present in the effluent from the other aquaria. Mollusks were most abundant in effluent from the 10 control aquaria.

DISCUSSION AND CONCLUSIONS

The polychlorinated biphenyl, Aroclor 1254. influenced the composition of animal communities that developed from planktonic larvae in sea water which entered the test aquaria. The primary influence was that the dominant species in the control aquaria was the amphipod, *C. volutator*, and the dominant species in aquaria receiving 10 μg/l PCB was the tunicate, *M. manhattensis.* Also, there were fewer species and the number of animals was markedly but not significantly fewer in the 10 μg/l aquaria. The abundance of arthropods, chordates, bryozoans and mollusks differed significantly but abundance of annelids, brachiopods, coelentrates, echinoderms or nemerteans apparently did not differ.

Differences in community structure that were apparent at the lowest concentration (0.1 μg/l) became more pronounced as the concentration of Aroclor 1254 increased to 10 μg/l. Control aquaria were dominated by arthropods (76 percent). with lesser numbers of animals from eight other phyla. Aquaria contaminated by 0.1, 1 or 10 μg/l contained fewer mollusks than did control aquaria; however, the percentage occurrence of mollusks was not altered by the PCB. Aquaria receiving 1 μg/l or 10 μg/l had more tunicates and fewer arthropods than did control aquaria. Aquaria receiving 10 μg/l were dominated by tunicates (80 percent) with lesser numbers of five other phyla; only 1% of the animals were arthropods. Animals most reduced in numbers at the highest PCB concentration included the amphipod, *C. volutator*; the xanthid crabs, *Eurypanopeus depressus* and *Neopanope texana*; the bryozoan, *M. tenuis*; the gastropod. *Bittium varium.* and the pelecypods, *Abra aequalis* and *Tellina alternata.*

Few of these changes could have been predicted from information from current literature, because only one species in this study had been challenged previously

with PCB and only a few are phylogenetically related to previously challenged species. Arthropods, particularly amphipods and crabs were sensitive to Aroclor 1254 in this experiment and in the experiments by Nimmo *et al.* (1971a) in which pink shrimp were killed by 1 μg/l. Juvenile blue crabs appeared resistant to 5 μg/l (Duke *et al.* 1970). In my experiment, the numbers of xanthid crabs was reduced by 10 μg/l indicating that larval stages may be particularly sensitive to PCBs. Sensitivity of crab larvae has been shown with the insecticides dieldrin (Epifanio 1971) and mirex (Bookhout *et al.* 1972). Aroclor 1254 was lethal to two estuarine fishes at 5 μg/l (Hansen *et al.* 1971) but in this study lower chordates (tunicates) seemed unaffected and were most abundant in the PCB-stressed communities. Lethal effects of PCBs on mollusks are not known. However, growth of oysters was reduced significantly without mortality by 5 μg/l of Aroclor 1254 (Parrish *et al.* 1972). In my experiment fewer mollusks occurred in all exposure concentrations possibly because the larval stages are sensitive or because of factors other than the presence of Aroclor. Studies on protozoans (Cooley, Keltner and Forester 1972) provided the only other data I am aware of on the sensitivity of estuarine animals of other phyla to PCBs.

The Shannon-Weaver species diversity index has been used as an indicator of the effects of some types of pollution on animal communities in estuaries, but in this experiment, the index was not decreased even though composition of the communities were greatly altered. This species diversity index did not decrease as the concentration of PCB increased because the index was proportional to the relative number of each species present in the aquaria, and communities that developed at each treatment concentration were dominated by one species of animal. If Aroclor 1254 affected the composition of established communities in an estuary as it did the developing communities in this experiment, this species diversity index could not be used to estimate effects of this pollutant in the environment.

One purpose of this experiment was to determine whether the effect of a toxicant on developing estuarine animal communities can be investigated in the laboratory. Preliminary experiments at this laboratory indicated that the structure of communities of organisms setting in aquaria was altered by presence of the insecticide Dursban® (J. I. Lowe, personal communication[2]). My analysis of his data indicated that replicate aquaria were necessary to separate the effects of a toxicant from the effects of an efficient predator or from the effects of an animal with great reproductive capacity. The use of ten replicate aquaria for each treatment in my experiment readily separated the effect of Aroclor 1254 from other factors that might influence community structure. My experiment also showed that small aquaria can be used, provided that larger animals can emigrate before they drastically affect community structure and thus mask effects of the toxicant. Emigrating animals must be caught and enumerated so the effects on them can be assessed.

[2] Mr. Jack I. Lowe, Environmental Protection Agency, Gulf Breeze, Fla. 32561.

® Registered trademark: Dow Chemical Co.

ACKNOWLEDGMENTS

I am grateful to Mr. Edward Matthews, who helped during the four-month experiment, and to Mr. Johnnie Knight, who prepared water and sediment samples for chemical analysis. I am particularly indebted to Dr. Nelson R. Cooley, because identification of the numerous species of animals would have been most difficult without his help.

LITERATURE CITED

ANONYMOUS. 1966. Report of a new chemical hazard. *New Scient.* **32:** 612.

BECHTEL, T. J. and B. J. COPELAND. 1970. Fish species diversity indices as indicators of pollution in Galveston Bay, Texas. *Contr. mar. Sci. Univ. Tex.* **15:** 103–133.

BOOKHOUT, C. G., A. J. WILSON, JR., T. W. DUKE and J. I. LOWE. 1972. Effects of Mirex on the larval development of two crabs. *Water, Air and Soil Pollut.* **1:** 165–180.

BROADHURST, M. G. 1972. Use and replaceability of PCBs. *Environ. Health Perspectives.* **2:** 81–102.

BUTLER, P. A. 1973. Organochlorine residues in estuarine mollusks—1965–1972. *Pestic. Monit. J.* **6**(4).

COOLEY, N. R., J. M. KELTNER, JR. and J. FORESTER. 1972. Mirex and Aroclor® 1254: Effect on and accumulation by *Tetrahymena pyriformis* strain W. *J. Protozool.* **19:** 636–638.

DUKE, T. W., J. I. LOWE and A. J. WILSON, JR. 1970. A polychlorinated biphenyl (Aroclor 1254®) in the water, sediment and biota of Escambia Bay, Florida. *Bull. Environ. Contam. Toxicol.* **5:** 171–180.

EPIFANIO, C. E. 1971. Effects of dieldrin in seawater on the development of two species of crab larvae, *Leptodius floridanus* and *Panopeus herbstii*. *Mar. Biol.* **11**(4): 356–362.

HANSEN, D. J., P. R. PARRISH, J. I. LOWE, A. J. WILSON, JR. and P. D. WILSON. 1971. Chronic toxicity, uptake and retention of Aroclor® 1254 in two estuarine fishes. *Bull. Environ. Contam. Toxicol.* **6:** 113–119.

LLOYD, M. and R. J. GHELARDI. 1964. A table for calculating the equitability component of species diversity. *J. Anim. Ecol.* **33:** 217–225.

NIMMO, D. R., R. R. BLACKMAN, A. J. WILSON, JR. and J. FORESTER. 1971a. Toxicity and distribution of Aroclor® 1254 in the pink shrimp *Penaeus duorarum*. *Mar. Biol.* **11**(3): 191–197.

NIMMO, D. R., P. D. WILSON, R. R. BLACKMAN and A. J. WILSON, JR. 1971b. Polychlorinated biphenyl absorbed from sediments by fiddler crabs and pink shrimp. *Nature, Lond.* **231:** 50–52.

PARRISH, P. R., J. I. LOWE, A. J. WILSON, JR. and J. M. PATRICK, JR. 1972. Effects of Aroclor® 1254, a PCB, on oysters, *Crassostrea virginica* (Bivalvia: Protobranchia: Ostreidae). *ASB Bull.* **19**(2): 90.

PIELOU, E. C. 1966. The measure of diversity in different types of biological collections. *J. Theor. Biol.* **13:** 131–144.

SHANNON, C. E. and W. WEAVER. 1963. *The mathematical theory of communication.* University of Illinois Press, Urbana.

SIEGAL, S. 1956. *Non Parametric Statistics for the Behavioral Sciences.* McGraw-Hill Book Co., Inc., N.Y. 312 p.

WILHM, J. L. and T. D. DORRIS. 1968. Biological parameters for water quality criteria. *BioScience* **18**(6): 477–481.

CONTRIBUTION NO. 165

ESTUARINE MICROBES AND ORGANOCHLORINE PESTICIDES (A BRIEF REVIEW)[1]

A. W. BOURQUIN

Gulf Breeze Environmental Research Laboratory[2]
U.S. Environmental Protection Agency
Gulf Breeze, Florida 32561

Little is known about microbiological degradation of organochlorine pesticides in the estuarine and oceanic environments. Since mircoorganisms are probably the main instruments of pesticide breakdown, and possibly offer an array of mechanisms by which pollution may be reduced, research is needed to learn the pathways of microbial degradation in the marine environment.

Table 1 lists a number of microorganisms, chiefly soil and aquatic, with demonstrated ability to partially degrade organochlorine pesticides in various environments. An excellent review of the interaction between halogenated pesticides and microorganisms has been given by Pfister and Matsumura (28). Lichtenstein and Schulz (18) found that soil bacteria converted aldrin to its more stable epoxide, dieldrin; the peak of dieldrin formation occurred 56 days after treatment. A bacterium, *Proteus vulgaris*, isolated from the gut of a mouse, converted DDT to DDD (4) and some soil actinomycetes degrade polychloro-nitrobenzene (PCNB) and dechlorinate DDT (8). Most of the reports listed in Table 1 were concerned with pure cultures and few involved more than one or two step transformations. However, other investigators have reported extensive degradation by soil microorganisms leading to speculation that biodegradation could result in mineralization of organochlorine compounds in the presence of certain microbial assemblages and environmental parameters. Bixby et al. (6) reported a soil fungus, *Trichoderma koningi*, which degraded dieldrin to carbon dioxide with cleavage of the chlorinated ring structure. Focht (10) reported that an aquatic fungus, isolated from sewage effluent, metabolized chlorinated bacterial degradation products to water, carbon dioxide, and hydrochloric acid.

Many soil microorganisms also occur in water, thus environmental relationships and microbial associations similar to those in soil may exist. The essential differences between terrestrial and aquatic environments relative to microbial activity appear to be: (a) usually fewer nutrients per unit mass and less biochemical activity are found in water than in soil, and (b) usually fewer adsorptive surfaces for microbial growth in water than in soil. Reports have indicated that fresh surface waters do not have a characteristic bacterial flora (12). However, certain microorganisms, such as *Beneckea* and *Caulobacter*, have been designated as typical marine or estuarine genera. Differences between estuarine, freshwater, and soil ecosystems make

---

[1] Gulf Breeze Contribution No. 165.

[2] Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon.

TABLE 1

Microorganisms Known to Metabolize Organochlorine Pesticides

Genera	Pesticides	Environment	Reference (see Literature Cited)
Bacteria			
Arthrobacter	Endrin, DDT	Soil-aerobic	Patil et al., 1970 (25)
Bacillus	Endrin, DDT	Soil-aerobic	Patil et al., 1970 (25)
Clostridium	Lindane	Aquatic-anaerobic	McRae et al., 1969 (19)
Escherichia	DDT	Aquatic-anaerobic	Mendel and Walton, 1966 (22)
Hydrogenomonas	DDT	Aquatic-anaerobic	Focht, 1972 (10)
Klebsiella	DDT	Aquatic-anaerobic	Wedemeyer, 1966 (32)
Micrococcus	Endrin, Aldrin, DDT	Soil-aerobic	Patil et al., 1970 (25)
Proteus	DDT	Aquatic-aerobic	Barker et al., 1965 (4)
Pseudomonas spp.	Endrin, Aldrin, DDT	Soil-aerobic	Patil et al., 1970 (25)
Pseudomonas spp.	Heptachlor	Aquatic-aerobic	Bourquin et al., 1971 (7)
Pseudomonas spp.	Dieldrin	Soil-aerobic	Matsumura et al., 1968 (21)
Unidentified	Dieldrin, Aldrin, Endrin, DDT	Marine-aerobic	Patil et al., 1972 (27)
Unidentified	Lindane, Aldrin	Soil-aerobic	Lichtenstein and Schulz, 1959 (18)
Actinomycetes			
Nocardia	DDT, PCNB	Soil-aerobic	Chacko et al., 1966 (8)
Streptomyces	PCNB	Soil-aerobic	Chacko et al., 1966 (8)
Fungi			
Aspergillus	PCNB	Soil-aerobic	Chacko et al., 1966 (8)
Fusarium	DDT	Aquatic-aerobic	Focht, 1972 (10)
Mucor	Dieldrin	Soil-aerobic	Anderson et al., 1970 (3)
Trichoderma	Dieldrin	Soil-aerobic	Bixby et al., 1971 (6)
Yeast			
Saccharomyces	DDT	Aquatic-anaerobic	Kallerman and Andrews, 1968 (16)
Algae			
Chlamydomonas	Lindane	Aquatic-aerobic	Sweeney, 1968 (31)
Chlorella and Dunaliella	Aldrin	Marine-aerobic	Patil et al., 1972 (27)

the estuarine area a unique environment for study of microbial degradation. Because of this uniqueness, data from soil and freshwater ecosystems cannot necessarily be extrapolated to estuarine systems. Therefore, research is needed to learn more about degradation pathways in the marine environment.

Several investigators have reported that degradation of pesticides by aquatic microorganisms is similar to degradation by soil microorganisms. Miles et al. (24) reported that soil microorganisms metabolized heptachlor to 1-hydroxy-2,3-epoxychlordene. Bourquin et al. (7) reported similar results, and proposed a pathway for microbial transformation of heptachlor in the aquatic environment. Metabolism of DDT occurs in soil, freshwater, and lake sediments (26). The similarity of transformations of these compounds may be due to similarity of microflora in the different environments. However, microbial differences as well as environmental factors exist between aquatic and terrestrial ecosystems. For example, most cultivated soils to which insecticides are applied are more aerobic than aquatic sediments. Although DDT is converted to DDD and other products in anaerobic systems, it is stable in aerobic systems (2,13).

Estuarine sediment is a reservoir for pesticides transported by rivers. Because organochlorine pesticides are strongly sorbed on soil and other particulate material (30), including microorganisms (17), they are found on suspended particulates in rivers and are incorporated into estuarine sediment (2). These sediments are often enriched by decomposing organic matter and are anaerobic beneath the surface. Although the rate of carbon turnover due to microbial activity in the sea may not be substantially different from that in fresh water, estuaries are areas of rapid microbial transformations (35). The latter play an important role in estuarine nutrition.

Biodegradation of organochlorine pesticides in estuarine or oceanic environments has been little studied despite the known persistence of the pesticides. Patil et al. (27) studied microbial metabolic transformations of DDT, dieldrin, aldrin, and endrin in samples of marine water, bottom sediments, and surface films. Transformations of DDT and cyclodiene insecticides occurred in samples with biological materials such as surface films, plankton, and algae, but not in waters from the open ocean. Pure cultures of marine microorganisms also metabolized the pesticides. In general, patterns of degradation that have been observed in terrestrial and aquatic ecosystems closely resemble those found for the marine environment (27). For example, production of 6,7-t-dihydroxy-dihydro-aldrin was the major metabolite found in soil fungi (20), in an aquatic bacterium (33), in algal cultures (5), and in pure cultures of marine algae, bacteria, and surface films (27). Similar results were obtained from aldrin, endrin, and DDT, except that algal cultures appeared to convert DDT to a "DDOH-like compound" (2,2-bis (p-chlorophenyl)). The strong degradation activity associated with surface films is significant. Surface films are areas of high biological activity (9) and concentrators of dissolved organics (34) and pesticides (29). Such films provide the environment necessary for selection of hydrocarbon-degrading microorganisms and a relatively high nutrient concentration for proliferation of cells. Presence of pollutants, crude oil or pesticides, in an already enriched area of microbial activity may select for hydrocarbon-degrading microorganisms.

The organically-enriched estuarine environment provides an opportunity for study of co-metabolism of pesticides by microorganisms. As noted by Focht

and Alexander (11), "Co-metabolism is the adventitious biological transformation of organic compounds which provides neither energy nor structural components to the organism." Many bacteria break down certain compounds while metabolizing other substrates but do not utilize the co-substrate as a source of energy or carbon for growth. Relevance of this phenomenon to natural soil ecosystems was noted by Horvath and Alexander (15). Focht and Alexander (11) demonstrated degradation of DDT by sewage bacteria that grew on diphenylmethane, an analogue of DDT. Co-metabolism has not been reported in the estuarine environment. Natural conditions, however, predispose estuaries to such metabolism because of the large microbial communities that exhibit a wide variety of physiological activities. These include degradation of relatively recalcitrant large molecular weight compounds such as complex polysaccharides and some petroleum products (1,23).

Synergism within the microbial ecosystem, including bacteria, yeasts and fungi, in the estuarine environment is another factor to consider when studying breakdown of pesticides. In the soil, microorganisms act synergistically to degrade molecules considered resistant to attack by single species (14). However, in estuarine and oceanic environments, such complex interrelationships among microorganisms have not been investigated adequately. Studies of interactions of estuarine microbial assemblages can provide important data for understanding microbial degradation in estuarine systems.

Estuaries, periodically flushed by tides, provide the near shore portions of the open ocean with many organic nutrients in solution or in the form of partially degraded organic detritus. Organic matter from estuaries and other biologically productive waters often forms a slick, or calm streak, on a rippled sea. As noted, surface slicks are areas of high biological activity and could provide nutrients necessary for co-metabolic transformation of pesticides in off-shore marine locales. Microbiology of naturally occurring surface slicks and oil slicks caused by spills or seepage is an important but neglected area of estuarine research.

Pathways of microbial attack upon chlorinated hydrocarbons in the estuarine environment need to be investigated since breakdown by microorganisms is probably the main natural process of pesticide degradation. Although microbiological processes might reduce environmental pollution attributed to use of persistent pesticides, detailed studies of degradative pathways are required to assess the degree of hazard caused by breakdown products. Required information on microbial degradation of organochlorine pesticides in estuaries will be supplied when we answer the following questions:

What types of microorganisms are involved in transformation of organochlorine pesticides? Are they the same types that predominate in organic detritus formation or are they species selected by exposure to pesticide pollution?

What is the degree of degradation of specific compounds?

Does co-metabolism occur in the estuary, and is it a means of degrading pesticides?

What effects do additional hydrocarbons, such as oil, have on microbial degradation of pesticides in the estuary?

Is synergistic activity within the estuarine microflora a factor in microbial degradation of pesticides?

What is the role of microbial intracellular accumulation and adsorption in biodegradation and/or biological magnification of pesticides?

What environmental factors in the estuary prevent or inhibit or accelerate microbial breakdown of pesticides?

What types of pesticides are easily degraded?

What are the effects of degradation products on estuarine macro- and microflora and fauna?

Considering all the above questions, are similar reactions, selections and effects occurring in the water column and in the sediments?

Such studies will provide data for an accurate picture of the role of estuarine microorganisms on the fate of organic pollutants. Such data are needed to formulate water quality criteria for pesticide regulation in the estuarine environment.

LITERATURE CITED

1. Ahearn, D. G., and S. P. Meyers. 1972. The role of fungi in the decomposition of hydrocarbons in the marine environment. *In* H. A. Walters and D. G. Hueck van der Plas (eds.), Biodeterioration of Materials, 2:12-18. Applied Science Publishers, Ltd., London.

2. Albone, E. S., G. Eglinton, N. C. Evans, J. M. Hunter, and M. M. Rhead. 1972. Fate of DDT in Severn Estuary sediments. Environ. Sci. Technol. 6:914-919.

3. Anderson, J. P. E., E. P. Lichtenstein, and W. F. Whittingham. 1970. Effect of *Mucor alternans* on the persistence of DDT and dieldrin in culture and in soil. J. Econ. Entomol. 63:1595-1599.

4. Barker, P. S., F. O. Morrison, and R. S. Whitaker. 1965. Conversion of DDT to DDD by *Proteus vulgaris*, a bacterium isolated from the intestinal flora of a mouse. Nature (London) 205:621-622.

5. Batterton, J. C., G. M. Boush, and F. Matsumura. 1971. Growth response of blue-green algae to aldrin, dieldrin, endrin and their metabolites. Bull. Environ. Contam. Toxicol. 6:589-594.

6. Bixby, M. W., G. M. Boush, and F. Matsumura. 1971. Degradation of dieldrin to carbon dioxide by a soil fungus *Trichoderma koningi*. Bull. Environ. Contam. Toxicol. 6:491-494.

7. Bourquin, A. W., S. K. Alexander, H. K. Speidel, J. E. Mann, and J. F. Fair. 1971. Microbial interactions with cyclodiene pesticides. Dev. Indust. Microbiol. 13:264-276.

8. Chacko, C. I., J. L. Lockwood, and M. Zabik. 1966. Chlorinated hydrocarbon degradation by microbes. Science 154:893-895.

9. Ewing, G. 1950. Slicks, surface films and internal waves. J. Mar. Res. 9:161-187.

10. Focht, D. D. 1972. Microbial degradation of DDT metabolites to carbon dioxide, water, and chloride. Bull. Environ. Contam. Toxicol. 7:52-56.

11. Focht, D. D., and M. Alexander. 1970. Bacterial degradation of diphenylmethane, a DDT model substrate. Appl. Microbiol. 20:608-611.

12. Frobisher, M. 1968. Fundamentals of Bacteriology, 8th ed. W. B. Saunders Co., Philadelphia.

13. Guenzi, W. D., and W. E. Beard. 1968. Anaerobic conversion of DDT to DDD and aerobic stability of DDT in soil. Soil Sci. Soc. Am. Proc. 32:522-524.

14. Gunner, H. B., and B. M. Zuckerman. 1968. Degradation of "Diazinon" by synergistic microbial action. Nature (London) 217:1183-1184.

15. Horvath, R. S., and M. Alexander. 1970. Co-metabolism of m-chlorobenzoate by an *Arthrobacter*. Appl. Microbiol. 20:254-258.

16. Kallerman, B. J., and A. K. Andrews. 1968. Reductive dechlorination of DDT to DDD by yeast. Science 141:1050.

17. Ko, W. H., and J. L. Lockwood. 1968. Accumulation and concentration of chlorinated hydrocarbon pesticides by microorganisms in soil. Canad. J. Microbiol. 14:1075.

18. Lichtenstein, E. P., and K. R. Schulz. 1959. Breakdown of lindane and aldrin in soil. J. Econ. Entomol. 52:118-124.

19. McRae, I. C., K. Raghu, and E. M. Bautista. 1969. Anaerobic degradation of the insecticide lindane by *Clostridium* sp. Nature (London) 221:859-860.

20. Matsumura, F., and G. M. Boush. 1967. Dieldrin: Degradation by soil microorganisms. Science 156:959-961.

21. Matsumura, G., G. M. Boush, and A. Tai. 1968. Breakdown of dieldrin in the soil by a microorganism. Nature (London) 219:965-967.

22. Mendel, J. L., and M. S. Walton. 1966. Conversion of p,p'-DDT to p,p'-DDD by intestinal flora of the rat. Science 151:1527.

23. Meyers, S. P., D. G. Ahearn, W. Gunkel, and F. J. Roth, Jr. 1967. Yeasts from the North Sea. Mar. Biol. 1:118-123.

24. Miles, J. R. W., C. M. Tu, and C. R. Harris. 1969. Metabolism of heptachlor and its degradation products by soil microorganisms. J. Econ. Entomol. 62:1334-1338.

25. Patil, K. C., F. Matsumura, and G. M. Boush. 1970. Degradation of endrin, aldrin, and DDT by soil microorganisms. Appl. Microbiol. 19:879-881.

26. Patil, K. C., F. Matsumura, and G. M. Boush. 1971. DDT metabolized by microorganisms from Lake Michigan. Nature (London) 230:325-326.

27. Patil, K. C., F. Matsumura, and G. M. Boush. 1972. Metabolic transformation of DDT, dieldrin, aldrin, and endrin by marine microorganisms. Environ. Sci. Technol. 6:629-632.

28. Pfister, R. M., and F. Matsumura. 1972. Interactions of halogenated pesticides and microorganisms: A review. *In* A. I. Laskin and H. Lechevalier (eds.), Critical Reviews in Microbiology, 2:1-33. CRC Press, The Chemical Rubber Co., Cleveland.

29. Seba, D. B., and E. F. Corcoran. 1969. Surface slicks as concentrators of pesticides in the marine environment. Pestic. Monit. J. 3:190-193.

30. Shin, Y. O., J. J. Chodan, and A. R. Wolcott. 1970. Adsorption of DDT by soils, soil fractions, and biological materials. J. Agric. Food Chem. 18:1129.

31. Sweeney, R. A. 1968. Metabolism of lindane by unicellular algae. Proc. 12th Conf. Great Lakes Research.

32. Wedemeyer, G. 1966. Dechlorination of DDT by *Aerobacter aerogenes*. Science 152:647.

33. Wedemeyer, G. 1968. Partial hydrolysis of dieldrin by *Aerobacter aerogenes*. Appl. Microbiol. 16:661-662.

34. Williams, P. M. 1967. Sea surface chemistry: organic carbon and organic and inorganic nitrogen and phosphorus in surface films and subsurface waters. Deep-Sea Res. 14:791-800.

35. Zhukova, A. R. 1963. On the quantitative significance of microorganisms in nutrition of aquatic invertebrates. *In* C. H. Oppenheimer (ed.), Symposium on Marine Microbiology, pp. 699-710. Charles C. Thomas, Springfield, Ill.

Reprinted from COPEIA, 1973, No. 1, March 5
pp. 140–141
Made in United States of America

A LARVAL TARPON, *MEGALOPS ATLANTICUS*, FROM PENSACOLA, FLORIDA.—A larval tarpon, *Megalops atlanticus*, an early Stage II as designated by Wade (1962), was collected in the upper reaches of East Bay, about 45 km from the Pensacola Inlet, Florida on 20 October 1970. It represents the second and most northern record of a larval tarpon from the Gulf of Mexico, and suggests late spawning in the Gulf.

Eldred (1968) reporting on the first capture of a tarpon larva (Stage I) in the Gulf, about 117 km due west of Sanibel Island, Florida on 6 July 1967, postulated that its presence could have resulted either from spawning in the Gulf or from being carried northward by transport currents from a more southerly spawning. Eldred's (1967) study on catch records of young indicated that tarpon spawn in the Florida Straits, the Gulf Stream, and the Caribbean Sea during spring and summer. The occurrence of the present early larva in the northern Gulf provides evidence of spawning in a Gulf locality.

The specimen from East Bay was collected unusually late in the year. This may indicate that spawning in the northern Gulf is later than in southern regions. Except for a larva

taken 12 November 1921 off French Guiana, all Stage I and II larvae have been captured from 17 May to 1 October (Wade, 1962; Eldred, 1967, 1968).

The larva was collected in a plankton tow near the entrance of East Bay River in water 1.5 m deep. Surface salinity of the water was 7.3 ‰; bottom, 11.5. Surface water temperature was 20.1° C; bottom, 21.6. Measurements (in mm) of the specimen are 28.5 total length, 25.8 standard length, and 2.6 head length. It has a total of 56 myomeres (39 predorsal, 41 preanal), 13 dorsal rays, and 20 anal rays.

I thank Dr. Thomas W. McKenney of the National Marine Fisheries Service Southeast Fisheries Center, Miami, Fla. for checking the measurements and meristic counts of the leptocephalus.

Literature Cited

Eldred, B. 1967. Larval tarpon, *Megalops atlanticus* Valenciennes, (Megalopidae) in Florida waters. Fla. Bd. Conserv. Mar. Lab., Leaf. Ser., IV, Pt. 1, No. 4:1–9.

———. 1968. First record of a larval tarpon, *Megalops atlanticus* Valenciennes, from the Gulf of Mexico. *Ibid.* IV, Pt. 1, No. 7:1–2.

Wade, R. A. 1962. The biology of the tarpon, *Megalops atlanticus*, and the ox-eye, *Megalops cyprinoides*, with emphasis on larval development. Bull. Mar. Sci. Gulf Carib. 12:545–622.

Marlin E. Tagatz, *National Marine Fisheries Service, Atlantic Estuarine Fisheries Center, Beaufort, North Carolina 28516. Present address: Environmental Protection Agency, Gulf Breeze Laboratory, Gulf Breeze, Florida 32561.*

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Mirex Residues in Selected Estuaries of South Carolina—June 1972 [1]

P. W. Borthwick,[2] G. H. Cook,[2] and J. M. Patrick, Jr.[2]

ABSTRACT

Estuarine sediments, crabs, shrimps, and fishes were collected in June 1972 at eleven stations two years after aerial applications of mirex bait for control of fire ants in coastal areas near Charleston, S.C. These stations had previously been monitored (October 1969 to June 1971) when levels of mirex in animal samples were: crabs, 0-0.60 ppm; shrimps, 0-1.3 ppm; and fishes, 0-0.82 ppm.

The recent study showed that mirex was present in three species of fishes (white catfish, 0.021 ppm; bluegill, 0.047 ppm; carp, 0.12 ppm) and blue crabs (0.026 ppm) at two freshwater stations. However, mirex was not detected in 36 animal samples, most of which were taken from nine saline stations in the estuaries after a period of restricted use of the pesticide. Analysis of bottom sediment samples at all stations detected no mirex. The lower limit of detection for mirex was 0.01 ppm.

Introduction

In June 1972 samples of estuarine sediments, crabs, shrimps, and fishes were collected at 11 stations near Charleston, S.C., where mirex fire ant bait had been applied aerially to coastal areas from October 1969 to December 1970. The United States Department of Agriculture supervised two applications of mirex, by fixed-wing aircraft, to several hundred thousand acres in the Charleston area. Treatments were terminated approximately 24 months before the June 1972 collections. However, 18 months lapsed since special applications were made by helicopter to 1200 acres at Toogoodoo Creek: Stations A, B, C, and D; and by hand seeder around a one-acre pond at Riverland Terrace: Station 1 (Fig. 1).

Since 1970 less extensive applications have been made for control of nuisance populations of fire ants. During the 1971-72 cooperative Federal State Control Program, mirex bait was applied aerially in South Carolina and seven other Southeastern states at property owners' requests.

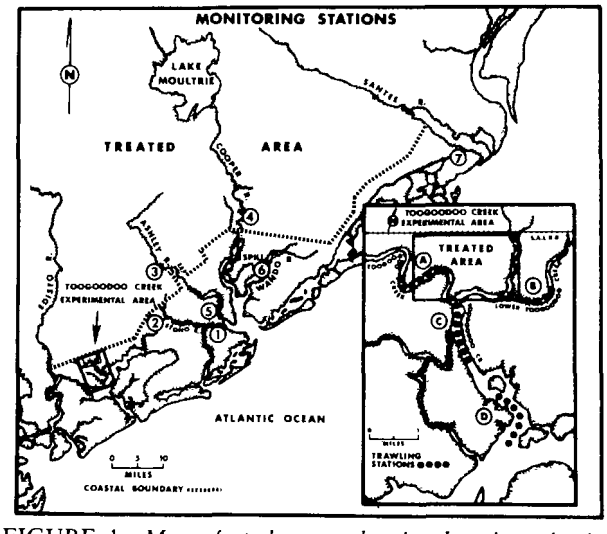


FIGURE 1—*Map of study area showing location of mirex sampling sites, South Carolina—June 1972*

[1] Contribution No. 168 from the Gulf Breeze Environmental Research Laboratory, United States Environmental Protection Agency, Gulf Breeze, Florida 32561; Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon.

[2] Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida 32561.

The use of mirex decreased when the U.S. Environmental Protection Agency (U.S. EPA) issued orders (*1*, *2*) to cancel the registration of products containing mirex, pending relabeling. The orders required that no mirex be applied aerially near estuaries and other aquatic areas, wildlife refuges, or heavily forested areas. The present study was implemented to determine how much mirex remained in the estuarine fishes and crustaceans following a period of restricted use of the pesticide.

The stations (Fig. 1) and analytical techniques were identical to those used in a more comprehensive study of the area (*3*). Samples were collected and analyzed by the United States Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida.

Results and Discussion

In June 1972, 24 months after large-scale aerial treatment of inland areas, mirex residues greater than 0.01 ppm were found in only 4 of 40 animal samples (Table 1). All four samples were collected at the two freshwater stations located on the Ashley and Cooper Rivers (Stations 3 and 4, Fig. 1) which drain watersheds within the treated area. Three species of freshwater fishes and blue crabs contained the following amounts of mirex (ppm):

white catfish	0.021
bluegill	0.047
carp	0.12
blue crabs	0.026

Except for the bluegill, these animals are omnivorous bottom-dwellers. Blue crabs are euryhaline; they occasionally enter brackish and fresh waters of estuaries.

Mirex was not detected (Table 1) in the remaining 36 animal samples, most of which were taken at 9 stations located on tidal creeks in salt marsh areas that support populations of finfish and crustaceans. Many of these animals are transient and spend only a portion of their lives in the estuary. No mirex was detected in bottom sediments sampled at each location.

The lower limit of detection for mirex with the method employed (*3*) was 0.01 ppm.

Between October 1969 and June 1971 mirex residues in economically important members of the estuarine food chain varied as follows: crabs, 0-0.60 ppm; shrimps, 0-1.3 ppm; and fishes, 0-0.82 ppm. Levels of mirex in these species diminished to less than 0.01 ppm over a period of 18 to 24 months after the last aerial broadcast treatments to coastal South Carolina.

LITERATURE CITED

(*1*) *Ruckelshaus, W. D. 1972.* Products containing the insecticide mirex; determination and order of the Administrator. Fed. Regist. 37(106): 10987-10988.

(*2*) *Ruckelshaus, W. D. 1972.* Products containing the insecticide mirex; determination and order. Fed. Regist. 37(130): 13299-13300.

(*3*) *Borthwick, P. W., T. W. Duke, A. J. Wilson, J. I. Lowe, J. M. Patrick, Jr., and J. C. Oberheu. 1972.* Accumulation and movement of mirex in selected estuaries of South Carolina, 1969-1971. Pestic. Monit. J. 7(1): 6-26.

TABLE 1.—*Whole body mirex residues, ppm, in estuarine animals, and sediments of South Carolina—June 1972*

Station Location:	Toogoodoo Creek				River-land Terrace Pond	Stono River	Upper [2] Ashley River	Cooper [2] River	Lower Ashley River	Wando River	South Santee River
Species / Station Identification:	A	B	C	D	1	2	3	4	5	6	7
CRABS											
Callinectes sapidus (blue crab)	—[1]	—	—	—	—	—	—	0.026	—	—	—
Uca pugilator (sand fiddler)			—								
SHRIMPS											
Penaeus aztecus (brown shrimp)	—	—	—	—	—	—		—	—	—	—
Palaemonetes pugio (grass shrimp)					—						
FISHES											
Leiostomus xanthurus (spot)	—		—	—	—	—	—	—	—	—	
Bairdiella chrysura (silver perch)		—						—			—
Ictalurus catus (white catfish)							0.021				
Cyprinus carpio (carp)								0.12			
Lepomis macrochirus (bluegill)							0.047				
MOLLUSKS											
Crassostrea virginica (oyster)			—								
Mercenaria mercenaria (hard clam)								—			
SEDIMENT	—	—	—	—	—	—	—	—	—	—	—

[1] — indicates <0.01 ppm mirex.
[2] Freshwater stations.

Short-term Effects of Organophosphate Pesticides on Cholinesterases of Estuarine Fishes and Pink Shrimp

by

DAVID L. COPPAGE and EDWARD MATTHEWS

U. S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, Fla. 32561

The esterase-inhibiting insecticides (organophosphates and carbamates) are now produced and enter the environment in greater quantities than the chlorinated hydrocarbon insecticides (ANONYMOUS, 1972; ANONYMOUS, 1971). These pesticides act as nerve poisons by blocking synaptic transmission in the cholinergic parts of the nervous system (HEATH, 1961; KARCZMAR et al. 1970; KOELLE, 1963; METCALF, 1971; O'BRIEN, 1967). The disruption of nerve impulse transfers is caused by excessive accumulation of the neurotransmitter acetylcholine (ACh) which is normally broken down by the enzyme acetylcholinesterase (AChE, EC 3.1. 1.7 acetylcholine acetyl-hydrolase). The organophosphates and carbamates bind to the active site of the AChE and prevent breakdown of ACh (ALDRIDGE, 1971; FUKUTO, 1971; KOELLE, 1963; METCALF, 1971). AChE inhibitors probably cause death in higher vertebrates by blocking neurotransmission in the respiratory center of the brain or neuromuscular junctions of the respiratory apparatus (DeCANDOLE et al. 1953; HEATH, 1961; KOELLE, 1963), but this has not been confirmed for fish. Inhibition of AChE is also believed to be the mode of action of these pesticides on arthropods (HEATH, 1961; KOELLE, 1963; O'BRIEN, 1960; O'BRIEN, 1967).

The possible hazards of AChE inhibiting pesticides in the aquatic environment should not be ignored. Over one hundred AChE inhibiting pesticides are produced and over 200 million pounds are manufactured annually in the United States (CASIDA, 1964; ANONYMOUS, 1972; ANONYMOUS, 1971). Aquatic organisms show a broad range of response to organophosphate pesticides, depending on the compound, exposure time, water conditions, and species (EISLER, 1970a). Short-term lethal concentrations in water range from a few parts per trillion to several parts per million (EISLER, 1970b; ANONYMOUS, 1963; ANONYMOUS, 1970).

---

[1] Gulf Breeze Environmental Research Laboratory Contribution No. 169

[2] Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon

Bulletin of Environmental Contamination & Toxicology,
Vol. 11, No. 5 © 1974 by Springer-Verlag New York Inc.

The organophosphate insecticides, with which we are concerned in this report, generally degrade more rapidly in the environment than do chlorinated hydrocarbon insecticides they are replacing. But, their presence and effects in the environment may be greater than expected because it may be necessary to apply the organophosphates more frequently and in greater quantity to control pests. The cholinesterases of vertebrates may remain inhibited for several weeks after exposure because of irreversible inhibition by extremely small quantities of dealkylated oxygen-analog metabolites of thiophosphates (COPPAGE and DUKE, 1972; HEATH, 1961; KOELLE, 1963; MACEK et al. 1972; O'BRIEN, 1960). Cumulative reduction of AChE by repetitive exposure has been demonstrated in some vertebrates (HEATH, 1961; KOELLE, 1963), and this may happen to fish subjected to similar repetitive exposure in the environment (HOLLAND and LOWE, 1966; ANONYMOUS, 1965; WEISS, 1958).

Recent studies have indicated AChE measurements are probably the best general index of organophosphate poisoning of fish in the environment (COPPAGE, 1972; MACEK et al. 1972; COPPAGE and DUKE, 1972). Also, if one considers the number of organophosphate compounds and the difficulty in detecting their highly toxic oxygen-analogs (McCULLY, 1972; PARDUE, 1971), AChE measurements in animals from the environment are probably the best general indicator of serious organophosphate pesticide pollution. The difficult task of detecting and interpreting residues alone in terms of effects on organisms is eliminated by measuring AChE in animals taken directly from the environment. A field study of three species of estuarine fishes from an area sprayed with organophosphate pesticide showed brain AChE inhibition was correlated with mosquito control operations with malathion (COPPAGE and DUKE, 1972). Brain AChE of fresh water fishes in ponds was also inhibited by application of Dursban® (MACEK et al. 1972). Inhibition of AChE in fish brains has been found below river outfalls of pesticide plants (COPPAGE, unpublished data; WILLIAMS and SOVA, 1966). Also, concentrations of malathion lethal to commercial shrimp may exist during mosquito control operations (CONTE and PARKER, 1971).

® Trademark: Dursban, Dow Chemical Co., Michigan. Mention of commercial products does not constitute endorsement by the U. S. Environmental Protection Agency.

We need more information on the relationship of AChE inhibition to poisoning and deaths of estuarine animals to aid in determining whether detrimental effects and "kills" in the environment are caused by organophosphate pesticide exposure. This report concerns AChE inhibitory effects of short-term laboratory exposures of four species of estuarine fishes and a commercial shrimp to lethal concentrations of malathion that may be found in the environment (CONTE and PARKER, 1971). In addition, AChE inhibitory effects of lethal exposure to naled, Guthion®, and parathion are reported for two of the fish species.

MATERIALS AND METHODS

Inhibition of AChE activity was used as an indicator of poisoning in brains of spot (Leiostomus xanthurus; 65-150 mm total length), pinfish (Lagodon rhomboides; 65-125 mm), Atlantic croaker (Micropogon undulatus; 85-150 mm), and sheepshead minnows (Cyprinodon variegatus; 45-70 mm), and in the ventral nerve cord (VNC) of pink shrimp (Penaeus duorarum; 78-122 mm). The acetylcholine hydrolyzing enzymes from fish brains were characterized and assayed as previously described (COPPAGE, 1971). The assay was carried out with a recording pH-stat at pH 7 and 22° C. We mixed 2 ml of brain homogenate containing 5 mg of tissue per ml with 2 ml of 0.03 M acetylcholine iodide and measured the acetic acid liberated by enzymatic hydrolysis of ACh by titrating with 0.01 N NaOH. Shrimp VNC was assayed similarly, except temperature was 25° C and the homogenate contained 2 mg of VNC per ml. AChE activity of both shrimp and fish was measured as micromoles of ACh hydrolyzed per hour per mg of tissue in the reaction vessel. Each AChE assay sample consisted of pooled organs from 4 to 6 animals that survived pesticide exposure at a designated time.

In each test, 10 fish or shrimp were exposed in 3-5 replicates to technical grade pesticide in 8-liter acrylic plastic aquaria that received a mixture of flowing seawater (400 ml per minute) and pesticide from a common source. The pesticide was dissolved in acetone or benzene and infused into seawater by means of syringe pumps. Solvent infusion never exceeded 2.5 parts per million in the water and did not affect AChE activity. Pesticide concentration in the water was expressed in theoretical parts per billion (ppb), but was not verified by residue analysis because our chosen criteria for toxic effects were only death and AChE inhibition. In quadruplicate tests comparing

---

®Trademark: Guthion, Chemagro Corp., Missouri.

different solvent (acetone vs. benzene) carrying the same quantity of malathion, there was no significant difference (Student's t-test, $P \leq 0.05$) in mortality or AChE inhibition. Temperature range was 18-23° C and salinity was 23-29 parts per thousand during the tests.

To determine the extent of AChE inhibition resulting from a near median kill, we assayed survivors in tests in which 40-60 percent of the test population was killed. The shrimp assayed had lost equilibrium (=moribund). Statistical comparisons of AChE activities of exposed animals were made with unexposed populations (Student's t-test, $P \leq 0.001$).

RESULTS AND DISCUSSION

AChE inhibition was great in surviving fish and moribund shrimp. Results of tests are summarized in Table 1.

TABLE 1.

AChE Inhibition in Fish and Shrimp by LC 40-60 of Organophosphates

Animal	Pesticide	Theoretical Conc. (ppb)	Hours Exposed	AChE Reduced (%) Mean	AChE Reduced (%) Range	Inhibition Significant at t 0.001
Spot	Malathion	1250	24	70	65-82	Yes
	Naled	75	24	85	82-89	Yes
	Guthion	20	24	96	93-98	Yes
	Parathion	10	24	88	87-89	Yes
Pinfish	Malathion	1000	24	88	87-89	Yes
	Naled	75	24	88	88-88	Yes
	Guthion	10	24	80	77-84	Yes
	Parathion	10	24	90	88-92	Yes
Croaker	Malathion	1000	24	86	79-90	Yes
Sheepshead Minnow	Malathion	200	24	96	90-99	Yes
Pink Shrimp (moribund)	Malathion	1000	48	75	72-82	Yes

Relatively consistent levels of AChE inhibition occurred in fishes even with different compounds and different species. The survivors of populations of fish in which 40-60 percent were killed by exposure to organophosphate pesticide had mean brain AChE reductions of 70-96 percent (Table 1). Mean AChE inhibitions in fishes were near or exceeded the "lethal threshold" of about 82 percent reduction indicated in a previous study of sheepshead minnows (COPPAGE, 1972), except inhibition of spot brain-AChE by malathion, which differs by only 12 percent. These inhibitions indicate that mean reductions in AChE activity of about 80 percent are critical in short-term organophosphate poisoning of the fishes tested and this may apply to fishes in general. Deaths may occur even at mean inhibition values of 70 percent in some cases, so the "lethal threshold" probably varies slightly among species. These specific levels of reduction of AChE show that it is unnecessary to rely on the dubious interpretation of residues alone to determine poisoning and cause of "kills" in the environment. Measurements of AChE activity and residue analysis or pesticide usage data would be especially helpful in cause and effect studies.

Reduction of activity of ACh hydrolyzing enzymes in the VNC of moribund shrimp was similar to that observed in fishes (Table 1). The large reduction (75 percent) of enzyme activity in moribund shrimp indicates that they too may be useful indicators.

REFERENCES

ALDRIDGE, W. N.: Bull. W. H. O. 44, 25 (1971).

ANONYMOUS: U. S. Environ. Prot. Ag. Tech. Studies Report: TS-00-72-04 (1972).

ANONYMOUS: U. S. Dep. Agric.: The Pesticide Review (1971).

ANONYMOUS: U. S. Fish Wildl. Serv. Circ. 167 (1963).

ANONYMOUS: U. S. Fish Wildl. Serv. Circ. 226 (1965).

ANONYMOUS: U. S. Bur. Sport Fish Wildl. Resour. Publ. 106 (1970).

CASIDA, J. E.: Science 164, 1011 (1964).

CONTE, F. S., and J. C. PARKER: Sea Grant Publ. No. TAMU-SG-71-211 (1971).

COPPAGE, D. L.: Bull. Environ. Contam. Toxicol. 6, 304 (1971).

COPPAGE, D. L.: Trans. Am. Fish. Soc. 101, 534 (1972).

COPPAGE, D. L.: Unpublished data.

COPPAGE, D. L. and T. W. DUKE, Proc. 2nd Gulf Coast Conf. Mosq. Suppr. Wildl. Manage. New Orleans, La., Oct. 20-22. 1971 (1972).

DeCANDOLE, C. A., W. W. DOUGLAS, K. E. V. SPENCER, R. W. TORRANCE, and K. M. WILSON: Br. J. Pharmacol. 8, 466 (1953).

EISLER, R.: U. S. Bur. Sport Fish. Wildl. Tech. Pap. 45 (1970a).

EISLER, R.: U. S. Bur. Sport Fish. Wildl. Tech. Pap. 46 (1970b).

FUKUTO, T. R.: Bull. W. H. O. 44, 31 (1971).

HEATH, D. F.: Organophosphorous Poisons. Pergamon Press, N. Y. (1961).

HOLLAND, H. T., and J. I. LOWE: Mosq. News 26, 383 (1966).

KARCZMAR, A. G., S. NISHI, and L. C. BLABER: Acta Vitaminol. Enzymol. 24, 131 (1970).

KOELLE, G. B., ed.: Cholinesterases and Anticholinesterase Agents. Springer-Verlag, Berlin. (1963).

MACEK, K. J., D. F. WALSH, J. W. HOGAN, and D. D. HOLTZ: Trans. Am. Fish. Soc. 101, 420 (1972).

METCALF, R. L.: Bull. W. H. O. 44, 43 (1971).

McCULLY, K. A.: J. Assoc. Of. Anal. Chem. 55, 291 (1972).

O'BRIEN, R. D.: Toxic Phosphorus Esters. Academic Press, N. Y. (1960).

O'BRIEN, R. D.: Insecticides. Academic Press, N. Y. (1967).

PARDUE, J. R.: J. Assoc. Of. Anal. Chem. 54, 359 (1971).

WEISS, C. M.: Ecology 39, 194 (1958).

WILLIAMS, A. K., and R. C. SOVA: Bull. Environ. Contam. Toxicol. 1, 198 (1966).

CONTRIBUTION NO. 170

Accumulation of Aroclor® 1254 in Grass Shrimp (*Palaemonetes pugio*) in Laboratory and Field Exposures

by

D. R. NIMMO, J. FORESTER, P. T. HEITMULLER, and G. H. COOK

U. S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, Fla. 32581

Results of several experiments indicate that aquatic invertebrates accumulate total body concentrations of polychlorinated biphenyls (PCB) thousands of times greater than that of the surrounding water. For example, SANDERS and CHANDLER (1972) showed that fresh water insects and crustaceans rapidly (1 day) accumulated PCB (Aroclor 1254) up to 24,000 times greater than the concentration in the water. Results of similar exposures conducted with estuarine animals showed oysters concentrating 85,000 (LOWE et al. 1972), shrimp 10,000 (NIMMO et al. 1971a), and fish 30,000 (HANSEN et al. 1971) times the amount of PCB in the water.

Although SANDERS and CHANDLER (1972) stated that PCBs entering the aquatic environment are below concentrations acutely toxic to invertebrates, we have noted that most of the accumulation studies conducted thus far by the investigators cited in the paragraph above have been at concentrations of 1.0 µg/ℓ and above, i.e., concentrations demonstratively toxic to test animals. Little is known about accumulation in marine invertebrates at extremely low concentrations, and with one exception (NIMMO et al. 1971a), no one to our knowledge has placed PCB-free animals in a natural environment known to have PCBs and followed accumulation with time.

We report here the results of several experiments on chronic toxicity of Aroclor 1254 to Palaemonetes pugio, an estuarine grass shrimp, as well as concentration and loss of the compound from the animals with time. We also exposed grass shrimp for up to 3 months to Aroclor 1254-contaminated sediments in Escambia Bay, near Pensacola, Florida.

METHODS AND MATERIALS

With one exception, all laboratory experiments were conducted in 30-ml chambers supplied with flowing water from Santa Rosa

[1] Gulf Breeze Environmental Research Laboratory Contribution No. 170

[2] Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon

Sound. Three sets of 5 chambers each received test concentrations: the fourth set was a control. Each chamber contained 4-10 shrimp, the number depending on the size of the animals. Water flowed continuously through each chamber at a rate of 1.0 l/hr. Aroclor 1254, dissolved in polyethylene glycol (mol. wt. 200), was metered into each mixing tank with a syringe pump before the water entered the test chamber. An equal amount of solvent was added to the water flowing to controls. David J. Hansen of this laboratory, found the sensitivity of a marine fish to Aroclor 1254 remained unchanged when he varied concentrations of polyethylene glycol used to deliver the toxicant (personal communication). The shrimp were fed daily a commercial molly-flake diet (<0.02 mg/kg organochlorine compounds).

The experiment to determine the concentration and loss from the tissues of P. pugio was also conducted in a flowing-water system. We constructed 18-liter aquaria with false floors of nylon screen (1/4-inch mesh) to hold shrimp above the detritus brought in with the water or produced by the animals. This modification was intended to prevent the animals from eating these particles with adsorbed Aroclor 1254. Consequently, we assume that shrimp obtained more of the chemical from the water by absorption through the gills rather than from ingestion of contaminated detritus. The shrimp were not fed during this experiment.

Concentrations of Aroclor 1254 in tissues by gas chromatography were determined using pooled samples of at least 10 shrimp each (NIMMO et al. 1971a).

P. pugio were exposed to Aroclor 1254-contaminated sediments in upper Escambia Bay from November 1971 to February 1972. The shrimp in specially-constructed cages (HEITMULLER and NIMMO, 1972) were exposed directly to the sediments. Average concentration of Aroclor 1254 in the uppermost two inches of sediment in November 1971 was 5.0 mg/kg (dry weight).

RESULTS OF LABORATORY EXPOSURES

Tests conducted in flowing water showed P. pugio to be susceptible to Aroclor 1254 (Table 1). In a 7-day exposure, 60% died at 9.1 µg/ℓ, but significant mortality did not occur at 0.17 and 0.62 µg/ℓ. In the second series of tests lasting 16 days, 4.0 and 12.5 µg/ℓ were toxic, but significant mortality did not occur in 1.3 µg/ℓ.

At the conclusion of several one-week exposures to a range of concentrations (0.17 to 9.1 µg/ℓ), surviving shrimp from each exposure were analyzed for whole-body residues. Ambient concentration of toxicant in the water and resultant residues in the shrimp were correlated (r=0.91, Table 2). In some cases, duplicate test concentrations produced biological accumulations that differed by a factor of 2. Concentration factors ranged from 3,000 to 11,000. These ranges were similar to those found in

TABLE 1. MORTALITY AND ACCUMULATION OF AROCLOR 1254 IN *Palaemonetes pugio**

Test Conc. (µg/ℓ)	Days Exposed	Average Mortality (%)**	Body Conc. (mg/kg)	Concentration Factor
CONTROL	7	4(0 - 20)	0.1	-----
0.17	7	8(0 - 40)	1.3	7600
0.62	7	4(0 - 20)	5.4	8700
9.1	7	60(20 - 80)***	65.0	7100
CONTROL	16	25(0 - 50)	<0.1	-----
1.3	16	40(0 - 100)	18.0	14000
4.0	16	45(25 - 50)***	27.0	6700
12.5	16	55(50 - 75)***	46.0	3700

*All exposures were conducted in flowing seawater: salinity and temperature ranges were 22 to 28‰ and 17 to 28° C.

**5 replicates per concentration: at least 4 shrimp per replication.

***Significant at P >0.05.

TABLE 2. ACCUMULATION OF AROCLOR 1254 BY *Palaemonetes pugio**

Test Conc. (µg/ℓ)	Body Conc. (mg/kg)	Concentration Factor
0.17	1.3	7600
0.62	5.4	8700
1.0	3.2	3200
2.3	25.0	11000
2.7	19.0	7000
3.2	15.0	4800
3.2	26.0	8100
5.2	29.0	5600
5.3	16.0	3000
5.3	30.0	5700
9.1	65.0	7100

*7-day exposures conducted in flowing seawater at salinity and temperature ranges of 22 to 28 and 17 to 28° C.

TABLE 3.

ACCUMULATION OF AROCLOR 1254 IN *Palaemonetes pugio* WITH TIME AFTER EXPOSURES TO THE CHEMICAL IN WATER AT THREE CONCENTRATIONS (µg/ℓ)
(Each value represents a composite sample of 10 animals)

Length of Exposure (hr/ days)	Control		0.04		0.09		0.62	
	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor
0	0.1	*	0.1	*	0.1	*	0.1	*
1	---	*	0.1	*	0.1	*	0.1	*
2	---	*	0.1	*	0.1	*	0.1	*
3	---	*	0.1	*	0.1	*	0.1	*
4	---	*	0.1	*	0.1	*	0.1	*
8	---	*	0.1	*	0.1	*	0.12	190
12	---	*	0.1	*	0.1	*	0.14	230
16	---	*	0.1	*	0.1	*	0.26	420
24 / 1	---	*	0.1	*	0.1	*	0.20	320
36 / 1.5	---	*	0.1	*	0.1	*	0.20	470
48 / 2	---	*	0.1	*	0.10	1100	0.37	600
72 / 3	---	*	0.1	*	0.14	1560	0.58	930
96 / 4	---	*	0.1	*	0.15	1670	0.40	650
154 / 6.5	0.1	*	0.1	1590	0.33	3670	1.28	2060
336 / 14	---	*	0.13	3250	0.43	4780	7.40	11930
504 / 21	0.1	*	0.15	3750	0.45	5000	6.67	10900
672 / 28	0.14	*	0.17	4250	1.57	17400	10.82	17450
840 / 35	0.10	*	0.21	5250	0.75	8330	16.48	26580
			PCB - STOPPED					
1176 / 49	0.1	*	0.1	*	0.12	*	3.24	*
1512 / 63	0.15	*	0.1	*	0.13	*	1.64	*

* Magnification factor not calculated.

tests using penaeid shrimp (NIMMO et al. 1971a), but were somewhat lower than those found by SANDERS and CHANDLER (1972), in tests using several invertebrate species in fresh water.

There appeared to be no threshold below which levels of the chemical added to water failed to produce residues in the tissues (Table 3) in our tests. Whole-body concentrations produced after 5 weeks exposure to 0.04, 0.09 and 0.62 $\mu g/\ell$ ranged from 200 to 26,000 times the concentrations in the test water. Concentrations did not reach equilibrium and from 60 to 90 percent of the Aroclor 1254 was lost from the shrimp within 4 weeks after exposure to the chemical was stopped. Test concentrations of the chemical were not significantly toxic to shrimp. Although accumulation increased with increasing concentration of toxicant in this test, this was not observed in earlier studies (see Tables 1 and 2). Implications are to be discussed elsewhere.

RESULTS OF FIELD EXPOSURES

Average whole-body residue of Aroclor 1254 in P. pugio after 1 month was 0.41 mg/kg (0.34 to 0.57); after 3 months, 0.42 mg/kg (0.37 to 0.50). There was no evidence that significant mortality occurred during the exposures of grass shrimp to contaminated sediments.

DISCUSSION

Concentrations of Aroclor 1254 in P. pugio, after exposure to contaminated sediments for 3 months was equivalent to a laboratory-exposure of 0.09 $\mu g/\ell$ in water for 2 weeks (Table 3). We expected residues to be higher in caged shrimp since we had found that fiddler crabs exposed in the laboratory accumulated residues equal to or greater than (wet-weight basis) that of the contaminated substratum (dry-weight basis) after 30 days (NIMMO et al. 1971b). Concentrations of Aroclor 1254 in caged shrimp exposed to contaminated sediments appeared to reach a plateau, but this was not the case in laboratory exposures (Table 3) where an equilibrium was not reached. Therefore, we believe that shrimp exposed to the sediments might have obtained PCB from the water or food singly, but shrimp exposed to Aroclor in the laboratory obtained chemical from two sources, water and food. It might also be more available in the laboratory than the field due to the carrier. In earlier laboratory studies with penaeid shrimp, both water and food appeared to be sources (NIMMO et al. 1971a).

No significant mortality was observed in caged shrimp and none would be predicted since residues produced in the field were similar to those found in shrimp after laboratory exposures which caused no death.

Penaeid shrimp spend only a fraction of their life cycle in an estuary, moving into oceanic waters after reaching maturity

(PEREZ FARFANTE 1969), but grass shrimp are endemic in estuaries. Therefore, in relation to time of exposure we would expect grass shrimp to accumulate a pollutant from a contaminated estuary to a greater degree than penaeid shrimps, nevertheless, this is not true. In August 1968, penaeid shrimps (Penaeus duorarum, P. setiferus, and P. aztecus) collected during a survey of Escambia Bay, Florida, had whole-body residues of Aroclor 1254 as high as 14.0 mg/kg (NIMMO et al. 1971b). In that survey and in subsequent collections, P. pugio had a maximum residue of only 1.4 mg/kg.

Lower residues in P. pugio from Escambia Bay may be due to amounts of PCB in bay sediments and behaviorial patterns of the animals. We noted earlier (NIMMO et al. 1971a) in species of penaeid shrimp were related to higher concentrations of Aroclor 1254 in sediments that predominate in upper Escambia Bay. We found that penaeid shrimp, as adults, usually are captured in deeper waters and burrow into silty or sandy substrates. In contrast, grass shrimp usually do not burrow, rather are found along shallow sandy beaches and grass beds, where they obtain food that is relatively uncontaminated with PCB.

REFERENCES

HANSEN, D. J., P. R. PARRISH, J. I. LOWE, A. J. WILSON, JR., and P. D. WILSON: Bull. Environ. Contam. and Toxicol., 6, 113 (1971).

HEITMULLER, P. T., and D. R. NIMMO: Prog. Fish-Cult., 34, 120 (1972).

LOWE, J. I., P. R. PARRISH, J. M. PATRICK, and J. FORESTER: Mar. Biol. (Berl.), 17, 209 (1972).

NIMMO, D. R., R. R. BLACKMAN, A. J. WILSON, JR., and J. FORESTER: Mar. Biol. (Berl.), 11, 191 (1971a).

NIMMO, D. R., P. D. WILSON, R. R. BLACKMAN, and A. J. WILSON, JR.: Nature, 231, 50 (1971b).

PEREZ FARFANTE, I.: U. S. Fish Wildl. Serv., Fish. Bull., 67, 461 (1969).

SANDERS, H. O., and J. H. CHANDLER: Bull. Environ. Contam. and Toxicol., 7, 257 (1972).

ENVIRONMENTAL RESEARCH 7, 363–373 (1974)

Aroclor 1016: Toxicity to and Uptake by Estuarine Animals[1,2]

D. J. HANSEN, P. R. PARRISH, AND J. FORESTER

U. S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561 (Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon)

Received June 24, 1973

Bioassays were conducted to determine the acute toxicities of the polychlorinated biphenyl (PCB) Aroclor 1016 in flowing sea water to American oysters (*Crassostrea virginica*), brown shrimp (*Penaeus aztecus*), grass shrimp (*Palaemonetes pugio*), and pinfish (*Lagodon rhomboides*), and to determine its chronic toxicity to, and uptake and retention by pinfish. Acute 96-hour EC50's or LC50's were: oysters, 10.2 μ/liter; brown shrimp, 10.5 μg/liter; grass shrimp, 12.5 μg/liter. The PCB was not toxic to pinfish at 100 μg/liter for 96 hours, but significant mortality occurred when pinfish were exposed to 32 μg/liter of Aroclor 1016 for 42 days. Pinfish exposed to 1 μg/liter for 56 days accumulated the chemical with maximum concentrations attained in whole-fish by 21 to 28 days. Maximum whole-body residue (wet weight) was 17,000 × the nominal concentration in test water. Tissue alterations, such as severe vacuolation in the pancreatic exocrine tissue surrounding the portal veins, occurred in pinfish exposed to 32 μg/liter of Aroclor 1016 for 42 days.

Polychlorinated biphenyls (PCB's) have been used industrially for over 40 years (Broadhurst, 1972) and recently there has been concern about their environmental impact. Because of this concern, manufacture and sale of most PCB's were discontinued, and sales restricted to uses that are not likely to produce environmental contamination. A new PCB, Aroclor 1016, is now manufactured in the United States for sale to capacitor manufacturers as a substitute for all other PCB's. This new PCB is similar to Aroclor 1242, except that amounts of isomers containing 5 or more chlorine atoms per biphenyl group have been considerably reduced. Domestic sales of Aroclor 1016 increased from about 3.3×10^6 pounds in 1971 to 20.9×10^6 pounds in 1972 (W. B. Papageorge, personal communication[3]).

Our study was conducted to determine the acute toxicity of Aroclor 1016 to the American oyster (*Crassostrea virginica*), brown shrimp (*Penaeus aztecus*), grass shrimp (*Palaemonetes pugio*), and pinfish (*Lagodon rhomboides*) and to determine its chronic toxicity to, and uptake and retention by pinfish.

[1] Aroclor is a registered trademark, Monsanto Company, St. Louis, MO. Mention of commercial products or trade names does not constitute endorsement by the Environmental Protection Agency.

[2] Contribution No. 172, Gulf Breeze Environmental Research Laboratory.

[3] W. P. Papageorge, Monsanto Industrial Chemicals Company. 800 N. Lindbergh Boulevard, St. Louis, MO 63166.

MATERIALS AND METHODS

Test Animals

Test animals were collected near the Gulf Breeze Laboratory and acclimated to laboratory conditions for at least 7 days before exposure. If mortality exceeded 1% in the 48 hours immediately preceding the test, or if abnormal behavior was observed during acclimation, the animals were not used. Oysters tested were 35–55 mm in height; brown shrimp, 11–26 mm rostrum–telson length; grass shrimp, 20–32 mm rostrum–telson length; and pinfish 27–84 mm standard length. Animals were not fed during acute toxicity tests but they could obtain plankton from the unfiltered sea water. In the chronic exposures and the uptake and retention test, pinfish were fed commercial fish food that contained no detectable PCB (<0.2 μg/g).

Acute and Chronic Tests

Acute toxicity to Aroclor 1016 was determined by exposing 10 individual animals to 1, 10, or 100 μg/liter for 96 hours in each of two 20 liter aquaria. Each experiment was conducted twice. The PCB was dissolved in acetone or polyethylene glycol 200 and metered at 30 or 0.1 ml/hour, respectively, into unfiltered sea water that entered each aquarium at 75 liters/hour. Two control aquaria received the same quantities of water and solvent. Temperature and salinity of the water flowing into aquaria in replicated tests were similar ($\pm 10\%$).

Chronic toxicity of Aroclor 1016 to pinfish was determined in 3 experiments, each lasting 42 days. In each experiment, 50 fish were placed in each 90 liter aquarium that received 140 liters/hour of sea water. The PCB, dissolved in polyethylene glycol, was metered into the water at 0.083 ml/hour in the first two experiments, and 1.04 ml/hour in the third experiment. Control aquaria received the same quantity of water and solvent.

The same exposure techniques that were used in the first two chronic toxicity experiments were used to determine: (1) the rate of uptake and retention of Aroclor 1016 in pinfish exposed to 1 μg/liter for 56 days and (2) the rate of depuration of Aroclor 1016 by pinfish in PCB-free water for 56 days.

Effect of Aroclor 1016 was assessed by measuring percentage reduction in shell growth of exposed oysters as compared to control oysters (Butler, 1962), by determining mortality in shrimps and fish, and by pathological examination of chronically exposed fish.

Histopathological Examination

Dr. J. A. Couch, pathobiologist at this laboratory, examined viscera from live pinfish from the third 42-day exposure. Viscera were fixed either in 10% neutral buffered formalin or in Davidson's fixative. Those fixed in Davidson's were stored in 70% ethyl alcohol until processed for paraffin sections (7 μm) and stained with Harris hematoxylin and eosin (HHE) or Periodic Acid Schiff's (PAS). Viscera fixed in 10% neutral buffered formalin were processed for frozen sections (12 μm) and stained with oil Red O and hematoxylin.

Chemical Analyses

Concentrations of Aroclor 1016 in water and animals were determined by electron capture gas chromatography. Unfiltered water samples from each concentration were analyzed once during the 96-hour exposures, and weekly during longer exposures. Concentrations in animals that survived the 96-hour exposures were determined as whole-body residues. At the conclusion of each chronic exposure, surviving pinfish were dissected and PCB residues in flesh, flesh and scaleless skin, and remaining tissue determined. Residues in all tissues were summed to compute concentrations of Aroclor 1016 in whole fish. The same procedure was followed in the uptake and retention study, except fish were removed for analysis at selected intervals during exposure and depuration. Also, at the end of the 56 day exposure, brain, gills, heart, and liver were removed from exposed fish for residue analysis. All fish samples were composites of 10 individuals.

Tissue samples that weighed more than 5 g were prepared for analysis by mixing them with anhydrous sodium sulfate in a blender. The mixture was extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated to approximately 10 ml and transferred in 3–4-ml portions to a 400 × 20 mm chromatographic column that contained 76 ml of unactivated Florisil. After each portion settled in the column, vacuum was applied until all solvent was evaporated. This was repeated with three 5-ml rinses. The residue was eluted from the column with 70 ml of a 9:1 mixture (v/v) of acetonitrile and distilled water. The eluate was evaporated to dryness and the residue transferred to a Florisil column (Mills *et al.*, 1963) with petroleum ether. Aroclor 1016 was eluted with 6% ethyl ether in petroleum ether.

Tissue samples that weighed less than 5 g were analyzed by a modification of the micromethod described in the Pesticide Analytical Manual, Volume III (U. S. Food and Drug Administration, 1970). The samples were weighed into a size 23 Duall tissue grinder and extracted 3 times with 5-ml portions of acetonitrile. The acetonitrile was flooded with 15 ml of 2% (w/v) sodium sulfate in distilled water and extracted with three 5-ml portions of hexane. The hexane was evaporated to approximately 1 ml and transferred to a 9 × 200 mm Chromaflex column with a 50 ml reservoir that contained 3.3 g of Florisil topped with 3.3 g of anhydrous sodium sulfate. Aroclor 1016 was eluted with 20 ml of 5% ethyl ether in hexane and adjusted to an appropriate volume for analysis.

Water samples were extracted with petroleum ether, the extracts dried with anhydrous sodium sulfate, and evaporated to approximately 1 ml. The concentrates were transferred to a size 7 Chromaflex column containing 1.6 g Florisil topped with 1.6 g anhydrous sodium sulfate. Aroclor 1016 was eluted with 20 ml of 1% ethyl ether in hexane and the eluates were adjusted to an appropriate volume for analysis.

All samples were analyzed by electron capture gas chromatography using a 15 × 3.2 mm glass column packed with 2% OV-101 on 100–120 Gas Chrom Q. Nitrogen flow rate was 25 ml/min, the oven temperature was 190°C, and the injector and detector temperature was 210°C.

Aroclor 1016 was quantitated by comparing the total height of all peaks in the

sample with the total height of all peaks in a standard of known concentration. Recoveries were greater than 80%; data were not adjusted for recovery. All tissue residues were determined on a wet-weight basis.

RESULTS AND DISCUSSION

Acute (96-hr) Exposure

Aroclor 1016 was acutely toxic to the estuarine organisms tested (Tables 1 and 2). Shell growth in oysters was inhibited greatly by exposure to 100 μg/liter for 96 hours. Sensitivities of brown shrimp and grass shrimp were similar, and pinfish was the least sensitive species. Acute toxicities of Aroclor 1016 to oysters, brown shrimp, and pinfish were similar to that of Aroclor 1242 to these species (P. R. Parrish, unpublished data), and Aroclor 1254 to oysters, pink shrimp (*Penaeus duorarum*), and pinfish (Duke *et al.*, 1970).

All animals accumulated Aroclor 1016 (Table 1). The quantities accumulated depended on the exposure concentrations and not on species. Whole-body concentrations in live animals ranged from 440 to 4200 × the nominal concentration in test water and 1200 to 6700 × the measured concentration in test water.

TABLE 1

ACUTE TOXICITY TO AND UPTAKE OF AROCLOR 1016 BY AMERICAN OYSTERS (*Crassostrea virginica*), BROWN SHRIMP (*Penaeus aztecus*), GRASS SHRIMP (*Palaemonetes pugio*), AND PINFISH (*Lagodon rhomboides*) IN 96-HOUR EXPOSURES[a]

Species	Test concentration (μg/liter)		Effect (%)	Whole-body residue (μg/g, wet weight)
	Nominal	Measured		
C. virginica	Control	ND[b]	0	ND[b]
	1	0.6	10	4.0
	10	7.2	38	32
	100	58	93	95
P. aztecus	Control	ND	0	ND
	1	0.9	8	3.8
	10	8.9	43	42
	100	33	100	—
P. pugio	Control	ND	8	ND
	1	0.4	33	1.1
	10	9.4	38	22
	100	38	93	44
L. rhomboides	Control	ND	2	ND
	1	0.8	5	2.2
	10	6.9	0	21
	100	56	18	65

[a] Effect is expressed as percent reduction in shell growth in oysters and death in shrimps and fish. Whole body residues are from animals alive at end of exposure period.

[b] ND, not detectable: <0.2 μg/liter in water; <0.2 μg/g in tissue.

TABLE 2
ACUTE TOXICITY OF AROCLOR 1016 TO AMERICAN OYSTERS (*Crassostrea virginica*), BROWN SHRIMP (*Penaeus aztecus*), AND GRASS SHRIMP (*Palaemonetes pugio*)[a]

Species	96-hour LC50 (μg/liter)	Temperature (C)		Salinity (0/00)	
	Nominal	Mean	Range	Mean	Range
C. virginica	10.2	30	25–32	29	26–31
P. aztecus	10.5	31	29–32	29	28–30
P. pugio	12.5	30	29–32	29	25–30

[a] EC50:concentration expected to cause a 50 percent reduction in shell growth in oysters.

Chronic (42-day) Exposure

Toxicity of Aroclor 1016 to juvenile pinfish was greater in tests lasting 6 weeks than in 96-hour exposures. Pinfish seemed unaffected by 10 μg/liter or less, but died in concentrations of 32 and 100 μg/liter (Table 3). Mortality began in the second week of exposure. Fifty percent mortality did not occur in the third exposure, for example, until the 33rd day at 32 μg/liter and the 18th day at 100 μg/liter. Delayed mortality of pinfish was also observed with Aroclor 1254 (Hansen *et al.*, 1971).

Most of the fish that died in the 42 day exposure exhibited symptoms of poisoning, such as changed appearance and behavior. Initially, their color darkened, they stopped feeding, and they swam erratically with bodies inclined downward.

TABLE 3
TOXICITY AND UPTAKE OF AROCLOR 1016 BY PINFISH (*Lagodon rhomboides*) EXPOSED FOR 42 DAYS IN THREE SEPARATE EXPERIMENTS

Test concentration (μg/liter)		Mortality (%)	Concentration in fish (μg/g, wet weight)		
Nominal	Measured		Flesh	Flesh and skin	Whole fish
Control	ND[a]	36	ND	ND	ND
0.1	ND	16	0.7	0.8	2.4
1.0	0.8	48	5.1	6.3	11
10.0	3.0	38	60	90	166
Control	ND	12	0.5	0.6	0.5
1.0	0.9	16	4.0	6.0	17
3.2	2.5	16	34	39	65
10.0	7.0	28	63	76	170
32.0	13	44[b]	140	180	620
Control	ND	6	ND	ND	ND
10.0	6.8	6	23	49	111
32.0[c]	21	50[b]	30	48	106
100.0[c]	59	50[b]	38	72	205

[a] ND, not detectable: <0.2 μg/liter in water; <0.2 μg/g in tissue.

[b] Mortality significantly greater than in control fish, $\alpha = 0.01$.

[c] Exposure terminated and tissues analyzed when 50% of the fish died: 33 days at 32 μg/liter and 18 days at 100 μg/liter.

Difficulty in swimming progressed until the fish swam with their tails and dorsal fins breaking the water surface. Finally, the fish lost equilibrium, swam upside down, and died. Affected fish became vulnerable to attack by other pinfish in the tank. In the third experiment, the majority of dying fish exposed to 32 and 100 μg/liter lost scales, skin and, finally, flesh in front of the dorsal fin. This formed lesions sometimes as deep as the neural spine. This condition did not occur in fish exposed to 32 μg/liter in the second experiment.

Hepatocytes in liver sections of 13 control fish showed no unusual characteristics when stained with HHE and PAS or oil Red O. The hepatocytes from 6 fish demonstrated only a moderate PAS-positive reaction, indicating moderate to light glycogen reserves. In 7 other control fish, liver sections stained with oil Red O also showed a broad range of lipid patterns. Structure of livers of control fish was normal, being tubulosinusoidal in nature, with disseminated pancreas prominent along the course of the portal vein (Fig. 1).

Eight fish exposed to 10 μg/liter of Aroclor 1016 for 42 days showed no pathologic microscopic visceral characteristics distinguishable from control fish. Half of the fish samples were paraffin-processed; half were prepared for frozen sections.

Pinfish exposed to 32 μg/liter of Aroclor 1016 had several liver and pancreatic alterations that distinguished them from control fish and fish exposed to 10 μg/liter. Tissues from 8 fish were examined; half were paraffin-processed and half

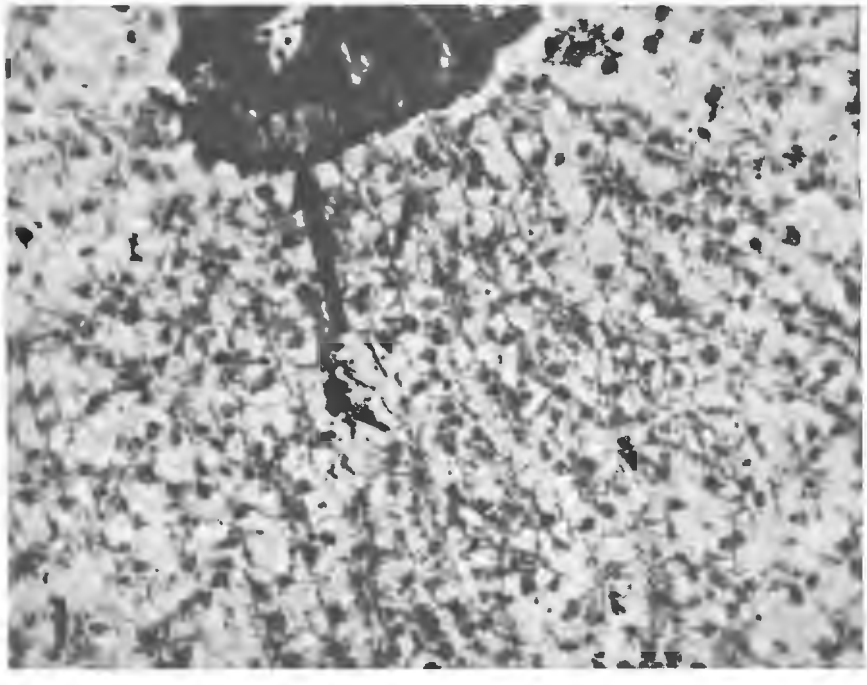


FIG. 1. Section of normal liver from control pinfish. The vacuoles in hepatocytes are results of extraction of lipid and/or glycogen during paraffin processing. Note the normal pancreatic exocrine tissue containing normal secretory deposite (arrows). (×450).

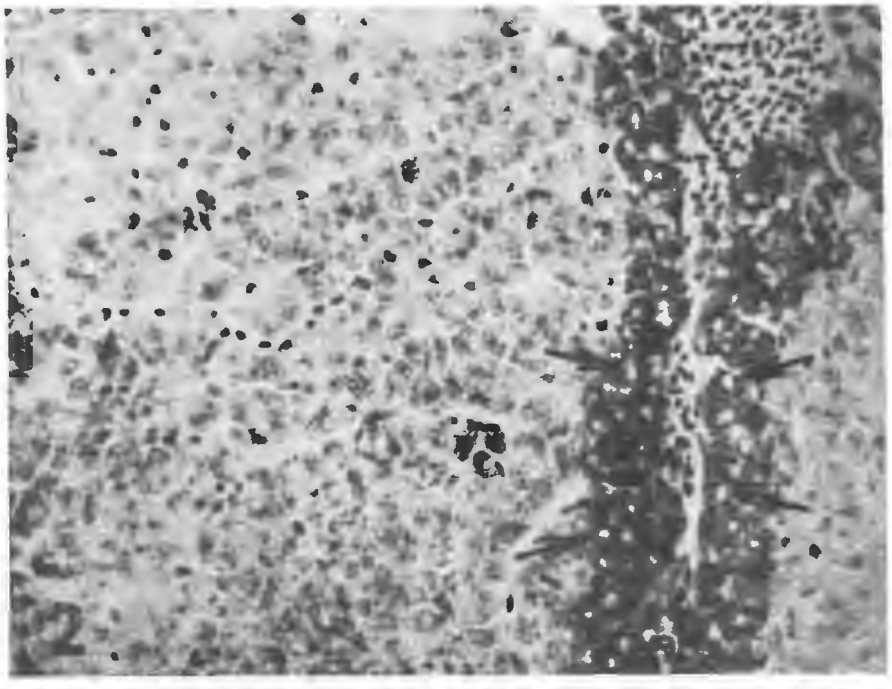


FIG. 2. Section of liver from pinfish exposed to 32 μg/liter Aroclor 1016 for 42 days. The hepatocytes appear relatively dense with more prominent nuclei than in Fig. 1. Note the lack of lipid or glycogen vacuoles in hepatocytes. Small, abnormal vacuoles in pancreatic exocrine tissue (arrows) distinguish fish exposed to 32 μg/liter from control fish. (×450).

were studied as frozen sections. Hepatocytes appeared slightly enlarged and more basophilic than in the control fish (Fig. 2). Normal liver cord orientation was somewhat altered and PAS-positive granules accumulated at the edge of the pancreatic acinar tissue in the liver (Fig. 3). Less lipid material existed in the livers of fish exposed to 32 μg/liter than in control fish. This contrasts with the heavy, abnormal accumulation of lipid in the livers of spot exposed to 6 μg/liter of Aroclor 1254 for 30 days (Couch, 1973). The most remarkable alteration in the pinfish was the occurrence of severe vacuolation in the pancreatic exocrine tissue surrounding the portal veins (Figs. 2 and 3). This vacuolation was distinguishable from normal secretory vacuoles and deposits in pancreatic tissue from control fish because those in exposed fish were small, abundant, and contained no secretory granules (Fig. 1).

After the first exposure, 16 pinfish from each control or Aroclor 1016-contaminated aquarium were held in PCB-free water, and the salinity reduced to determine if ability of pinfish to survive osmotic stress had been impaired by exposure to sublethal concentrations of Aroclor 1016. (Aroclor 1254 reduced the ability of pink shrimp (*Penaeus duorarum*) to withstand stress of decreased salinity, D. R. Nimmo, personal communication.[4]) Salinity was lowered from an

[4] D. R. Nimmo, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida 32561.

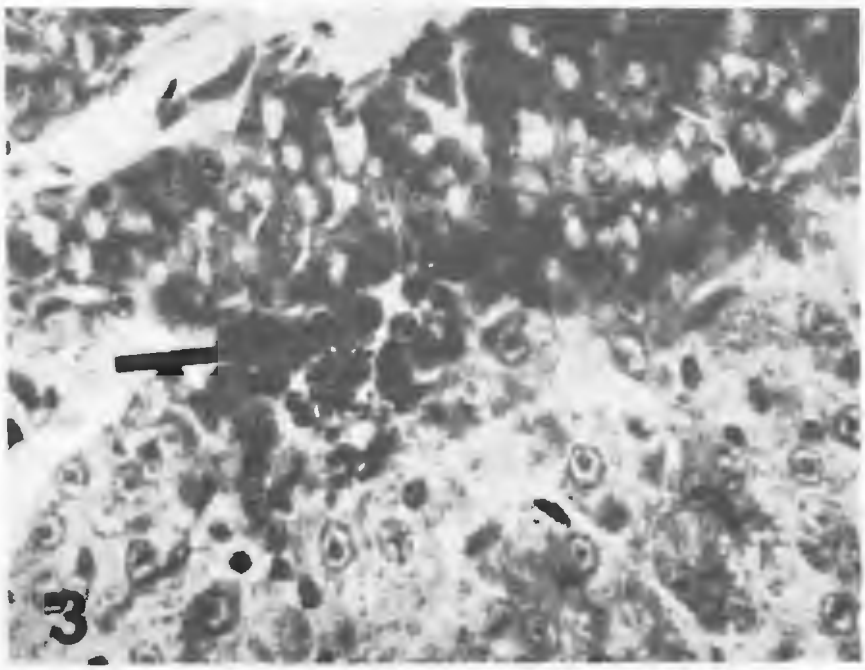


FIG. 3. Pigment deposition (PAS-positive) between pancreatic exocrine tissue and liver parenchyma (arrow). Note nature of small vacuoles in pancreatic tissue. This tissue is from fish exposed to 32 μg/liter Aroclor 1016. (×1000).

initial 28 ‰ by 50% on each of four consecutive days (14, 7, 3.5 and 1.8 ‰). None of the fish died until the salinity fell below 2 ‰. At that salinity, mortalities of control and PCB-exposed fish were similar.

Pinfish exposed for 42 days to concentrations of Aroclor 1016 between 0.1 and 32 μg/liter stored the chemical in proportion to the concentration in test water (Table 3). Concentrations in whole fish ranged from 11,000 to 24,000 × the nominal concentration in the test water and 14,000 to 55,000 × the measured concentration in test water, whereas the concentration factor in spot (*Leiostomus xanthurus*) exposed for 42 days to 1 μg/liter of Aroclor 1254 was 30,000 × the nominal concentration (Hansen *et al.*, 1971).

The concentration of Aroclor 1016 in edible tissue was less than that in whole fish. Concentrations in whole fish averaged 2.1 times those in flesh and scaleless skin and 2.8 times those in flesh. Pinfish exposed to 1 μg/liter of Aroclor 1016 stored quantities that exceeded the Food and Drug Administration's provisional action-level for all PCB's (5 μg/g) in edible tissues (Table 3).

Chronic (56-day) Exposure

Pinfish exposed to 1 μg/liter of Aroclor 1016 for 56 days accumulated the chemical, maximum concentrations in whole fish being attained in 21–28 days (Table 4). In a similar study with Aroclor 1254, whole-body concentrations of spot stabilized at about the same time, 14–28 days (Hansen *et al.*, 1971). Maximum

TABLE 4
CONCENTRATIONS OF AROCLOR 1016 (μG/G WET WEIGHT) IN PINFISH (*Lagodon rhomboides*) EXPOSED TO 1 μG/LITER OF THIS PCB (EACH SAMPLE CONSISTED OF TISSUES FROM 10 FISH)

Days of exposure	Concentration		
	Flesh	Flesh and skin	Whole fish
0	ND[a]	ND	ND
3	0.8	0.9	1.6
7	1.6	3.4	3.9
14	2.3	3.3	6.5
21	3.2	4.3	9.7
28	3.0	6.8	25
42	4.0	6.0	17
56	5.9	8.3	17
Depuration			
14	4.1	7.8	13.5
28	3.5	6.6	9.3
56	2.2	3.9	6.6

[a] ND, not detectable; <0.1 μg/g.

whole-body residue in pinfish was 17,000 × the nominal concentration in test water. Increases in concentrations in edible tissues were also rapid (Table 4), but may not have reached a maximum even after 56 days of exposure.

The quantity of Aroclor 1016 accumulated by pinfish differed in the various tissues and organs. Fish exposed to 1 μg/liter for 8 weeks accumulated 17 μg/g whole-body residue. Concentrations (μg/g) in other tissues or organs were: gills, 23; skin, 19; liver, 16; brain, 8.7; muscle, 5.9; and remaining tissues, 22.

Aroclor 1016 was lost from the tissues after pinfish were placed in PCB-free water (Table 4). After 56 days of depuration, concentrations in whole fish decreased by 51%. In an earlier study, spot which had accumulated Aroclor 1254 lost 66% after 56 days in PCB-free water (Hansen *et al.*, 1971).

We examined chromatograms to compare the proportions of 9 peaks of Aroclor 1016 in reference standards (Fig. 4) with those peaks from water and pinfish tissue samples (Table 5). Chromatograms of standards, tissue spikes, and water samples were similar, while chromatograms of standards and tissue samples were dissimilar. Chromatograms of Aroclor 1016 from different pinfish tissues (flesh, flesh and skin, and rest) were similar. Chromatograms from all tissues had smaller early eluting peaks, numbers 1, 2, and 3. Reduction in early eluting peaks of Aroclor 1254 from shrimp and fish tissues was noted by Nimmo *et al.* (1971). The relative proportions of the 9 peaks found in chromatograms from pinfish tissues early in the 56-day exposure, late in the exposure, and throughout the 56-day depuration period were similar. We do not know whether differences between chromatograms from reference standards and chromatograms from tissue samples reflect alterations in Aroclor 1016 molecules, differential solubility, or other factors. It is unlikely, however, that the PCB molecules were altered, because no change in relative proportions of peaks was noted throughout the 112 day experiment.

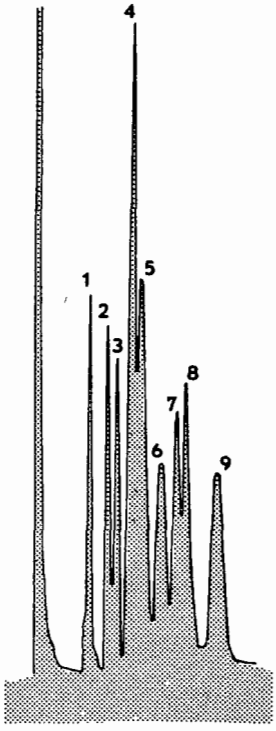


FIG. 4. Chromatogram of 9 peaks of Aroclor 1016 reference standard. Operating conditions: gas flow nitrogen 25 ml/min; injection and detector temperature 210°C; oven temperature 190°C; ^3H electron capture detector; 152.4 × 0.32 cm glass column packed with 2% OV-101 on 100–120 Gas Chrom Q.

TABLE 5

PERCENTAGES OF THE 9 MEASURED PEAKS FROM CHROMATOGRAMS OF AROCLOR 1016 REFERENCE STANDARDS AND FROM TISSUES OF PINFISH EXPOSED TO 1 μG/LITER OF AROCLOR 1016 FOR 56 DAYS AND THEN HELD IN PCB-FREE WATER FOR 56 DAYS

	Percentage of peak[a]									Chromatograms
	1	2	3	4	5	6	7	8	9	
Reference standard	12.4	11.3	10.0	23.5	13.3	6.4	8.1	8.8	6.2	8
All tissues through 14 days of exposure	2.4	5.3	1.8	39.6	7.7	10.3	12.1	9.4	11.4	7
All tissues, days 21–56 of exposure	2.5	5.0	2.1	33.2	7.8	11.9	11.3	10.3	15.9	12
All tissues during 56 day depuration	1.0	1.7	0.3	38.2	2.8	15.0	12.4	11.9	16.7	7

[a] Determined as $\frac{\text{peak height}}{\text{sum of nine peaks}} \times 100$.

The potential environmental hazard of a chemical is dependent upon its likelihood of entering the environment and its potential hazard to organisms. Aroclor 1016 is similar to other PCB's in its toxicity to, and uptake and retention by estuarine animals. Therefore, its substitution for other PCB's reduces environmental hazard only if the policy of restricting sales to uses not likely to produce environmental contamination is continued.

ACKNOWLEDGMENTS

We thank Dr. J. A. Couch for histopathological examination of the pinfish, S. S. Foss for preparing illustrations, and the Monsanto Company for providing Aroclor 1016.

REFERENCES

BROADHURST, M. G. (1972). Use and replaceability of PCB's. *Environ. Health Perspect.* **2**, 81–102.

BUTLER, P. A. (1962). Reaction of some estuarine mollusks to environmental factors. *In* "Biological Problems in Water Pollution" Third Seminar. U. S. Dept. of Health, Education and Welfare, Public Health Serv. Publ. No. 999-WP-25, 1965: 92–104.

COUCH, J. A. (1973). Pathologic effects of pesticides and related chemicals on the livers of fishes. *Proc. Fish. Path. Symp. AFIP.* Univ. Wis. Press. In press.

DUKE, T. W., LOWE, J. I., AND WILSON, A. J., JR. (1970). A polychlorinated biphenyl (Aroclor 1254®) in the water, sediment and biota of Escambia Bay, Florida. *Bull. Environ. Contam. Toxicol.* **5**, 171–180.

HANSEN, D. J., PARRISH, P. R., LOWE, J. I., WILSON, A. J., JR., AND WILSON, P. D. (1971). Chronic toxicity, uptake and retention of Aroclor® 1254 in two estuarine fishes. *Bull. Environ. Contam. Toxicol.* **6**, 113–119.

NIMMO, D. R., BLACKMAN, R. R., WILSON, A. J., JR., AND FORESTER, J. (1971). Toxicity and distribution of Aroclor® 1254 in pink shrimp, *Penaeus duorarum*. *Mar. Biol. (Berlin)* **11**, 190–197.

MILLS, P. A., ONLEY, J. F., AND GAITHER, R. A. (1963). Rapid method for chlorinated pesticide residues in nonfatty foods. *J. Assoc. Agric. Chem.* **46**, 186–191.

U. S. FOOD AND DRUG ADMINISTRATION. (1970). "Pesticide Analytical Manual." U. S. Dept. of Health, Education and Welfare.

CONTRIBUTION NO. 174

AROCLOR® 1254, DDT AND DDD, AND DIELDRIN: ACCUMULATION AND LOSS BY AMERICAN OYSTERS *(CRASSOSTREA VIRGINICA)* EXPOSED CONTINUOUSLY FOR 56 WEEKS [1]

Patrick R. Parrish

U.S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, Florida

Separate populations of oysters were exposed continuously for 56 weeks to 0.01 μg/l of Aroclor® 1254, p,p' -DDT and DDD, or dieldrin and sampled at 8-week intervals for residues. Maximum concentrations based on body weight (μg/g) occurred after 8 weeks of exposure, but maximum concentrations based on absolute amount of toxicant accumulated (μg) occurred after 56 weeks of exposure. After 8 weeks, average whole-body residues (wet weight) from five oysters analyzed individually were: Aroclor 1254, 1.65 μg/g, 4.0 μg; DDT (and metabolites DDD and DDE), 0.46 μg/g, 1.0 μg; and dieldrin, 0.08 μg/g, 0.2 μg. After 56 weeks, residues were: Aroclor 1254, 0.89 μg/g, 25.7 μg; DDT and metabolites, 0.37 μg/g, 7.0 μg; and dieldrin, 0.03 μg/g, 0.6 μg. Seasonal patterns of accumulation and loss of the three toxicants were similar. Residues based on body weight (μg/g) decreased 45%-81% in early July and late October, apparently as the result of spawning, and increased following these periods. This shows that the life history of oysters must be considered when evaluating residue data from monitoring programs. Growth rate (height and in-water weight) of exposed oysters was not different from that of control oysters (Student's t-test; $a = 0.01$) Mortality was not significant in any group.

---

[1] Contribution No. 174, Gulf Breeze Environmental Research Laboratory.

® Registered trademark, Monsanto Company, St. Louis, MO. Mention of commercial products or trade names does not constitute endorsement by the Environmental Protection Agency.

Proc. National Shellfisheries Assoc.
64: 7. (1974).

Effects of Aroclor® 1254 on Laboratory-Reared Embryos and Fry of Sheepshead Minnows (*Cyprinodon variegatus*)

Steven C. Schimmel, David J. Hansen and Jerrold Forester

Made in United States of America
Reprinted from Transactions of the American Fisheries Society
Vol. 103, No. 3, July 1974
pp. 582–586

Effects of Aroclor® 1254 on Laboratory-Reared Embryos and Fry of Sheepshead Minnows (*Cyprinodon variegatus*)[1]

STEVEN C. SCHIMMEL, DAVID J. HANSEN AND JERROLD FORESTER
U. S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, Florida 32561
(Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon)

ABSTRACT

Eggs of the sheepshead minnow (*Cyprinodon variegatus*) were artificially fertilized and maintained at temperatures from 15 to 35 C and in salinities from 0 to 35‰ to determine efficient culture conditions. Fertilization was not affected by temperature or salinity ranges chosen, but hatching success was greatest (χ^2; $\alpha = 0.01$) at a temperature range of 24 to 35 C and a salinity range of 15 to 30‰.

Artificially fertilized sheepshead minnow eggs were exposed to logarithmic concentrations of Aroclor 1254 (10.0 to 0.1 μg/liter) in seawater averaging 30 C and 24‰ in a flow-through bioassay. Fertilization was not affected but significantly fewer embryos developed in the 10.0 μg/liter concentration, and fewer fry survived in concentrations greater than 0.1 μg/liter. Fry were more susceptible to Aroclor 1254 than were embryos, juveniles, or adults.

Polychlorinated biphenyls (PCB's) occur in estuaries in many states (Butler 1973), and the occurrence of one, Aroclor 1254, in nearby Escambia Bay, Florida and its acute toxicity to estuarine animals has been documented (Duke, Lowe and Wilson 1970). Hansen, Parrish and Lowe (1971) found 5 μg/liter of Aroclor 1254 toxic to the juvenile estuarine fishes, pinfish (*Lagodon rhomboides*) and spot (*Leistomus xanthurus*), in 14- to 45-day bioassays. Because Escambia Bay is a nursery ground for many marine species of fish, it is important to determine the effect of Aroclor 1254 on the early life stages of these fish.

The sheepshead minnow (*Cyprinodon variegatus*) is found in brackish waters from Cape Cod, Massachusetts to Brownsville, Texas (Hildebrand 1917). It is important in estuarine food chains as a voracious omnivore and as food for predators such as croakers (*Micropogon undulatus*) and spotted seatrout (*Cynoscion nebulosus*) (Darnell 1958). The size, hardiness, high fecundity, and generation time of the sheepshead minnow make it a nearly ideal laboratory test fish.

In this research, we determined water temperatures and salinities suitable for the culture of embryos and fry of the sheepshead minnow and observed the effect of Aroclor 1254 in water on the various life stages of this fish.

METHODS AND MATERIALS

Adult fish were acclimated for 1 wk in salt water averaging 25 C and 20‰. The following week, acclimated female fish were given three intraperitoneal injections of 50 I.U. human chorionic gonatrophic hormone at 48-hr intervals to induce egg maturation. Over 70% of the fish produced viable eggs. Eggs were manually stripped and deposited in 40 ml of filtered seawater. Only large, round, clear eggs were selected for fertilization. Testes from 7–10 acclimated males were excised, macerated in 20 ml of filtered seawater and mixed with eggs in a 100 ml beaker. Thirty minutes were allowed for fertilization. Each experiment lasted as long as was required for embryos to hatch (4 to 15 days) plus an additional 2 wk to determine survival of fry. *Artemia salina* nauplii, with no detectable levels of PCB, were fed to the fry daily.

We investigated the effect of temperature on hatching by withdrawing 25 eggs from the 100-ml beaker with a wide-bore pipette and placing them in two 1-liter dishes containing 25 C, 20‰ seawater. A pair of dishes was partially submerged in a bath at 15, 20, 25, 30, or 40 C in one temperature

[1] Contribution No. 175, Gulf Breeze Environmental Research Laboratory.

® Registered trademark, Monsanto Co., St. Louis, Mo. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

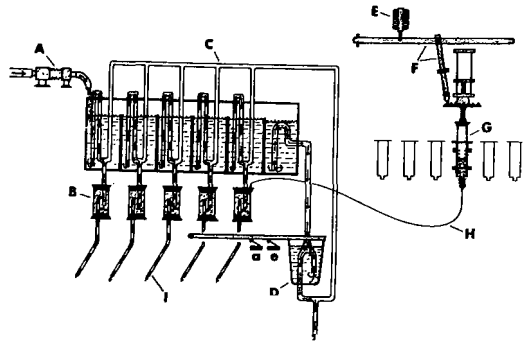


FIGURE 1.—Dosing apparatus: A—oscillating pump; a—pump micro-switch; B—mixing bottles; C—vacuum lines; D—receiving bucket; E—solenoid; e—solenoid micro-switch; F—injector lever apparatus; G—50cc. syringe; H—toxicant delivery tubing; I—water delivery tube.

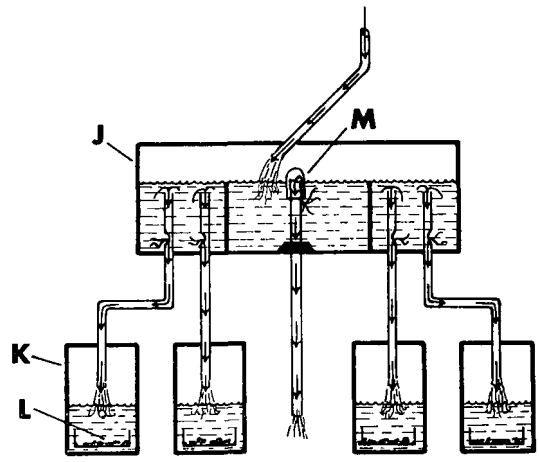


FIGURE 2.—Distribution apparatus: J—distribution box; K—egg/larvae trays; L—Petri dishes; M—siphon.

test and 22, 24, 26, or 28 C in another. One hour later, when temperatures in the dishes equaled the bath temperature, fertilization was confirmed microscopically. The criterion for fertilization was cleavage. Dishes were checked daily during the 3-wk static tests and dead or nonfertile eggs and larvae were removed. The criteria for embryo death was an opaque, white, fungal growth and for the larvae, a white coloration of the trunk musculature. Temperatures were monitored continuously and were within 1 C of the desired temperatures. Salinity alterations due to evaporation were compensated for daily by addition of distilled water.

Fertilization procedures for the salinity study were identical to those of the temperature study except that the number of eggs in each salinity varied from 35 to 75, depending on egg availability, and the temperature was 30 C. Filtered seawater from Santa Rosa Sound, Florida was diluted to give salinities of 0, 5, 10, 15, 20, 25, 30, and 35‰. The 30 and 35‰ concentrations were attained by adding Rila® salts to the water from the Sound. Salinities, checked daily with a TS® refractometer, were within 2‰ of the desired level.

An Aroclor 1254, flow-through bioassay was accomplished using culture conditions determined suitable in the temperature and salinity tests. Eggs were fertilized in control water and placed in four Petri dishes, 20 eggs per dish, in each PCB concentration and control. Fertilization was confirmed after 1 hr. Temperatures in both tests averaged 29 C (range 27–31 C) and salinity averaged 24‰ (range 16–32‰). The salinity fluctuated with that of Santa Rosa Sound. The toxicant dosing system used in this test was a modification of the apparatus of Brungs and Mount (1970). In our system, the toxicant and carrier were injected into the delivery tube leading to each exposure tank (Fig. 1). Our apparatus allowed us to retain the advantages of the Mount and Brungs (1967) dosing apparatus and to select any concentration of toxicant while maintaining the same concentration of carrier in each toxicant concentration.

Seawater used in this bioassay was pumped from Santa Rosa Sound into a constant head box in the laboratory. An oscillating pump (1A), regulated by a rheostat, pumped water from the head box through a 20-μ-pore polypropylene filter and into the compartments in the dosing apparatus. After all compartments were filled, the self-starting siphon in the last compartment emptied into a receiving bucket (1D). The weight of the bucket being filled operated two micro-switches. One switch

® Registered trademark, Rila Products, Teaneck, N. J.
® Registered trademark, American Optical Corp., Buffalo, N. Y.

TABLE 1.—*Effect of temperature on fertility, time-to-hatch, hatch success and survival of fry for 2 wk following hatching of sheepshead minnows* (Cyprinodon variegatus). *Temperatures varied ± 0.5 C; salinity averaged 20‰, ± 2‰*

Temperature (°C)	Eggs		Hatching		Fry survival %
	Number	Fertile %	Days #	Survival %	
15	50	92	No hatch	0[b]	0[b]
20	50	98	No hatch	0[b]	0[b]
22	50	100	15.0	28.0[b]	100
24	50	96	9.0	50.0	100
25	50	98	7.0	83.7	80
26	25[a]	92	7.0	78.0	100
28	50	90	6.0	55.5	92
30	50	94	4.5	82.9	90
35	50	94	4.0	70.2	100
40	50	94	4.0	8.5[b]	50

[a] Loss of 25 eggs due to spillage.
[b] Significantly less than the greatest fertility or survival (χ^2; $\alpha = 0.01$).

TABLE 2.—*Effect of salinity on fertility, time-to-hatch, hatch success and survival of fry for 2-wk following hatching of sheepshead minnow* (Cyprinodon variegatus). *Temperature 30 ± 2 C; salinity varied ± 1‰*

Salinity ‰	Eggs		Hatching		Fry survival %
	Number	Fertile %	Days #	Survival %	
0	60	35	No hatch	0[a]	0
5	35	46	7.0	12[a]	50
10	75	35	7.0	35[a]	89
15	35	51	5.5	67	92
20	75	44	6.0	67	100
25	35	54	5.5	53	90
30	75	49	5.5	73	93
35	35	37	6.0	30[a]	50

[a] Significantly less than the greatest fertility or survival (χ^2; $\alpha = 0.01$).

(1a) shut off the oscillating pump and the other (1e) activated a solenoid (1E) that raised the lever of the injection apparatus (1F). This rising lever was connected to gears that forced the barrels of six 50-ml syringes (1G) equal distances. Mixing bottles (1B) received injections from a control syringe containing the carrier (polyethylene glycol 200).

Stock solutions of the carrier and Aroclor 1254 were mixed to give concentrations 0.1, 0.32, 1.0, 3.2 and 10.0 μg of Aroclor per liter of water. The water then flowed to the distribution boxes (Fig. 2) containing one large and four small compartments, each with a standpipe or siphon. Each compartment filled completely during each cycle and the siphon delivered water to the aquarium in which adults and juvenile fish were held. Water remaining in the four small compartments flowed slowly out through submerged holes in the standpipes into each of four containers holding eggs or fry. Each container received 100 ml of water per cycle. The number of cycles per day ranged from 100 to 140 and was sufficient to replace water in each egg container at least three times each day.

Aroclor 1254 concentrations in test and control water were determined weekly by gas chromatography. Methods were the same as those of Nimmo et al. (1971), except that an OV-101 column was used and all peak heights were averaged for PCB quantification. Measured PCB concentrations in test water were typically 35% to 60% of nominal concentrations; control water contained no detectable PCB (< .03 μg/liter). Recovery efficiency of Aroclor 1254 was greater than 60%. Measured concentrations were not corrected for percentage recovery.

Dissolved oxygen was determined weekly by the modified Winkler method (Strickland and Parsons 1968). Concentrations seemed adequate and above 50% saturation.

The chi-square (χ^2; $\alpha = 0.01$) test was used in the statistical analysis of the data. In temperature and salinity studies the maximum positive response was compared with all other responses to determine the most efficient culture conditions. Data in the two temperature experiments were analyzed separately. Probit analysis was applied to the data to determine the LC50 of the Aroclor 1254.

RESULTS AND DISCUSSION

Temperature influenced the number of days required for hatching (time-to-hatch) and affected the survival of embryos and fry of the sheepshead minnow (Table 1). Sheepshead minnow eggs hatched at water temperatures from 22–40 C but none hatched at lower temperatures. Hatching success was greatest at temperatures ranging from 24 to 35 C. Fry survival did not differ in the range of 22 to 35 C. The longest time-to-hatch was 15 days at 22 C, and decreased rapidly, leveling off at about 4 days at temperatures ranging from 30 to 40 C.

Salinity affected survival of embryos and fry but did not affect time-to-hatch (Table

TABLE 3.—*Effect of Aroclor® 1254 on fertility, time-to-hatch, hatch success and survival of fry for 2 wk following hatching of sheepshead minnow* (Cyprinodon variegatus). *Temperature averaged 30 C (range 27–31 C); salinity averaged 24‰ (range 16–32‰)*

Concentration (μg/liter)		Eggs		Hatching		Fry
Nominal	Measured	Number	Fertile %	Days #	Survival %	Survival %
Control	< 0.03 μg/liter	160	86	7.0	79	89
0.1	0.06	160	86	6.5	69	95
0.32	0.16	160	86	7.0	73	62[a]
1.0	0.36	160	91	7.0	82	63[a]
3.2	1.04	160	85	7.0	75	40[a]
10.0	3.48	160	91	6.5	57[a]	8[a]

[a] Significantly different from control fish (χ^2; $\alpha = 0.01$).

2). Survival to hatching was greater at salinities from 15 to 30‰ and fry survival did not differ in that range. Under natural conditions, adult and juvenile sheepshead minnows occur in a wide salinity range (Simpson and Gunter 1956). Our laboratory data show that embryos and fry also can survive in a wide salinity range.

Aroclor 1254 affected the survival of both embryos and fry of the sheepshead minnow but had no effect on time-to-hatch. Embryos developed and hatched at all PCB concentrations, but hatching succes at 10 μg/liter was significantly less than that of control eggs (Table 3). After the eggs hatched, survival of fry was significantly less than control fry at all PCB concentrations except 0.1 μg/liter. Mortality increased with an increasing concentration of PCB. Three-week LC50 in exposed embryos and fry was estimated at 0.93 μg/liter (S.E. = 0.17 μg/liter). Fry did not die immediately after hatching but were killed over most of the 2-wk period of post hatch exposure. Many of the dying fish developed fin rot as previously described in other PCB exposed fishes (Hansen et al. 1971).

Aroclor 1254 was more toxic to fry of sheepshead minnows than it was to juveniles, adults or to the fertilization of eggs (Table 4). In 3-wk exposures to the same concentrations of this PCB, mortality of the juveniles was significantly greater (24%) in the 10 μg/liter concentration. Adult fish were not killed in a 3-wk exposure but became lethargic and exhibited fin rot. The concentration of PCB in the fry compared to that in the water (concentration factor) was nearly identical with that of adult fish. Concentration factors in fry ranged from 1.6 to 3.2×10^4 and those for adults ranged from 1.1 to 3.2×10^4. A 24-hr static test in 10.0 μg/liter PCB showed no inhibition of fertilization of sheepshead minnow eggs. The greater sensitivity of early life stages of this fish to Aroclor 1254 stresses the need for bioassays designed to assess the effects of such chemical pollutants on these stages.

The relationship between Aroclor 1254 contamination in Escambia Bay, Florida and the effects of the PCB on various life stages of *C. variegatus* in our bioassay is not clear. Water and fish samples (12 species not including *C. variegatus*) were taken 1 to 28 mo after the reported spill that led to the Bay's contamination. PCB residues, found in 10 of the 37 water samples, ranged from nondetectable (< 0.03 μg/liter) to 0.07 μg/liter. Concentrations in the Bay water were less than those found lethal in our bioassays. Fish in the estuary can accumulate the Aroclor from the water, food, and sediment. Concentrations in the fish ranged from 0.29 to 20 μg/g (average 4.0 μg/g) whole body wet weight. Adult sheepshead minnows averaged 9.3 μg/g when exposed to 0.32 μg/liter PCB

TABLE 4.—*Relative susceptibility of various life stages of sheepshead minnows* (Cyprinodon variegatus) *to Aroclor® 1254 in a flow-through system. Criteria are infertility of eggs and death of embryos, fry, juveniles, and adults*

Life stage	Exposure (days)	Concentration (μg/liter) Maximum not affecting	Minimum affecting
Egg fertilization[a]	1	10.0	—
Embryos	7	3.2	10.0
Fry	21	0.1	0.32
Juveniles	21	3.2	10.0
Adults	21	10.0	—

[a] 24-hr static test.

for 4 wk in the laboratory (Hansen, Schimmel and Forester In press). Eggs produced by these exposed fish were fertilized and although adults seemed normal and embryos appeared to develop normally, survival of fry in the first week after hatching was significantly reduced compared to that of unexposed fry.

ACKNOWLEDGMENTS

We thank Gary Cook and Dennis Knight for chemical analyses of water samples from the Aroclor 1254 test and Steve Foss for preparing the illustrations.

LITERATURE CITED

BRUNGS, W. A., AND D. I. MOUNT. 1970. A water delivery system for small fish-holding tanks. Trans. Amer. Fish. Soc. 99(4): 799–802.

BUTLER, P. A. 1973. Organochlorine residues in estuarine mollusks, 1965–1972. National Pesticide Monitoring Program. Pestic. Monit. J. 6(4): 238–362.

DARNELL, R. M. 1958. Food habits of fishes and larger invertebrates of Lake Pontchartrain, Louisiana, an estuarine community. Publ. Inst. Mar. Sci. Univ. Tex. 5: 354–416.

DUKE, T. W., J. I. LOWE, AND A. J. WILSON, JR. 1970. A polychlorinated biphenyl (Aroclor 1254®) in the water, sediment, and biota of Escambia Bay, Florida. Bull. Environ. Contam. Toxicol. 6: 171–180.

HANSEN, D. J., P. R. PARRISH, J. I. LOWE, A. J. WILSON, JR., AND P. D. WILSON. 1971. Chronic toxicity, uptake, and retention of Aroclor® 1254 in two estuarine fishes. Bull. Environ. Contam. Toxicol. 6: 113–119.

———, S. C. SCHIMMEL, AND J. FORESTER. In press. Aroclor® 1254 in eggs of sheepshead minnows: Effect on fertilization success and survival of embryos and fry. Proc. 26th Annu. Conf. Southeast. Ass. Game Fish. Comm.

HILDEBRAND, S. F. 1917. Notes on the life history of the minnows (*Gambusia affinis* and *Cyprinodon variegatus*). Rep. U. S. Comm. Fish., 1917: 1–14.

MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21–29.

NIMMO, D. R., R. R. BLACKMAN, A. J. WILSON, JR., AND J. FORESTER. 1971. Toxicity and distribution of Aroclor® 1254 in the pink shrimp *Penaeus duorarum*. Mar. Biol. (Berlin) 11(3): 191–197.

SIMPSON, D. G., AND G. GUNTER. 1956. Notes on habitats, systematic characters and life histories of Texas salt water cyprinodontes. Tulane Stud. Zool. 4: 115–134.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. Fish. Res. Board Can. Bull. 167: 21–26.

CONTRIBUTION NO. 176

PROCEEDINGS
OF THE
NATIONAL SHELLFISHERIES ASSOCIATION

OFFICIAL PUBLICATION OF THE NATIONAL
SHELLFISHERIES ASSOCIATION;
AN ANNUAL JOURNAL DEVOTED TO
SHELLFISHERY BIOLOGY

VOLUME 64

Published for the National Shellfisheries Association, Inc. by
Economy Printing Co., Inc., Easton, Maryland

JUNE 1974

Proceedings of the National Shellfisheries Association
Volume 64 1974

TRENDS IN PESTICIDE RESIDUES IN SHELLFISH

Philip A. Butler [1]
U.S. ENVIROMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES PROGRAMS
GULF BREEZE, FLORIDA

ABSTRACT

The National Estuarine Monitoring Program, a cooperative effort between the State and Federal Governments, collected and analyzed shellfish samples for persistent synthetic pesticides at monthly intervals during the years 1965-1972 in 15 coastal states. The recently completed study of the 8000-plus analyses demonstrates that: (1) the residues found, primarily DDT and its metabolites, were universally too low to have human health significance, (2) areas of both high and low residues were clearly defined geographically, (3) in some areas there has been a trend towards a wider distribution of smaller residues, and (4) there has been a marked decline generally in DDT residues since 1968 when peak levels in molluscs were detected.

INTRODUCTION

During the period 1965-1972, samples of oysters and other bivalve molluscs were collected at monthly intervals at about 180 estuarine locations to determine the incidence and magnitude of pesticide residues along the Atlantic, Pacific and Gulf of Mexico coasts. More than 8000 samples were screened for the presence of 12 of the more persistent chlorinated pesticides. In the later years, chlorinated biphenyls or PCB's were included in the analytical procedures. This report briefly summarizes the implications of some of the principal findings. A detailed report of the sample collections and analyses has been published recently (Butler, 1973).

BACKGROUND

Oysters exposed to varying concentrations of pesticides under controlled conditions in the laboratory demonstrate their sensitivity to these pollutants. In aquaria with flowing unfiltered seawater, for example, as little as 1.0μg/kg (ppb) of DDT inhibits oyster shell growth by about 20 percent in a 4-day period. One μ g/g (ppm) inhibits shell deposition completely at water temperatures of about 17-20 C (62-68 F) (Butler, 1966).

Concentrations as high as these were not anticipated in the natural environment and so it was of importance in the development of a proposed monitoring program to discover that oysters were sensitive to the presence of DDT in ambient water at levels as low as 10×10^{-12} (10 parts per trillion). Exposure of oysters for 7 days to this extremely low concentration led to the formation of DDT residues in the tissues of about 70 μ g/kg, a biological magnification of 70,000x. DDT levels of this magnitude might be anticipated in the marine environment since it is less than the solubility of DDT in water. Further laboratory experiments demonstrated that oysters and other molluscs would be reliable as biological tools to monitor estuarine ecosystems because of this tendency to concentrate persistent chemicals (Table 1).

Additional experimentation showed that contaminated oysters cleansed themselves of resi-

[1] Contribution No. 176, Gulf Breeze Environmental Research Laboratory, U.S. Environmental Protection Agency, Gulf Breeze, FL 32561.

TABLE 1. *Uptake of DDT by eastern oysters maintained in flowing seawater. Exposure period 7-15 days in different tests. (Butler, 1968)*

Concentration in water	Residue in oyster	Biological magnification
(µg/kg) or (ppb)	(µg/g) or (ppm)	(x1000)
10.0	150.0	15
1.0	30.0	30
0.1	7.0	70
0.01	0.72	70
0.0001	0.07	0
control	0.06	

dues when returned to clean water. The disappearance time or biological half-life of the residues in molluscs was short; a matter of days as compared to months or years in fish and other vertebrates. Consequently, when oysters were sampled at about 30-day intervals, it was possible to estimate when pollution entered the estuary and thus gain some insight as to its source.

FINDINGS

Analyses of monthly collections of oysters in an estuarine complex near Pensacola, Florida revealed a seasonal pattern of DDT residues later found to be typical of estuaries in many coastal areas. In the period February through May there was a gradual increase in residue magnitude to a seasonal high in late spring. This was followed by a decline to background' levels typical of the remainder of the year. It seems reasonble to assume that this picture results primarily from the occurrence of seasonal rains and surface water run-off which carry soil eroded from agricultural lands through the river basin and into the estuary. In contrast to this picture, there was a second seasonal peak of DDT residues during the winter months in samples from the South Texas coast. This bimodal cycle probably reflected the double cropping of farm lands and the associated multiple applications of pesticides in this sub-tropical area.

A more obvious result of the seasonal agricultural use of DDT was indicated by residues in oysters monitored in the Caloosahatchee River Basin in southwest Florida. Here, peaks in DDT residues in oysters appeared soon after the seasonal application of DDT to maturing crops of sweetcorn and sugarcane. In 1967-68, the early spring residues were nearly ten times the level of residues found during the other months of the two-year monitor period (Fig. 1). In some instances, seasonal and annual patterns of pesticide accumulation in estuarine oysters could be associated with the dumping of industrial effluents or with the control of noxious insect populations. The declining use of DDT in stable-fly control in northeast Florida, for example, was clearly indicated by annual decreases in DDT residues in local oysters in the period 1965-1968. DDT residues were no longer

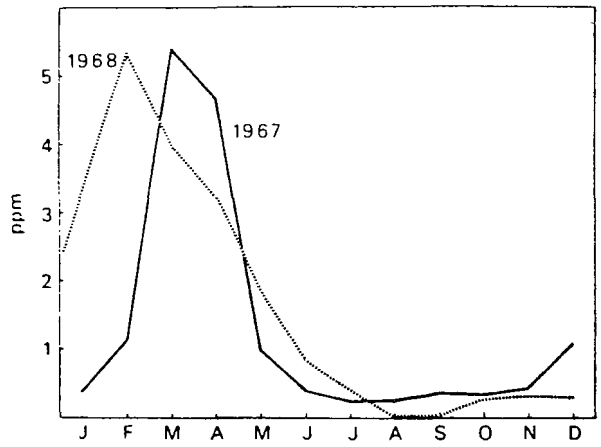


FIG. 1. *DDT residues in the eastern oyster from the Caloosahatchee River Basin, Lee County, Fla., by month of collection (Butler, 1973).*

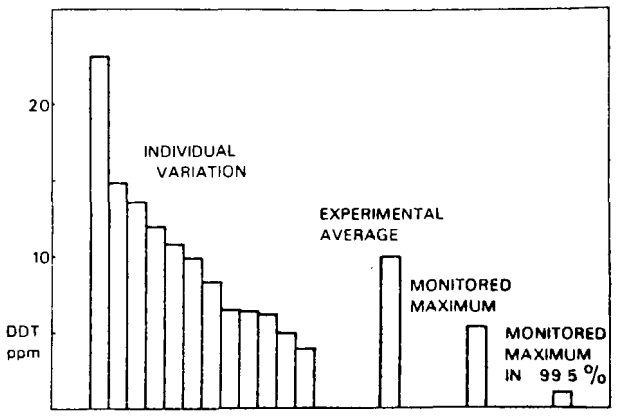


FIG. 2. *DDT residues in experimental and field-collected oysters in the period 1965-1972. See text for explanation.*

identified after the substitution of methoxychlor, a less persistent compound, for fly control in 1969. More importantly, methoxychlor was not detected in the monitor samples in succeeding years.

The significance of DDT residues in field samples may be judged to some extent by the magnitude of DDT residues observed in laboratory experiments. Market-size eastern oysters were exposed to 1.0 μ g/kg of DDT in flowing seawater for a 10-day period and then 12 were individually analyzed. The sum of DDT and its metabolites found as residues ranged from a low of 3.9 to a high of 23.2 μg/g with an arithmetic average of 10.1 μg/g (ppm) for the group. This value is about twice the largest DDT residue observed (5.39 ppm) in all of the molluscan samples collected in the 7-year monitoring period. It should be noted further that DDT residues were less than 1.0 μg/g in 99.5 percent of the 8000+ monitoring samples analyzed. It appears that despite the build-up of large residues in higher carnivores DDT pollution of estuarine waters generally has been at levels below 1.0 μg/kg (Fig. 2).

It must be emphasized that the observed levels of DDT residues in molluscs were too low to have human health significance or to have demonstrable effects on the oysters themselves. Only in isolated areas were DDT residues high enough to indicate that some elements of the estuarine fauna might have been damaged by the magnification and accumulation of DDT residues in the food web.

With these observations in mind, the overall findings of the monitoring data may be summarized geographically. The lowest average incidences of DDT positive samples were found, in order, in Washington, Georgia and Maine. Highest incidence rates were observed in New Jersey, Alabama, North Carolina and California. However, the *largest* residues of DDT and its metabolites were found in samples collected in the estuaries of Florida, California and Texas.

There has been a well-defined but gradual decline in both the incidence and magnitude of DDT residues in oysters during the monitoring period in most areas. In some coastal estuaries this trend is obscured by the lack of uniformity in the timing of sample collections or by variations in the kind of mulluscs collected. Despite erratic fluctuations in magnitude and the fact that individual residues were never very high, it is clear that DDT pollution in estuaries was at peak levels in 1966-1967 and gradually declined thereafter. This 1966 peaking in the magnitude of residue data parallels, not unexpectedly, the findings of peak DDT levels in fresh water monitoring samples in 1966 followed by sharp declines in 1967 and 1968 (Lichtenberg, et al., 1970).

Data demonstrating the overall decline in the magnitude of DDT residues in estuarine molluscs are summarized in Fig. 3. This dia-

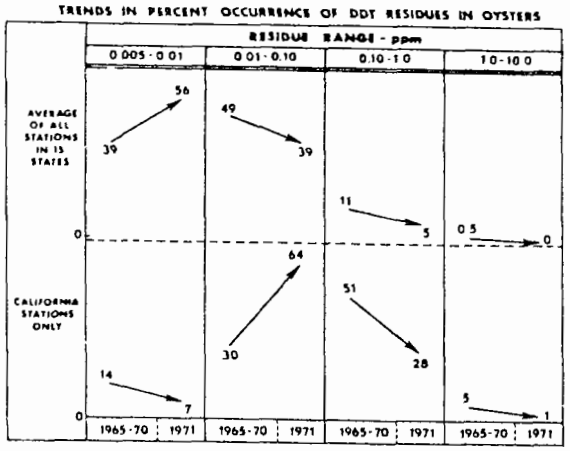


FIG. 3. *Percentage occurrence of DDT residues in estuarine bivalves in the period 1965-1970 as compared to 1971. Data summarize about 7000 analyses of more than 75,000 animals. See text for explanation.*

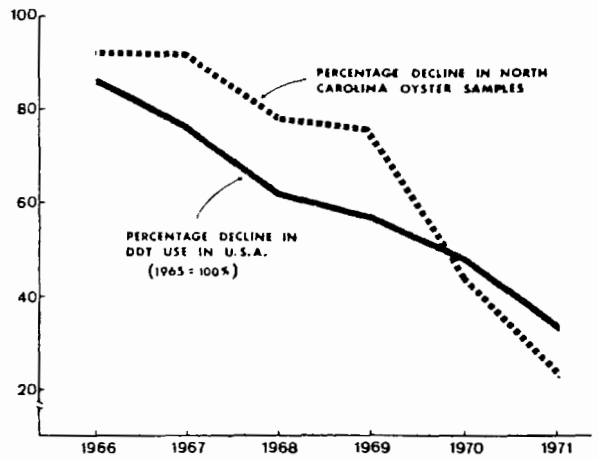


FIG. 4. *Percentage decline in DDT residues of more than 10 μ g/kg in North Carolina oysters as compared to the decline in the consumption of DDT in the entire United States in the period 1965-1971.*

gram shows that, in the period 1965-1970, 39% of all samples contained negligible DDT residues, less than 0.01 ppm. while in 1971 this value increased to 56%. Conversely, in these same years the percentage of samples containing larger residues declined sharply. In California and a few other isolated locations there was an exception to this generalized picture in that the number of samples with DDT residues in the 0.01-0.10 ppm range increased during the monitoring period but the percentage of samples with high residues decreased sharply as in other coastal areas. Apparently in these drainage basins, there was an increased cycling of DDT in the trophic web accompanied by a diminution of the amount present in individual animals. In other words, DDT residues were distributed more thinly among more members of the biota.

At ten monitoring stations in North Carolina, where the continuity of sample collections was especially good, the data provide a clear picture of annual trends in DDT pollution levels. Fig. 4 shows the decline in the percentage of samples having measurable DDT residues as compared with the approximate percentage decline in the domestic use of DDT throughout the United States after 1965. DDT supplies in that year have been arbitarily designated as 100% for the basis of this comparison (USDA, 1967-72). These data demonstrate the progressive loss of residual DDT from at least one segment of an estuarine ecosystem following the generalized curtailment in the agricultural use of DDT, and controvert the widespread belief that environmental problems with DDT would be longlasting regardless of how soon its use was terminated.

SUMMARY

These monitoring data show that the domestic use of DDT resulted in only nominal residues in estuarine molluscs in the United States in the period 1965-1972. By extrapolation from laboratory data, we may infer that these residues were too small to have a deleterious effect on the growth and productivity of estuarine bivalves. Despite the chemical stability of DDT, curtailment in its use was almost immediately reflected by declines in the magnitude of residues in estuarine molluscs. The data establish a baseline for levels of DDT pollution in estuaries during the monitored period, and suggest that despite the stability of a synthetic organic compound it may become biologically unavailable soon after its widespread use is discontinued.

LITERATURE CITED

Butler, P. A. 1966. Pesticides in the marine environment. J. Appl. Ecol. 3(Suppl.):253-259.

Butler, P. A. 1968. Pesticide residues in marine molluscs. *In* Proc. Natl. Symp. Estuarine Pollut., Stanford Univ., Stanford, Calif. 1967. p. 107-121.

Butler, P. A. 1973. Organochlorine residues in estuarine molluscs, 1965-1972 — National Pesticide Monitoring Program. Pestic. Monit. J. 6: 238-362.

Lichtenberg, J. L., J. W. Eichelberger, R. C. Dressman and J. E. Longbottom, 1970. Pesticides in the surface waters of the United States — a 5-year summary, 1964-68. Pestic. Monit. J. 4:71-86.

USDA, Agricultural Stabilization and Conservation Service. The Pesticide Review. 1967-1972. Washington, D. C.

CONTRIBUTION NO. 177

Reprinted from the proceedings of the 27th Annual Conference of the Southeastern Association of Game and Fish Commissioners, 1973.

AROCLOR® 1254 IN EGGS OF SHEEPSHEAD MINNOWS: EFFECT ON FERTILIZATION SUCCESS AND SURVIVAL OF EMBRYOS AND FRY[1]

David J. Hansen, Steven C. Schimmel and Jerrold Forester
U. S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, Florida 32561
(Associate Laboratory of the
National Environmental Research Center, Corvallis, Oregon)

ABSTRACT

The effect of the polychlorinated biphenyl (PCB), Aroclor 1254, in eggs of the sheepshead minnow, *Cyprinodon variegatus*, on fertilization success and survival of embryos and fry was investigated. Adult fish were exposed for four weeks to 0.1, 0.32, 1.0, 3.2 or 10.0 ug/*l* of PCB, then injected twice with 50 IU of human chorionic gonadotrophin to stimulate egg production. The eggs were fertilized, placed in PCB-free flowing seawater and observed for mortality. Fertilization success was unimpaired by concentrations in eggs as high as 201 ug/g but survival of embryos and fry was reduced. Usually, fry from eggs containing 7.0 ug/g or more began dying 24-48 hours after hatching. If this PCB affects other species similarly, then populations of fish that presently have comparable concentrations in their eggs may be endangered.

INTRODUCTION

Polychlorinated biphenyls (PCB's) have been found frequently in estuarine organisms from many states (Butler, 1973) and in an estuary near the Gulf Breeze Laboratory (Duke et al., 1970). PCB's in seawater are toxic to and accumulated by juvenile shrimp, crabs, oysters and fishes (Nimmo, et al., 1971; Lowe, et al., 1972 and Hansen, et al., 1971). The relationship between the amount accumulated by fish and subsequent effects is poorly understood. However, PCB's in eggs may decrease fertility and survival in early stages of embryonic development in Atlantic salmon, *Salmo salar* (Johannsson et al., 1970), and PCB's have been implicated in poor reproductive success of striped bass, *Morone saxatilis* (Anonymous, 1971). Because reproductive success with both fishes varied, the exact relationship of success to concentration of PCB in eggs remains unclear.

Our study was conducted to determine the effect of one PCB, Aroclor 1254, on fertilization success of eggs of sheepshead minnows, *Cyprinodon variegatus*, and on survival of embryos and fry. Aroclor 1254 was selected because we found eggs from striped bass that exhibited decreased reproductive success contained a PCB whose chormatograms closely resembled Aroclor 1254. Sheepshead minnows were selected because they can be readily exposed in the laboratory and reproductive success is excellent.

MATERIALS AND METHODS

Test fish

Adult sheepshead minnows were seined from ponds on laboratory grounds and acclimated to laboratory conditions for four days before exposure. During

[1]Contribution No. 177, Gulf Breeze Environmental Research Laboratory. ®Registered trademark, Monsanto Company, St. Louis, Mo. Mention of commercial products or trade names does not constitute endorsement by the Environmental Protection Agency.

acclimation, mortality was less than 1% and no abnormal behavior was observed. Females averaged 42.5 mm standard length, range 35-52 mm, and males averaged 42.8 mm, range 35-52 mm. During acclimation and exposure, fish were fed commercial fish food that contained no detectable PCB (0.01 ug/g).

Adult exposure

We exposed 20 female and 10 male fish in aquaria containing none, 0.1, 0.32, 1.0, or 3.2 ug/l of Aroclor 1254 and exposed 25 females and 15 males to 10 ug/l for four weeks in an intermittent-flow bioassay. The apparatus used was a modification of that of Brungs and Mount (1970). In our modification, Aroclor 1254 and carrier, polyethylene glycol 200, were injected into seawater each time the apparatus cycled. Each cycle siphoned water to six 80l test aquaria. The injection device was operated by a solenoid that raised a lever each cycle turning gears on six injectors and pushing the plungers of six 50cc syringes. Each of the approximately 150 daily cycles delivered 1.5l of filtered 30C seawater, 11 ug of carrier and appropriate amounts of PCB to each aquarium. Water and carrier without PCB were delivered to the control aquarium. Salinity of water averaged 17 o/oo, range 5 to 28 o/oo.

Egg fertilization, embryo and fry survival

The effect of Aroclor 1254 in eggs was determined by enhancing egg production in exposed fish by hormonal injection, fertilizing the eggs artificially and monitoring their development in flowing PCB-free seawater. Female sheepshead minnows were injected intraperitoneally with 50 I. U. human chorionic gonadotrophic hormone on exposure-days 25 and 27. On day 28, eggs were stripped manually from five females from each aquarium and those from each female placed in individual beakers containing 40 ml of filtered 30C seawater. Ninety-three of 96 females that survived produced eggs. Eggs from a female were fertilized with excised macerated testes from a male from the same aquarium. In addition, eggs from five control fish and two fish surviving exposure to 10 ug/l PCB were fertilized by males exposed to 1.0 ug/l. Twenty-five eggs from each fish were placed in Petri dishes to which a nine cm high collar of 500u nitex mesh was glued. Dishes were submerged 7 cm in the 80l aquarium which received approximately 225l of filtered PCB-free seawater per day; average salinity was 18 o/oo, range 10 - 27 o/oo. Success of fertilization was confirmed by checking microscopically for cleavage 1.5 hours after fertilization. Thereafter, dishes were checked daily to determine survival of embryos and fry. Dishes remained in the aquarium for 34 days. Fry were fed brine shrimp nauplii or dry commercial fish food daily.

Chemical analyses

Concentrations of Aroclor 1254 in water, eggs and fish were determined by electron capture gas-chromatography. Unfiltered water samples from each aquarium were analyzed weekly during the four-week exposures of adults. At the end of the adult exposure, concentrations were determined in the fertilized eggs from each fish and in surviving adult males and females. Also, fry that hatched from these eggs and survived for four weeks in PCB-free water were analyzed for Aroclor 1254 content. Analytical methods for water, eggs and fish were the same as those of Nimmo et al. (1971), except than an OV-101 column was used and all peak heights were summed for PCB quantification. Recovery efficiency of Aroclor 1254 exceeded 80%. Measured concentrations were not corrected for percentage recovery.

[2]Manufactured by George Frazer, 4528 Pitt Street, Duluth, Minn. 55804.

Statistical analysis

Probit analysis was used to determine whether increasing concentration of PCB in eggs increased the effect on fertilization success and on survival of embryos and fry. The χ^2 test for independent samples was used to compare data for eggs from individual unexposed and exposed fish. Differences were considered real at $\alpha = 0.05$ for probit analysis and $\alpha = 0.01$ for χ^2 tests.

RESULTS AND DISCUSSION

Aroclor 1254 in water was toxic to and accumulated by adult sheepshead minnows exposed for four weeks (Table 1). Mortality of fish was negligible, except in the aquarium receiving 10 ug/*l*. Dying fish in this aquarium typically became lethargic, ceased feeding, and some developed fin rot. Fish accumulated the PCB in direct proportion to the concentration in the water and concentrations in fish ranged from 15,000 to 30,000 X the nominal concentration in the water. Concentrations in males and females were similar. Concentrations of the chemical in eggs from exposed adult fish were proportional to the concentrations in the fish and concentrations in female fish were 1.8 to 2.3 times greater than the concentrations in their eggs. The PCB exposure apparently did not alter the percentage of females producing eggs or their fecundity.

Fewer embryos and fry from eggs of exposed fish survived than did embryos and fry from eggs of control fish (Table 2). The percentage of the eggs fertilized was not affected, but survival of embryos to hatching was less in eggs from fish exposed to 10 ug/*l*. Survival rate of fry in the first week following hatching was less in eggs from fish exposed to 0.32 to 10.0 ug/*l* than in eggs from unexposed fish. The estimated LC50 was 6.1 ug/g; 95 percent confidence limit equals 3.5 to 11.8 ug/g. Fry typically began to die one or two days after hatching, about the time they started feeding. If fry survived the first week, there seemed to be no additional mortalities related to PCB during three weeks of additional observation. Concentrations of Aroclor 1254 in surviving fry were similar, 0.26-0.56 ug/g, and not proportional to concentrations in eggs.

Embryo survival decreased at the highest concentration of PCB in eggs and fry survival decreased with increasing concentration of PCB in eggs. The amount in eggs was critical because it was the sole source of PCB for the embryos and fry reared in PCB-free water. PCB in milt was probably not critical to fertility and survival because when eggs from control fish were fertilized with milt from either control or 1.0 ug/*l* exposed males, survival rate of embryos and fry was not altered (Table 2). Survival rate of fry hatched from eggs containing 7.0 ug/g or more of PCB was significantly less than the lowest survival rate of eggs from any of the five control fish (Table 3).

If the effect of PCB in eggs of other fishes is similar to that found with sheepshead minnows — and we have no data to support this view — then variations in published information concerning the chemicals relation to spawning success could be explained. Atlantic salmon eggs containing up to 1.9 ug/g of PCB had decreased fertility and survival of early embryos, but survival of late embryos and sac fry was unimpaired (Johannsson et al., 1970). Chesapeake Bay striped bass eggs containing PCB's had decreased fertility and survival of newly hatched fry (Anonymous, 1971). Our analysis of eggs from eleven striped bass from the Eastern shore of Chesapeake Bay showed that the eggs contained about 2.5 to 8.7 ug/g of a PCB resembling Aroclor 1254. Because concentrations of PCB in eggs of sheepshead minnows as high as 201 ug/g were not accompanied by decreased fertility and only minimal embryo mortality, it seems unlikely that decreased fertility and embryo survival in Atlantic salmon and striped bass could be related solely to PCB in their eggs. Diminished survival of newly hatched striped bass fry, however, could be PCB-related since concentrations in

their eggs were similar to those in sheepshead minnow eggs which produced fry whose survival was poor.

LITERATURE CITED

Anonymous. 1971. The striper — this century's dinosaur. Stripers Unlimited 1971 Directory and Guidebook. pp. 11-62.

Brungs, W. A. and D. I. Mount. 1970. A water delivery system for small fish-holding tanks. Trans. Am. Fish. Soc. 99(4):799-802.

Butler, P. A. 1973. Organochlorine residues in estuarine mollusks, 1965-1972. National Pesticide Monitoring Program. Pestic. Monit. J. 6(4):238-362.

Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor 1254®) in the water, sediment and biota of Escambia Bay, Florida. Bull Environ. Contam. Toxicol. 5(2):171-180.

Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr. and P. D. Wilson. 1971. Chronic toxicity, uptake and retention of Aroclor® 1254 in two estuarine fishes. Bull. Environ. Contam. Toxicol. 6(2):113-119.

Johannsson, Nils, S. Jensen and M. Olsson. 1970. PCB-indicators of effects on fish. *In* "PCB Conference, Wenner-Gren Center", Sept. 29, 1970. pp. 59-68. Natl. Environ. Prot. Bd., Stockholm.

Lowe, J. I., P. R. Parrish, J. M. Patrick, Jr. and J. Forester. 1972. Effects of the polychlorinated biphenyl Aroclor® 1254 on the American oyster (*Crassostrea virginica*). Mar. Biol. (Berl.) 17(3):209-214.

Nimmo, D. R., R. R. Blackman, A. J. Wilson, Jr. and J. Forester. 1971. Toxicity and distribution of Aroclor® 1254 in the pink shrimp (*Penaeus duorarum*). Mar. Biol. (Berl.) 11(3):191-197.

Table 1. Toxicity and uptake of Aroclor® 1254 by adult sheepshead minnows (*Cyprinodon variegatus*) exposed for 28 days in an intermittent-flow bioassay. Thirty fish were tested per concentration. Residue analyses are for a minimum of 7 male and 17 female fish and eggs from 5 fish.

TEST CONCENTRATION (ug/l)		MORTALITY %	CONCENTRATION IN FISH (ug/g, wet weight)			FEMALES GRAVID %	AVERAGE FECUNDITY No.
Nominal	Measured		Males	Females	Eggs		
Control	ND*	7	0.64	0.47	0.52	100	97
0.1	0.09	13	2.5	1.9	0.88	89	121
0.32	0.14	7	9.7	9.3	5.1	100	110
1.0	0.39	10	---	25.	11.	100	127
3.2	1.1	3	49.	49.	27.	94	152
10.0	5.6	95	---	---	170.**	100	138

*ND = not detectable, < 0.03 ug/l
**Eggs from two fish.

Table 2. Success of fertilization of eggs from sheepshead minnows exposed to Aroclor® 1254 for four weeks, survival of embryos from fertile eggs until hatching and survival of hatched fry. Eggs are from five fish per concentration (except two fish from 10 ug/*l*). Percentages are in parentheses.

CONCENTRATION		EGGS			FRY	
Adult Exposure	Eggs Average	Tested	Fertile	Hatched	Survival Week 1	Survival Weeks 2,3,4
(ug/*l*)	(ug/g)					
Control	0.52	125	125 (100)	116 (93)	111 (95)	106 (96)
0.1	0.88	125	120 (96)	106 (88)	103 (97)	98 (95)
0.32	5.1	126	120 (95)	107 (80)	82 (77)*	76 (93)
1.0	11.	126	121 (96)	118 (98)	31 (26)*	26 (96)
3.2	27.	126	118 (94)	100 (85)	23 (23)*	19 (83)
10.0	170.	50	46 (92)	33 (72)*	0 (0)*	0 (–)
Control F and 1.0 ug/*l* M	--	128	119 (93)	113 (95)	112 (99)	111 (99)

*Significantly less than control hatching or one week fry survivals (X^2; $\alpha = 0.01$).

Table 3. Comparison of concentration of Aroclor® 1254 in eggs (wet weight) from sheepshead minnows exposed to the PCB for four weeks and success of fertilization of eggs and survival of embryos and fry.

Concentration in Eggs	EGGS			FRY		Adults from Aquaria
	Tested	Fertile	Hatched	Week 1	Weeks 2,3,4	
(ug/g)						(ug/*l*)
0.41	25	25	23	23	22	Control
0.44	25	25	24	19	19	"
0.45	25	25	25	25	24	"
0.53	25	25	24	24	23	"
0.57	25	24	19	18	18	0.1
0.76	25	25	20	20	18	Control
0.84	25	23	21	21	19	0.1
0.91	25	25	25	23	23	"
0.98	25	23	17	17	15	"
1.1	25	25	24	24	23	"
3.7	25	23	22	21	20	0.32
4.1	26	26	23	22	21	"
5.4	25	24	19	13	12	"
5.4	25	24	22	21	19	"
7.0	25	23	21	5	4	"
7.1	26	23	22	2	2	1.0
9.5	25	24	23	7	7	"
10.8	25	25	25	8	5	"
13.2	25	25	25	9	7	"
13.3	25	24	23	5	5	"
23.6	25	23	23	4	4	3.2
25.7	25	24	16	6	6	"
27.9	25	25	23	0	0	"
28.6	25	25	25	5	3	"
28.7	26	21	13	8	6	"
145.	25	23	15	0	0	10.0
201.	25	23	18	0	0	"

CONTRIBUTION NO. 178

Reprinted from the proceedings of the 27th Annual Conference of the Southeastern Association of Game and Fish Commissioners, 1973.

DIELDRIN: EFFECTS ON SEVERAL ESTUARINE ORGANISMS[1]

Patrick R. Parrish,[1] John A. Couch, Jerold Forester, James M. Patrick, Jr. and Gary H. Cook
U. S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Islnad, Gulf Breeze, Florida 32561
(Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon)

ABSTRACT

Tests were conducted to determine (1) the acute toxicity of dieldrin in flowing sea water to American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus* duorarum), grass shrimp (*Palaemonetes pugio*) and sheepshead minnows (*Cyprinodon variegatus*) and (2) the rate of dieldrin uptake and depuration by spot (*Leiostomus xanthurus*). Acute (96-hour) EC50's were: oysters, 12.5 ug/l; pink shrimp, 0.9 ug/l; grass shrimp, 11.4 ug/l; and sheepshead minnows 23.6 ug/l. Spot exposed to 0.0135, 0.075, 0.135, 0.75 or 1.35 ug/l for 35 days accumulated the chemical with maximum concentrations attained in 11 to 18 days. Maximum whole-body residue (wet-weight) was 6,000X the concentration in test water. Spot contained no detectable dieldrin residues at the end of a 13-day depuration period in dieldrin-free water. Tissue alterations, such as subepithelial edema in gill lamellae and severe lysis and sloughing of the small intestine epithelium, occurred in spot exposed to 1.35 ug/l for four days.

INTRODUCTION

The effects of dieldrin on estuarine organisms were investigated because this toxicant is present in most of this nation's estuaries. Dieldrin was the second most commonly detected organochlorine compound in molluscs from 15 coastal states during the period 1965-1972 (Butler, 1973).

Dieldrin, a chlorinated hydrocarbon insecticide, is acutely toxic to certain non-target estuarine animals under field conditions (Harrington and Bidlingmayer, 1958). Dieldrin is also acutely toxic to several estuarine animals exposed for 48 hours under laboratory conditions (Lowe, personal communication[1]).

This study was conducted to determine (1) the acute (96-hour) toxicity of dieldrin to American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum),* grass shrimp (*Palaemonetes pugio*) and sheeshead minnows (*Cyprinodon variegatus*) and (2) the rate of uptake and depuration in spot (*Leiostomus xanthurus*).

MATERIALS AND METHODS

Test animals

All test animals except pink shrimp were collected near the Gulf Breeze Laboratory and acclimated to laboratory conditions for at least ten days before exposure. Pink shrimp were purchased from a local bait dealer and acclimated similarly. If mortality in a specific lot of animals exceeded 1% in the 48 hours immediately preceding the test or if abnormal behavior was observed during acclimation, those animals were not used. Oysters tested were from 24 to 43 mm in

[1]Contribution No. 178, Gulf Breeze Environmental Research Laboratory
[1]Jack I. Lowe, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, FL 32561.

height; pink shrimp, 52 to 81 mm rostrum-telson length; grass shrimp, 18 to 24 mm rostrum-telson length; sheepshead minnows, 11 to 14 mm standard length; and spot, 22 to 38 mm standard length. Animals were not fed during acute toxicity tests but they could obtain food (plankton and other particulate matter) from the unfiltered sea water in which they were maintained. In the uptake and depuration study, spot were fed commercial fish food that contained no pesticide or polychlorinated biphenyl contaminants detectable by gas chromatographic analysis.

Test Conditions

Acute toxicity of dieldrin was determined by exposing ten animals per aquarium to different concentrations for 96 hours. Two 20 l aquaria were used for each concentration. Technical grade dieldrin (92% active ingredient) was dissolved in reagent grade acetone and metered at 0.14 ml/hr into unfiltered sea water that entered each aquarium at 75 l/hr. Two control aquaria received the same quantities of water and solvent but no dieldrin.

The rate of uptake and depuration of dieldrin by spot was determined by exposing 35 animals per aquarium in duplicate 20 laquaria to 0.0135, 0.075, 0.135, 0.75, or 1.35 ug/l for 35 days and then placing them in dieldrin-free water for 14 days. Sea water flow rate was 75l/hr per aquarium. Technical grade dieldrin (92% active ingredient) was dissolved in reagent grade acetone and metered at 15 ml/hr into the unfiltered sea water.

Effect of dieldrin was assessed by measuring reduction of shell growth of oysters (Butler, 1962), by determining mortality in shrimps and fish, and by examining for pathological changes fish from the uptake and depuration exposure.

Histopathological examination

Gills and viscera from live fish from the uptake and retention exposure were examined. Tissues were fixed in Davidson's fixative, stored in 70% ethyl alcohol and then processed for paraffin sections (7 u). Sections were stained with Harris hematoxylin and eosin. Six fish from each concentration were removed for tissue preparation after 4 days of exposure. Six fish from concentrations of 0.135, 0.075, and 0.0135 ug/l were removed at the end of the 35-day exposure, and six fish from concentrations of 0.135 and 0.075 ug/l and control were removed after the 13-day depuration in dieldrin-free water.

Chemical analyses

Concentrations of dieldrin in water and animals were determined by electron capture gas chromatography. Unfiltered water samples from each concentration were analyzed once during the 96-hour exposures and weekly during the uptake and depuration exposure. Concentrations in animals that survived the 96-hour exposures were determined as whole-body residures. In the uptake depuration exposure, six fish were removed from each connentration after 4, 11 18, 25 and 35 days and after 13 days in dieldrin-free water. Concentrations were determined for pooled samples of liver, muscle (all muscle above lateral line on left side of fish with scalless skin), and remaining tissues. Results from the two pooled samples of each tissue from each concentration were averaged. Residues in all tissues were summed to compute concentrations of dieldrin in the whole fish.

Tissue samples that weighed more than 5 g were prepared for analysis by mixing with anhydrous sodium sulfate in a blender. The mixture was extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated to approximately 10 ml and transferred in 3- to 4-ml portions to a 400 x 20 mm chromatographic column that contained 76 ml of unactivated Florisil. After

each portion settled in the column, vacuum was applied until all solvent was evaporated. This was repeated with three 5-ml rinses. The residue was eluted from the column with 70 ml of a 9:1 mixture (v/v) of acetonitrile and distilled water. The eluate was evaporated to dryness and the residue transferred to a Florisil column (Mills, et al., 1963) with petroleum ether. Dieldrin was eluted in the 15% ethyl ether-in-petroleum ether fraction.

Tissue samples that weighed less than 1 g were analyzed by the micro method described in the Pesticide Analytical Manual, Volume III (U. S. Food and Drug Administration, 1970).

Water samples were extracted with petroleum ether, the extracts dried with anhydrous sodium sulfate and evaporated to approximately 1 ml. The concentrates were transferred to a size 7 Chromaflex[1] column containing 1.6 g Florisil topped with 1.6 g anhydrous sodium sulfate. Dieldrin was eluted with 20 ml of 10% ethyl ether in hexane and the eluates were adjusted to an appropriate volumn for analysis.

All samples were analyzed by electron capture gas chromatography using a 182 cm x 2 mm ID glass column packed with 2% OV-101 on 100-120 mesh Gas Chrom Q. Nitrogen flow rate was 25 ml/min, the oven temperature was 190° C, and the injector and detector temperature was 210° C. Recovery exceeded 85%; data were not adjusted for recovery. All tissue residues were determined on a wet-weight basis.

Statistical analyses

Data from the acute (96-hour) exposures were analyzed statistically. Oyster shell growth data were analyzed by unweighted least squares and shrimp and fish mortality data were analyzed by maximum liklihood profit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Acute (96-hr) exposures

Dieldrin was acutely toxic to the estuarine organisms tested (Tables 1 and 2). Shell growth in oysters was appreciably inhibited by exposure to 32 ug/l for 96 hours. Pink shrimp were more sensitive to dieldrin than were grass shrimp, but significant numbers of both these crustaceans died when exposed to concentrations in the low parts-per-billion (ug/l) range.

All animals accumulated dieldrin (Table 1). The quantities accumulated depended on the species and the exposure concentration. In live oysters, whole-body (meats only) concentrations ranged from 2,000 to 5,000X nominal concentrations in test water and 2,400 to 21,500X measured concentrations. In an earlier experiment at this laboratory, oysters chronically exposed to 0.01mg/l of dieldrin accumulated 8,000X the concentration in test water after 8 weeks exposure (Parrish, 1973). In live pink shrimp, whole-body concentrations ranged from only 240 to 250X nominal concentrations in test water and 280 to 420X measured concentrations. In live grass shrimp, whole-body concentrations ranged from 330 to 660X nominal concentrations in test water and from 470 to 750X measured concentrations. In live sheepshead minnows, whole-body concentrations ranged from 2,000 to 4,000X nominal concentrations in test water and from 3,500 to 7,300X measured concentrations.

[1]Mention of commercial products or trade names does not constitute endorsement by the U. S. Environmental Protection Agency.

[1]Present Address: Bionomics Marine Laboratory, Route 6, Box 1002, Pensacola Fl. 32507.

Uptake and depuration

Spot exposed to 0.0135, 0.075, 0.135, 0.75 or 1.35 ug/l of dieldrin for 35 days accumulated the chemical, maximum concentrations being attained in 11 to 18 days (Table 3). Fish in some concentrations began to lose dieldrin after body concentrations had peaked, even though the exposure continued and dieldrin concentrations in test water remained constant (Table 4). Unlike our findings, DDT concentrations in pinfish (*Lagodon rhomboides*) and Atlantic croaker (*Micropogon undulatus*) exposed to 0.1 and 1.0 ug/l increased for 14 days, then remained relatively constant for 21 days (Hansen and Wilson, 1970).

Dieldrin was accumulated in greatest quantity in the liver of spot, where maximum concentration was 113,000X that in test water. Maximum concentration in muscle was 11,000X that in test water and maximum concentration in whole-body was 6,000X that in test water.

Spot lost all detectable dieldrin residues after a 13-day depuration period in dieldrin-free sea water (Table 3). Pinfish lost 87% of DDT residues and Atlantic croaker lost 78% of accumulated DDT when held in pesticide-free water for 56 days (Hanson and Wilson, 1970). Similarly, goldfish (*Carassius auratus*) have been reported to eliminate ^{14}C-dieldrin from various tissues more rapidly than DDT (Grzenda et al., 1972). Thus, the flushing rate of dieldrin in fish appears to be faster than that of DDT.

Fish exposed to 1.35 ug/l showed degenerative changes in gill and visceral tissue after 4 days of exposure. Gill lamellae from three of six fish exhibited subepithelial edema (Fig. 1). A similar condition was observed in gills of cutthroat trout (*Salmo clarki*) exposed chronically to endrin (Eller, 1971) and in gills of goldfish exposed chronically to mirex (Van Valin et al., 1968). Alteration of visceral tissue included severe lysis and sloughing of the mucosal epithelium of the anterior small intestine (Fig. 1) and apparent inflamation of the underlying lamina propria in three of six fish.

Fish examined at the end of the exposure (from concentrations of 0.135, 0.075, and 0.0135 ug/l) and at the end of the depuration (from concentrations of 0.135 and 0.075 ug/l) showed no significant differences from control fish.

Dieldrin is a persistent chlorinated hydrocarbon insecticide (Wurster, 1971) and, as shown by our study, is acutely toxic to an estuarine mollusc, two crustaceans and a fish. Concentrations of dieldrin shown by our study to be acutely toxic to estuarine animals, as well as concentrations which are chronically toxic, should be kept out of the estuarine environment.

Table 1. Acute toxicity of dieldrin to and uptake by American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), and sheepshead minnows (*Cyprinodon variegatus*) during 96-hour exposures. Effect is expressed as percentage reduction in shell growth for oysters and death for shrimps and fish. Whole-body residues are from animals alive at end of exposure.

SPECIES	WATER CONCENTRATION (ug/l)		EFFECT (%)	WHOLE-BODY RESIDUE
	Nominal	Measured		(ug/g, wet weight)
C. virginica	Control	<0.01	0	0.022
	1.0	0.23	18	4.95
	3.2	5.8	0	13.85
	10.0	6.7	24	20.0
	32.0	13.0	61	80.5
P. duorarum	Control	<0.01	0	0.016
	0.01	0.014	0	<0.01
	0.32	0.19	25	0.08
	1.0	0.9	55	0.25
	3.2	2.5	70	0.76
	10.0	11.4	100	
P. pugio	Control	ND[1]	0	0.09
	3.2	2.8	20	2.1
	10.0	7.1	30	3.3
	32.0	27.1	85	
	100.0	57.4	100	
	320.0	65.7	100	
C. variegatus	Control	<0.01	0	1.1
	1.0	0.52	0	3.8
	3.2	2.2	0	12.8
	10.0	6.0	10	34.0
	32.0	17.6	65	62.4
	100.0	13.1	100	

[1]Not detectable; <0.005 ug/l.

Table 2. Acute toxicity of dieldrin to American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), and sheepshead minnows (*Cyprinodon variegatus*). Effect is expressed as percentage reduction in shell growth for oysters and death for shrimp and fish. Confidence limits (95%) are in parentheses.

SPECIES	96-HOUR EC50 (ug/l)		TEMPERATURE (°C)		SALINITY (o/oo)	
	Nominal	Measured	Mean	Range	Mean	Range
C. virginica	12.50 (4.80-20.2)	31.20 0.60-61.80)	16.6	14.5-19.0	30.8	30.0-32.5
P. duorarum	0.93 (0.52-1.48)	0.70 (0.39-1.15)	19.6	18.2-21.0	26.0	22.0-30.0
P. pugio	11.39 (7.47-16.71)	8.64 5.92-12.05)	22.5	21.4-23.5	30.8	28.5-33.0
C. variegatus	23.57 (17.47-32.03)	10.00	13.8	12.0-15.5	31.5-33.0	

Table 3. Uptake and depuration of dieldrin by spot (*Leiostromus xanthurus*) exposed to 0.135, 0.075, 0.135, 0.74 or 1.35 ug/l in flowing sea water. Residue concentrations (wet-weight) are the average of two samples of pooled tissue from three fish.

LIVER	DAYS	CONCENTRATION, ug/g					
	Exposure	Control	.0135	.075	.135	.75	1.35
	4	ND	0.08	0.52	0.98	1.8	10.2
	11	ND	-[b]	3.90	15.3	12.9	
	18	ND	0.42	1.10	2.0	17.5	
	25	ND	0.15	1.20	1.4	5,8[c]	
	35	ND	0.31	0.55	0.47		
	Depuration						
	13	ND		ND	ND		
MUSCLE	Exposure						
	4	ND	0.029	0.07	0.16	0.81	2.6
	11	ND	-[b]	0.44	1.45	1.40	
	18	ND	0.029	0.12	0.15	1.20	
	25	ND	0.029	0.11	0.24	0.81[c]	
	35	ND	0.030	0.15	0.20		
	Depuration						
	13	ND		ND	ND		
	Exposure	Control	.0135	.075	.135	.75	1.35
WHOLE-BODY	4	ND	0.029	0.43	0.63	1.2	2.9
	11	ND	-[b]	0.15	0.52	1.9	
	18	ND	0.031	0.07	0.23	2.0	
	25	ND	0.033	0.15	0.29	0.6[c]	
	35	ND	0.045	0.12	0.27		
	Depuration						
	13	ND		ND	ND		

[a] Not detectable; <0.005 ug/g.

[b] Sample lost.

[c] Analysis of one sample only.

Table 4. Concentration (ug/l) of dieldrin in test water on days 4, 11, 18, 25 and 35 of uptake and depuration exposure of spot (*Leiostomus xanthurus*).

NOMINAL	MEASURED					AVERAGE
Control	ND	ND	ND	ND	ND	ND
0.0135	0.015	0.019	0.010	0.016	0.016	0.015
0.075	0.075	0.052	0.070	0.057	0.067	0.064
0.135	0.11	0.12	0.13	0.15	0.11	0.12
0.75	0.55	0.45	0.50	0.68		0.55
1.35	0.70					0.70

Figure 1. Photomicrographs of tissues from spot (*Leiostomus xanthurus*) exposed to dieldrin. 1: Normal gill tissue from fish exposed 35 days to 0.075 ug/l. (X450) 2: Gill tissue from fish exposed 4 days to 1.35 ug/l. Note subepithelial edema in lamellae. (X450) 3. Normal small intestine tissue from fish exposed 35 days to 0.075 ug/l. (X450) 4: Small intestine tissue from fish exposed 4 days to 1.35 ug/l. Note severe lysis and sloughing of mucosal epithelium. (X450).

LITERATURE CITED

Butler, Philip A. 1973. Organochlorine residues in estuarine mollusks, 1965-72 -- National Pesticide Monitoring Program. Pestic. Monit. J. 6(4): 238-362.

1962. Reaction of some estuarine mollusks to environmental factors. *In*: Biological problems in water pollution. Third Seminar. U. S. Dept. Health, Educ., Welfare, Public Health Serv. Publ. No. 999-WP-25, 1965: 92-104.

Eller, Lafayette L. 1971. Histopathologic lesions in cutthroat trout (*Salmo clarki*) exposed chronically to the insecticide endrin. Am. J. Pathol. 64(2): 321-336.

Finney, D. J. 1971. Probit analysis. (Third ed.) Cambridge Univ. Press, Cambridge, England. 333 p.

Grzenda, A. R., W. J. Taylor, and D. F. Paris. 1972. The elimination and turnover of ^{14}C - dieldrin by different goldfish tissues. Trans. Amer. Fish. Soc. 101(4): 686-690.

Hansen, David J. and A. J. Wilson, Jr. 1970. Significance of DDT residues from the estuary near Pensacola, Fla. Pestic. Monit. J. 4(2): 51-56.

Harrington, R. W. and W. L. Bidlingmayer. 1958. Effects of dieldrin on fishes and invertebrates of a salt marsh. J. Wildl. Manage. 22(1): 76-82.

Mills, P. A., J. F. Onley and R. A. Gaither. 1963. Rapid method for chlorinated pesticide residues in non-fatty foods. J. Assoc. Offic. Agric. Chem. 46(2):

Parrish, Patrick R. 1973. Aroclor^{R} 254, DDT and DDD, and dieldrin: accumulation and loss by American oysters (*Crassostrea virginica*) exposed continuously for 56 weeks. (Abstract) Proc. Natl. Shellfish. Assoc. (In Press).

U. S. Food and Drug Administration. 1970. Pesticide Analytical Manual. U. S. Dept. of Health, Educ., Welfare, Sect. H212.

Van Valin, Charles C., A. K. Andrews, and L. L. Eller. 1968. Some effects of mirex on two warm-water fishes. Trans. Amer. Fish. Soc. 97(2): 185-196.

Wurster, Charles F. Aldrin and dieldrin. Environment 13(8): 33-45.

Reprinted from MOSQUITO NEWS, Vol. 34, No. 3, September, 1974

EFFECTS OF GROUND APPLICATIONS OF MALATHION ON SALT-MARSH ENVIRONMENTS IN NORTHWESTERN FLORIDA [1]

M. E. TAGATZ, P. W. BORTHWICK, G. H. COOK AND D. L. COPPAGE

U. S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561
(Associate Laboratory, National Environmental Research Center, Corvallis, Oregon)

ABSTRACT. Effects of thermal fog {6 wt. oz/acre (420 g/ha)} and ULV aerosol spray {0.64 fl. oz/acre (57 g/ha)} applications of malathion 95 (o,o-dimethyl phosphorodithioate of diethyl mercaptosuccinate) on salt-marsh environments near Pensacola Beach, Florida, were investigated. Studies were conducted on selected plots after each of three treatments using a portable thermal fogger and three ultra low volume (ULV) sprays with a truck-mounted generator. The ULV sprays were typical of usual mosquito-control operations. The foggings were on a small scale and results should be considered as indicative of what may occur under usual conditions. Deaths due to malathion were not observed among confined blue crabs, *Callinectes sapidus;* grass shrimps, *Palaemonetes vulgaris* and *P. pugio;* pink shrimp, *Penaeus duorarum;* or sheepshead minnows, *Cyprinodon variegatus.* Brain acetylcholinesterase activity was not reduced in confined *C. variegatus* exposed to one or more treatments. Confined animals and the snail, *Littorina irrorata,* contained no measurable malathion at our limit of detectability. The chemical was not detected in sediment, but concentrations as high as 4.10 parts per million (ppm) were found in *Juncus* sp., trace amounts persisting as long as 14 days (>0.05 but <0.10 ppm). Highest concentration in marsh water after fogging was 5.2 parts per billion (ppb); after ULV spraying, 0.49 ppb. For each method of application, only trace amounts (>0.1 but <0.3 ppb) persisted in marsh water as long as 1 day.

INTRODUCTION

The organophosphate insecticide malathion (O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate), applied in thermal fogs or as ultra low volume (ULV) non-thermal mist sprays, is commonly used in marsh areas to control adult mosquitoes. Both methods are effective, but ULV mist sprays require less insecticide than fog applications and cause less environmental contamination (Taylor and Schoof, 1971; Fultz *et al.,* 1972).

Few field studies have been conducted on effects of malathion on aquatic environments, particularly estuaries. Malathion formulated in fuel oil and sprayed by airplane at the rate of 8 wt. oz/acre (560 g/ha) on tidal marshes in Delaware was toxic within 4 hours to ocellated killifish, *Fundulus ocellaris,* held in tubs (Darsie and Corriden, 1959). Aerial application of 3 fl. oz/acre (256 g/ha if technical grade chemical) to marsh embayments in Texas killed 14 to 80 percent of commercial shrimp, *Penaeus aztecus* and *P. setiferus,* held in live boxes, within 49 hours (Conte and Parker, 1971). Residues of malathion up to 48 hours after spraying ranged from 0.8 to 3.2 ppm (parts per million) in the water and from 0.28 to 2.67 ppm in tissues of living shrimp. Significant inhibition of brain acetylcholinesterase in fishes was associated with aerial ULV application of 3 fl. oz/acre of malathion in Louisiana (Coppage and Duke, 1971). ULV (3 fl. oz/acre) applications of technical malathion over towns in Hale County, Texas had no authenticated adverse effect on various freshwater fishes in reservoirs and ponds (Hill *et al.,* 1971).

The objectives of our study were to evaluate effects of thermal fog and ULV ground applications of malathion on some estuarine animals in saltwater marshes in northwestern Florida and to determine occurrence and persistence of the chemical in various components of the marshes.

---

[1] Contribution No. 179, Gulf Breeze Environmental Research Laboratory.

MATERIALS AND METHODS

Each thermal fog or ULV spray was applied near the time of low tide to permit maximum settling and retention in the marsh and near sunset, when sprays are usually applied to coincide with the greatest activity of mosquitoes and with optimum winds and temperatures which provide for the spray to remain close to the ground. Salinity, water temperature and pH were measured at the time of the second fogging and each of the ULV sprayings.

Water, grass, sediment and animals were analyzed by gas chromatography with a flame photometric detector in the phosphorus mode to determine concentrations of malathion. Based on our levels of detection, the terms nondetectable (N. D.) and trace (Tr.) amounts of malathion in the estuarine components sampled are defined as follows: 1.5l water sample, not above 0.1 parts per billion, ppb (N. D.) and >0.1 but <0.3 ppb (Tr.); 20 g sample of sediment or grass, not above 0.05 ppm (N. D.) and >0.05 but <0.10 ppm (Tr.); and 5 g animal sample, not above 0.2 ppm (N. D.) and >0.2 but <0.3 ppm (Tr.). Samples spiked with known amounts of malathion in the laboratory were recovered with efficiencies greater than 80%, but sample concentrations were not corrected for percentage recovery. Water was extracted in the field with petroleum ether; animals, grass and sediment were placed on ice or dry ice to inhibit degradation of malathion during transport from field to laboratory.

I. THERMAL FOG APPLICATIONS. Malathion (Cythion®[2] Technical 95% formulated in fuel oil) was applied to a *Juncus* sp.-dominated salt-marsh with a Sears, Roebuck and Co. portable gas-engine fogger, Model '71–14871. Maximum volume output of the hand-held fogger was 17,500 cu. ft. (495 m^3) per minute.

The treated and control plots were in a marsh at Range Point, about 1 mile (1.6 km) east of Pensacola Beach, Florida. The marsh was connected to Santa Rosa Sound by an inlet that allowed tidal exchange. One area, 85 ft. x 115 ft. (26 m x 35 m), was fogged with malathion in diesel fuel oil and served as the treated plot, and an area of the same size 100 yards (91.4 m) distant was fogged with diesel fuel oil and served as the control plot. Tidal canals, 10-ft. (3.0 m) wide and 1- to 4-feet (0.3- to 1.2-m) deep, bordered one side of the *Juncus* plots and were included in the treated areas.

Malathion was applied to the treated plot at approximately 2-week intervals (September 18, October 3 and 16, 1972) at a theoretical rate of 6 wt. oz/acre (420 g/ha). The maximum thermal fog recommendation is 8 wt. oz/gal (60 g/l) at 40 gph (151 l/hr) at 5 mph (8 km/hr), equivalent to 1.3 fl. oz/acre (111 g/ha).[3] An employee of the Escambia County Mosquito Control Department, Pensacola, Florida, walked the plot along fog-swath transects until the content of the insecticide tank {1.35 wt. oz (38.3 g) malathion and 3 qts. (2.8 l) fuel oil} was dispensed (about 15 minutes). The control plot was treated in a similar way with fuel oil carrier.

Prior to the first application, animals were placed in cages in the tidal canal portions of the plots, and all but shrimp were held for the three foggings. In each plot, 10 juvenile blue crabs, *Callinectes sapidus,* were held in each of 2 cages; adult or near-adult sheepshead minnows, *Cyprinodon variegatus,* in 3 cages (20 per cage); and adult grass shrimp, *Palaemonetes vulgaris,* in 2 cages (16 per cage). Shrimp were held near the surface of the water in small cages

[2] ® Registered trademark of American Cyanamid Company, Princeton, N. J. Mention of commercial products or trade names does not constitute endorsement by the Environmental Protection Agency.

[3] Personal communication, C. B. Rathburn, Jr., West Florida Arthropod Research Laboratory, Panama City, Florida 32401.

made of nylon screen over acrylic frames; crabs and fish, in large cages made of hardware cloth over wooden frames. To prevent cannibalism, shrimp and crabs were confined in individual compartments. After the first application, unaccountably high mortality of grass shrimp occurred in treated and control groups after 7 days; therefore, juvenile pink shrimp, *Penaeus duorarum,* were used in the second treatment. Shrimp studies were terminated 3 days after the second fogging because of vandalism of some pink shrimp cages. Data on mortalities of grass shrimp were obtained only during 7 days after the first fogging; and data for pink shrimp, 3 days after the second fogging. Mortalities among crabs and fish were recorded prior to the second and third treatments; survivors 6 hours after the third treatment were analyzed for malathion residues. Shrimp were not analyzed for residues.

Sheepshead minnows (300 in each of two cages) were held in the treated plot for a study on activity of brain acetylcholinesterase (AChE) in fish exposed to one or more applications of the chemical. Using procedures reported in Coppage (1972), 25 fish (representing five samples) were used to measure AChE activity at each of the following times: 1, 4 and 7 days after the first fogging, 12 hours and 3 days after the second fogging, and 6 and 12 hours after the third fogging. Coppage (1972) found that reduction of AChE activity in sheepshead minnows below a specific level (about 18% of normal) indicates death or impending death from organophosphate poisoning.

Samples of sediment, *Juncus,* marsh water (at base of *Juncus*), and canal water were analyzed for malathion at 6 hours, 12 hours, 1, 3, 7 and 14 days after each fogging operation or until malathion was not detected. Except for canal water, each type of sample was a composite of material from three locations. These locations were selected randomly from a grid pattern of 54 divisions per plot.

II. ULV AEROSOL SPRAYS. ULV malathion (Cythion® Technical 95%) was applied to a *Juncus*-dominated salt-marsh by a truck-mounted Leco HD ULV cold aerosol generator.[4] Discharge was toward the rear at an upward angle of 45°. Actual discharge rate was 0.5 gallon per hour at 2½ mph (1.9 l/hr at 4 km/hr) which is equivalent to 2.0 gph at 10 mph (7.6 l/hr at 16 km/hr), the maximum allowable rate for ground ULV application in Florida. Pressure in the insecticide tank was 4 pounds/square inch (0.28 kg/cm ^{2}). Discharge rate for the flowmeter setting used was calibrated for temperature. Volume discharged was 160 ml and spray time was 5 minutes.

Three sprays were applied (May 15, June 11 and 25, 1973) to approximately 8½ acres (3.4 ha) of marsh by employees of the West Florida Arthropod Research Laboratory, Panama City, Florida. The rate was equivalent to 0.64 fl. oz/acre (57 g/ha) based on a swath of 330 feet (100.6 m). For the first and second treatments, the Range Point marsh served as the control plot and a similar *Juncus*-dominated marsh about 5 miles (8 km) east of Range Point was the treated plot. Range Point was selected as the treated area for the third spray because of a more favorable wind direction for chemical drift; the marsh east thereof was the control. Both sites were connected to Santa Rosa Sound by inlets that allowed tidal exchange. Wind velocities during the three sprayings averaged 6.3, 6.0 and 10.2 mph (10.1, 9.6 and 16.4 km/hr).

Prior to each spray, grass shrimp (adult *P. pugio*), blue crabs (15–25 mm wide) and sheepshead minnows (25–40 mm total length) in 18 in. (45.7 cm) diameter polyethylene tubs containing 25 liters of water were placed in the marshes. In each marsh, tubs were positioned in two rows of three adjacent tubs. The rows were 50 ft. (15.2 m) apart, and animals in each row consisted of 25 shrimp in

[4] Lowndes Engineering Company, Inc., Valdosta, Georgia.

one tub, 15 crabs in a second tub, and 20 fish in a third tub. Immediately after treatment, screens were placed over tubs to keep out predators. Mortalities were determined 1 and 3 days after spray. One day after treatment, duplicate living samples of 15 shrimp, 5 crabs or 10 fish were removed for chemical analyses.

Seventy sheepshead minnows in a 5.4 sq. ft. (0.5 m^2) polyethylene pool containing 91 liters of water were centered between the rows of tubs in the treated marsh. AChE activities in the fish were measured at 6 hours and 1 day after each spray.

At selected intervals after treatment samples of sediment, *Juncus,* water from the marsh, water from tubs, and snails (*Littorina irrorata* collected from *Juncus*) were analyzed for malathion. A composite water sample (1.5 l) was obtained from each of the two groups of tubs; composite samples of marsh water and other materials were obtained randomly in the vicinity of the tubs.

To determine the effectiveness of our ULV sprays for mosquito control, caged mosquitoes were placed on 5-ft. (1.5 m) poles in the marsh for the third spraying by personnel of the West Florida Arthropod Research Laboratory. One hundred forty *Aedes taeniorhynchus* in six cages and 131 *Culex nigripalpus* in six cages were used in the treated plot, and as a check, approximately the same numbers of mosquitoes were held in 12 cages away from the treated plot.

RESULTS

I. THERMAL FOG APPLICATIONS. Physical and chemical characteristics of canal water in the treated and control plots for the second fogging were: temperature, 24.5°–25.0° C; salinity, 27.5–28.0 ppt (parts per thousand); and pH, 7.2–7.6.

No effects of malathion on caged animals were observed. Mortality of crabs and fish did not differ greatly between control and treated groups after the first and second foggings (Table 1). Treated crabs (average width 83.2 mm, range 44–115) and control crabs (77.0 mm, 41–113 mm) each molted seven times in the 28-day period. In our limited shrimp studies, single deaths of grass shrimp occurred in each plot after 7 days, and no deaths of pink shrimp occurred after 3 days. Fish and crabs obtained 6 hours after the third treatment contained no detectable malathion. No decided inhibition of AChE activity in brains of sheepshead minnows was detected after any of the three treatments.

No deaths that could be attributed to the treatments were observed among resident populations of shrimp, crabs, and fish.

Malathion did not persist for long in sediment, *Juncus* or water after each application. The chemical was not detected in sediment after 6 hours. However, trace amounts occurred in samples of *Juncus* after 14 days (Table 2). Malathion was not detected in water after 1

TABLE 1. Mortality of confined animals in salt-marshes after thermal fog applications of malathion 95 at 6 wt. oz/acre (420 g/ha).

	14 days after 1st treatment		14 days after 2nd treatment		Total	
Animal	No. dead	%	No. dead	%	No. dead	%
Blue crabs						
Control	3	15	5	25	8	40
Treated	3	15	2	10	5	25
Sheepshead minnows						
Control	4	7	3	5	7	12
Treated	5	8	6	10	11	18

day. After 6 and 12 hours, residues of the toxicant ranged from <0.1 to 5.2 ppb in marsh water and from <0.1 to 0.42 ppb in canal water. Malathion was not found in sediment, *Juncus* or water from the control plot, except for trace amounts in the water 6 hours after the second application.

II. ULV AEROSOL SPRAYS. Ranges of various water properties in both marshes at the time of ULV sprayings were: water temperature in tubs and marsh, 24.5°–32.5° C; salinity of tub water, 14–16 ppt, of marsh water, 13.5–20.0 ppt; and pH, 7.5–7.8.

We observed no effects of malathion on

TABLE 2. Malathion residues (ppm) in samples of *Juncus* from a salt-marsh after thermal fog applications of malathion 95 at 6 wt. oz/acre (420 g/ha).

Treatment	Time elapsed after treatment					
	6 hrs.	12 hrs.	1 day	3 days	7 days	14 days
1	0.31	0.21	N.D.[1]	0.16	N.D.	N.D.
2	1.21	4.10	2.10	....	Tr.[2]	Tr.
3	0.45	1.20	N.D.	0.71	Tr.	Tr.

[1] N.D. (non detectable)=not above 0.05 ppm.
[2] Tr. (trace)=>0.05 but <0.10 ppm.

TABLE 3. Deaths of confined animals in salt-marshes after ULV sprays of malathion 95 at 0.64 fl. oz/acre (57 g/ha).

Spray	Animal	Number start	Deaths 0–1 day	Number 1 day[1]	Deaths 1–3 days
1	Blue crabs				
	Control	30	2	18	0
	Treated	30	2	18	1
	Sheepshead minnows				
	Control	40	0	20	0
	Treated	40	0	20	0
	Grass shrimp[2]				
	Control	50	1	19	8
	Treated	50	0	20	0
2	Blue crabs				
	Control	30	1	19	5
	Treated	30	0	20	0
	Sheepshead minnows				
	Control	40	0	20	0
	Treated	40	0	20	0
	Grass shrimp[2]				
	Control	50	0	20	6
	Treated	50	0	20	3
3	Blue crabs				
	Control	30	0	20	2
	Treated	30	4	16	2
	Sheepshead minnows				
	Control	40	0	20	0
	Treated	40	1	19	0
	Grass shrimp[2]				
	Control	50	0	20	2
	Treated	50	0	20	0

[1] Number of animals remaining after 1 day upon removal of dead animals and living samples (10 crabs, 20 minnows or 30 shrimp) for residue analyses.
[2] Most shrimp listed as dead were not found; some may have escaped or been eaten.

animals. Few deaths occurred among treated animals (Table 3). Some deaths of crabs in both marshes were due to cannibalism. The bodies of most shrimp listed as dead were not found; some may have escaped or been eaten. No deaths of resident crabs, fishes, and shrimps were noted after any treatment. Confined crabs, fish and shrimp and free-living snails obtained 1 day after each spray contained no detectable malathion. AChE activities in brains of sheepshead minnows were not altered by any of the three sprays.

Residues of malathion in sediment, water, or *Juncus* 3 days following ULV applications generally were low or not detected (Table 4). Malathion was not detected in sediment 1 or 6 hours after treatments. None was detected in water or from *Juncus* after the first spray. After the third spray, however, it persisted at least 3 days at concentrations up to 0.34 ppb in tub water and 0.28 ppm in *Juncus* samples. Malathion was not detected in control samples.

Deaths of caged mosquitoes after the third spraying were 100% treated and 0.1% check for *A. taeniorhynchus,* and 89.3% treated and 0% check for *C. nigripalpus*. Mortalities were within the range found in other field tests using caged mosquitoes and ULV ground equipment (Rathburn and Boike, 1972).

TABLE 4. Malathion in samples of tub water (from each of two rows of tubs), marsh water and *Juncus* from salt-marshes after ULV sprays of malathion 95 at 0.64 fl. oz/acre (57 g/ha).

Sample and time elapsed	Spray 1	Spray 2	Spray 3
Tub water 1			
1 hour	N.D.[1]	Tr.[1]	1.52 ppb
6 hours	N.D.	Tr.	0.58 ppb
12 hours	N.D.	Tr.	0.73 ppb
1 day	N.D.	Tr.	0.48 ppb
3 days	N.D.	N.D.	Tr.
Tub water 2			
1 hour	N.D.	Tr.	0.32 ppb
6 hours	N.D.	N.D.	Tr.
12 hours	N.D.	N.D.	0.36 ppb
1 day	N.D.	N.D.	0.32 ppb
3 days	N.D.	....	0.34 ppb
Marsh water			
6 hours	N.D.	N.D.	N.D.
12 hours	N.D.	0.49 ppb	N.D.
1 day	N.D.	Tr.	N.D.
3 days	N.D.	N.D.	N.D.
Juncus			
6 hours	N.D.[2]	Tr.[2]	N.D.
12 hours	N.D.	Tr.	N.D.
1 day	N.D.	N.D.	0.41 ppm
3 days	N.D.	N.D.	0.28 ppm

[1] N.D. (non detectable)=not above 0.10 ppb; Tr. (trace)=>0.10 but <0.30 ppb.
[2] N.D. (non detectable)=not above 0.05 ppm; Tr. (trace)=>0.05 but <0.10 ppm.

DISCUSSION

No adverse effects of malathion on confined animals or on the salt-marsh environment were observed under the conditions of these studies. In addition, no deaths were noted among resident crabs, fishes and shrimps after any of the treatments. Malathion was not detected in animals or sediment. In general, when found in plant samples or water, concentrations were low and did not persist. We found trace amounts of the chemical in *Juncus* samples for as long as 14 days after treatment. Although Bender (1969) found that carp, *Cyprinus carpio,* accumulated malathion when exposed to relatively high concentrations (1.0–7.5 ppm), he reported that the average half-life of the chemical, calculated from his data, was only 12 hours. The highest concentration we detected in marsh water was 5.2 ppb; only trace amounts persisted as long as 1 day. Guerrant *et al.* (1970) reported that 0.5 ppm malathion, the highest concentration found in waters of Hale County, Texas after ULV aerial spraying, was completely decomposed in 1 day.

In our field studies, the applications of thermal fog were on a small scale, but the ULV aerosol sprays were typical of usual operations for mosquito control. Even so, the fogging studies indicate what might occur in typical operations

with truck-mounted equipment over larger areas.

ACKNOWLEDGMENTS

Mr. Arthur Peagler and Mr. Howard Shinton, Escambia County Mosquito Control, Pensacola, Florida, together with Dr. Andrew Rogers, Dr. Carlisle Rathburn, Jr., and staff members, West Florida Arthropod Research Laboratory, Panama City, Florida, contributed outstanding cooperation in the study. Mrs. Dana Tyler-Schroeder and Mr. Terrence Hollister aided in the field work. The American Cyanamid Company kindly supplied malathion.

References Cited

Bender, M. E. 1969. Uptake and retention of malathion by the carp. Prog. Fish-Cult. 31(3): 155–159.

Conte, F. S. and Parker, J. C. 1971. Ecological aspects of selected crustacea of two marsh embayments of the Texas coast. Texas A & M Univ., Sea Grant Publ. No. TAMU–SG–71–211: 184 p.

Coppage, D. L. 1972. Organophosphate pesticides: Specific level of brain AChE inhibition related to death in sheepshead minnows. Trans. Amer. Fish. Soc. 101(3):534–536.

Coppage, D. L. and Duke, T. W. 1971 Effects of pesticides in estuaries along the Gulf and Southeast Atlantic Coasts. Proc. 2nd Gulf Conf. Mosq. Suppr. Wildl. Manage., N. O. La., Oct. 20–22:24–31.

Darsie, R. F., Jr. and Corriden, F. E. 1959. The toxicity of malathion to killifish (*Cyprinodontidae*) in Delaware. J. Econ. Entomol. 52(4): 696–700.

Fultz, T. O., Jr., McDougal, M. L. and Thrift, E. C. 1972. Observations on ground ULV applications in Chatham County, Georgia. Mosq. News 32(4):501–504.

Guerrant, G. O., Fetzer, L. E., Jr. and Miles, J. W. 1970. Pesticide residues in Hale County, Texas, before and after ultra-low volume aerial application of malathion. Pestic. Monit. J. 4(1):14–20.

Hill, E. F., Eliason, D. A. and Kilpatrick, J. W. 1971. Effects of ultra-low volume applications of malathion in Hale County, Texas. III. Effect on nontarget animals. J. Med. Entomol. 8(2): 173–179.

Rathburn, C. B., Jr. and Boike, A. H., Jr. 1972. Ultra-low volume tests of malathion applied by ground equipment for the control of adult mosquitoes. Mosq. News 32(2):183–187.

Taylor, R. T. and Schoof, H. F. 1971. The relative effectiveness of malathion thermal aerosols and ground-applied ULV aaginst three species of mosquitoes. Mosq. News 31(3):346–349.

CONTRIBUTION NO. 180

Criteria for Determining Importance and Effects of Pesticides on the Marine Environment: A Brief Overview

THOMAS W. DUKE

Scientists continually emphasize the importance of pesticide residues that occur in the marine environment by assigning these residues high priority in terms of needed surveillance and research. In most instances, the analytical technology for determining residues of pesticides in the parts per trillion range is available, but we often do not fully understand the significance of these levels in relation to biological and ecological effects.

Much time and effort are being devoted to developing meaningful criteria for assessing the impact of pesticides on organisms and their environment. One approach to developing these criteria is to assess the effect of pesticides through laboratory research, experimental ecosystems, and environmental studies:

I. Laboratory Research
   1. Standard "Bioassay"
      A. Acute
      B. Chronic
      C. Criteria for Effects
      D. Indicator Organisms
      E. Application Factor

II. Environment Ecosystems
   1. Compartmental Analysis
   2. Community Bioassay

III. Environmental Studies
   1. Baselines
   2. Impact of Pesticide Application in the Environment

Laboratory Research

The first step in assessment after subjecting a candidate pesticide and its degradation products to chemical characterization is to perform a "standard" bioassay. Known amounts of the pesticide are administered to selected test organisms maintained in static or flowing water systems for a given period of time. Periodically, test organisms are examined and compared with "control" organisms to determine if an effect has occurred.

These tests are often termed acute because they are conducted for a short period of time in relation to the life span of the organism. For example, when fish whose life span is normally a year or more are subjected to a test for 96 hours, it is considered an acute bioassay. The objective of these tests is to provide the researcher with information on toxicity levels which subsequently can be used to conduct more comprehensive bioassays. Chronic tests are actually an extention of the acute tests and are conducted through a reproductive cycle or through some developmental stage for a relatively long period of time.

In the past, mortality of the test population or a part of the population was considered the prime criterion for an effect, but this is no longer considered adequate. Other criteria, including oxygen evolution (by phytoplankton), changes in growth rate, respiration rate, shell deposition, blood protein configuration, inhibition of enzyme systems and pathological changes, give the researcher more complete information on an effect. Efforts are being made by investigators in the field to compile acceptable procedures for bioassay tests incorporating these criteria so that a more flexible bioassay procedure will be available to marine scientists.

Care Required

Special care must be taken in performing even preliminary bioassay tests. The organisms used should be in good physiological condition and representative numbers should be analyzed for background levels of contaminants, especially the pesticide being tested. The quality of the water, including pesticide content, must be known before it enters the test apparatus. In addition, the amount of pesticide in the water of the test apparatus must be checked to insure that the desired concentration is present. Accurate chemical analysis of the pesticide content of water and test organisms is imperative.

Representative organisms from several trophic levels should be employed in the bioassay test when possible and should include organisms known to be sensitive to a specific group of compounds. Crustaceans, particularly shrimp and crabs, are usually more sensitive to organochlorines than are other marine organisms. Larval and juvenile stages are generally more sensitive than adult forms. Unfortunately, we are unable at the present time to culture a wide array of test organisms for saltwater bioassays, and in many instances, cannot maintain in the laboratory sensitive life stages of needed test organisms. As a result, we often have data on the acute effects of the chemical with adults only and must extrapolate to determine what the effect might be over a longer period of time on more sensitive stages.

An application factor is useful to those involved in setting standards for pesticide levels in marine waters. An application factor is a ratio of a safe concentration of a pesticide to the acutely lethal concentration. One can estimate a value for an "acceptable" level of a pesticide in marine waters by multiplying the lethal concentration determined in acute bioassays by the appropriate application factor. In many cases an arbitrary application factor of 0.01 is used when the necessary scientific data has not yet been developed. Much more effort should be devoted to obtaining necessary

scientific data for determining application factors for specific pesticides. Also, users of application factors should be made aware that this ratio does not include a safety factor as such, but is only a fractional factor applied to a lethal concentration. Additional information on the development of application factors is available in the literature (1), (2).

Experimental Ecosystems

Pesticides entering into the natural environment can affect not only individual animals but communities of animals and ecosystems. The interactions of the various communities with each other and with their physical environment could be affected by a pesticide. One approach to quantify such effects is to construct an experimental ecosystem in which several species of organisms and their substrates can be subjected to the pesticide. Quantitative information on rates, routes and reservoirs of accumulation can be obtained. Bioaccumulation data are easily obtained by residue analysis of individual organisms in such environments. These studies could lead to predictive models on the effect of pesticides.

Sophisticated equipment is not necessarily required to obtain information on the effect and movement of pollutants in experimental environments (3). This investigator observed how Aroclor 1254, a polychlorinated biphenly, affected the composition of communities of estuarine animals in water that flowed through test aquaria. Communities of planktonic larvae that developed in control aquaria and aquaria that received one-tenth of a microgram of PCB per liter in the water were dominated (greater than 75 percent) by arthropods. The number of arthropods decreased and the number of chordates (tunicates) increased as the concentration increased from one to ten micrograms per liter; over 75 percent of the animals in 10 micrograms per liter aquaria were tunicates. Although species diversity was not altered, numbers of phyla, species and individuals were decreased by this PCB.

Environmental Studies

Much more information is needed on the manner in which an "unstressed" marine system operates before we can properly assess the impact of a pesticide or other chemicals on such systems. We require integrated scientific studies leading to the development of predictive models that could assess possible effects of specific environmental stresses. Often, it is necessary to make observations on the routes, rates and reservoirs of pesticides used in large scale applications in the environment after application is made. Such studies should not be termed "ecological" because time will not permit baseline data to be developed before the pesticide is applied.

Surveillance and analysis of residues in and near the application area can, however, give some insight concerning effect of the pesticide on non-target species. Such a study was made in Louisiana during aerial application of malathion to control mosquito vectors of Venezuelan equine encephalomyelitis (4). Fish were collected from spray areas a short time before, during and after the spraying operation. Acetylcholinesterase (AChE) activity in the brains of these fish was used as an indicator of the occurrence of malathion in the fish's environment. Levels of inhibition of AChE activity in fish from Lake Prien, Louisiana approached levels associated with death of fish in laboratory bioassay studies. However, the AChE level of this species of fish returned to normal within 40 days after the application of malathion. This is an example of how laboratory bioassays and field observations can be used to better understand the impact of a particular pesticide on the marine environment.

Future Challenge

The kinds and amounts of pesticides used in this country are changing and the change is reflected in production figures. Recently, production of herbicides and organophosphate insecticides has exceeded that of organochlorine insecticides. Pesticide applicators are substituting organophosphates and carbamates for more persistent chemicals, such as DDT. Also, much effort is being devoted to developing an integrated pest control procedure whereby biological and other control methods will play just as important a role as chemicals. Several companies are developing biological control organisms, such as viruses, and isolating juvenile insect hormones to be used in control programs for certain agricultural pests. This commendable strategy could result in a much "cleaner" environment.

We must be prepared to assess control methods before they gain widespread usage in the environment. In many instances, we can no longer depend upon routine monitoring methods to detect the presence of these biological control agents and new chemicals. Our concern must include the potential effect of the new agents on an environment that already contains residues of organochlorines and other persistent chemicals. □

Gulf Breeze Contribution No. 180

References

Brungs, William A. 1969. "Chronic Toxicity of Zinc to the Fathead Minnow, *Pimephales promelas* Rafinesque." *Trans. Am. Fish. Soc.*, 98(2): 272-279.

Mount, Donald I. 1968, "Chronic Toxicity of Copper to Fathead Minnows *Pimephales promelas* Rafinesque." *Water Research*, 2(3): 215-223.

Hansen, David J. (In press – Contributions in Marine Science, University of Texas). Aroclor 1254: Effect on Composition of Estuarine Animal Communities in the Laboratory.

Coppage, D. L. and T. W. Duke. 1972. Effects of Pesticides in Estuaries Along Gulf and Southeast Atlantic Coasts. *Proceedings of the 2nd Gulf Coast Conference on Mosquito Suppression and Wildlife Management*, New Orleans, La., October 20-22, 1971.

THOMAS W. DUKE is presently director of the Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida. The laboratory's mission is to study the effects of toxic organics, particularly pesticides, on marine organisms and their environment. Information developed at the laboratory is used in EPA's pesticide registration process and in setting water quality criteria for the marine environment. Dr. Duke has been employed by the Federal government since 1961. He received his Ph.D. in Oceanography from Texas A. & M. University and worked with the A. & M. Research Foundation for a year before joining the Federal government. Dr. Duke's research interest is in the field of estuarine ecology–particularly pollution ecology which includes studies of the movement of radioactive materials and pesticides in the estuarine environment.

CONTRIBUTION NO. 181

Avoidance of Aroclor® 1254 by Shrimp and Fishes[1]

by D. J. HANSEN, S. C. SCHIMMEL, and E. MATTHEWS

U.S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, Fla. 32561
(Associate Laboratory of the National Environmental Research Center, Corvallis, Ore.)

The polychlorinated biphenyl (PCB) Aroclor 1254 was found in Escambia River and Bay, which are parts of the estuary near our laboratory (DUKE et al. 1970). In laboratory experiments this chemical was toxic to certain mollusks (LOWE et al. 1972), arthropods (NIMMO et al. 1971) and fishes (HANSEN et al. 1971). Some invertebrates (HANSEN et al. 1973 and PORTMAN 1970) and fishes (HANSEN 1969; HANSEN et al. 1972; and SPRAGUE and DRURY 1969) possess the ability to avoid other toxic pollutants in water. Because it could be an advantage to mobile organisms in the river and bay if they could avoid toxic concentrations of Aroclor 1254, we conducted laboratory studies to determine if pink shrimp (Penaeus duorarum), grass shrimp (Palaemonetes pugio), pinfish (Lagodon rhomboides), sheepshead minnows (Cyprinodon variegatus) and mosquitofish (Gambusia affinis) could avoid water contaminated with 0.001, 0.01, 0.1, 1 or 10 mg/ℓ of the PCB.

Methods and Materials

Test animals were collected from local waters not contaminated by Aroclor 1254 and were acclimated to laboratory conditions for at least seven days before testing. If mortality exceeded 5% or abnormal behavior was observed in the 48 hours before a test the animals were not used. Pink shrimp were between 13 and 75 mm rostrum-telson length; grass shrimp 10-40 mm rostrum-telson length; mosquitofish and pinfish 20-50 mm standard length. Animals were not fed for 24-hours prior to testing.

The ability of these animals to avoid Aroclor 1254 was tested in a black plastic apparatus (HANSEN et al. 1972) in which animals could move from a holding area into either (1) a section that contained water with Aroclor 1254 or (2) a section that contained water without the PCB. Water maintained at 20° C, entered the upper end of each of the two sections at the rate of 400 ml/min and flowed to the drain in the holding area. Freshwater was used to test mosquitofish and 20 °/oo saltwater was used to test the other species. PCB dissolved in acetone was metered through stopcocks at 0.5 ml/min into the water entering one of the two sections. The same amount of solvent without PCB was metered into the other section.

Each species was tested at each concentration at least four times; twice with the PCB entering one section of the apparatus

[1] Contribution No. 181, Gulf Breeze Environmental Research Laboratory.

Bulletin of Environmental Contamination & Toxicology, Vol. 12, No. 2 © 1974 by Springer-Verlag New York Inc.

and twice with it entering the opposite section. For each of the four replications, 50 animals (except pink shrimp, when 25 animals were used for each replicate test) were placed in the holding area with a closed gate located at the junction of the holding area and the two sections. After 30 minutes the gate was opened, allowing access of animals to both sections. One hour later the gate was closed and the number of animals in each section was recorded. The apparatus was covered with black plastic during each test to shield the animals from external disturbances.

Concentrations of Aroclor 1254 selected were not lethal to any animal during the 1 1/2 hour avoidance study but 0.01 mg/ℓ was toxic to pink shrimp, (NIMMO et al. 1971) grass shrimp, (NIMMO et al. In press) sheepshead minnows (SCHIMMEL In press) and pinfish (HANSEN et al. 1971) in chronic bioassays conducted for longer periods of time at this laboratory.

Some of the concentrations in the sheepshead minnow and grass shrimp tests were checked by chemical analysis. Methods of chemical analyses were identical to those used by NIMMO et al 1971.

The ability of these animals to avoid Aroclor 1254 was evaluated by the chi-square test on the assumption that if there was no avoidance response to the PCB, animals that left the holding area would enter each section with equal frequency. Avoidance was considered significant if the probability that observed distributions would occur by chance was 0.01 or less. Animals remaining in the circular holding area after a test was completed were not included in the statistical analyses.

Results and Discussion

Grass shrimp, pinfish and mosquitofish avoided at least one concentration of Aroclor 1254 but pink shrimp and sheepshead minnows did not avoid any of the concentrations tested (Table 1). Grass shrimp and pinfish avoided 10 mg/ℓ and mosquitofish 0.1, 1 and 10 mg/ℓ of the PCB. In mosquitofish tests, PCB was added to fresh instead of salt water. Therefore, it is not known if the fish had a greater ability to avoid or if the response was affected by the test water.

This study demonstrates that some animals can avoid Aroclor 1254 in laboratory tests but we can only speculate on the possibility of avoidance of PCB's in the estuary. Concentrations of Aroclor avoided by mosquitofish in the laboratory have been found in the Escambia River near the source of a leak of this chemical (DUKE et al. 1970). Concentrations measured in water from other localities in the river and Escambia Bay never approached concentrations avoided by grass shrimp, pinfish or mosquitofish in the laboratory. If animals avoid because they sense the PCB in

TABLE 1

Capacity of aquatic animals to seek water free of the polychlorinated biphenyl, PCB, Aroclor 1254

Test Species	Aroclor 1254 Concentration (mg/ℓ)	Number of Animals* In PCB-Free Water	Number of Animals* In Water with PCB	Percentage In PCB-Free Water
Palaemonetes pugio	0.001	60	64	48.4
	0.01	55	64	46.2
	0.1**	65	56	53.7
	1.0**	57	62	47.9
	10.0**	91	51	64.1***
Penaeus duorarum	0.001	22	33	40.0
	0.01	34	34	50.0
	0.1	41	31	56.9
	1.0	33	36	47.8
	10.0	37	32	53.6
Cyprinodon variegatus	0.001	51	44	53.7
	0.01**	42	40	51.2
	0.1**	48	48	50.0
	1.0	49	48	50.5
	10.0**	43	55	43.9
Lagodon rhomboides	0.001	66	76	46.5
	0.01	55	48	53.4
	0.1	67	69	49.3
	1.0	121	119	50.4
	10.0	84	44	65.6***
Gambusia affinis	0.01	38	34	52.8
	0.1	66	27	71.0***
	1.0	66	26	71.7***
	10.0	43	13	76.8***

* Does not include animals in holding area at end of test.

** Nominal Concentration = Average Measured Concentration: 10.0 = 5.7, 1.0 = 0.48, 0.1 = 0.054, 0.01 = 0.033.

*** Statistically significant; χ^2-test, $\alpha = 0.01$.

water, it seems unlikely that contamination by this chemical altered the movements of these animals except immediately adjacent to the site of the leak. Animals may avoid because they sense the toxic effect of a PCB and move to reduce this effect. If so, concentrations that can be avoided may possibly be much lower than shown in these tests where fish were in the PCB for a maximum of only 1 1/2 hours.

References

DUKE, T. W., J. I. LOWE and A. J. WILSON, JR.: Bull. Environ. Contam. Toxicol. 5, 171 (1970).

HANSEN, D. J.: Trans. Amer. Fish. Soc. 98, 426 (1969).

HANSEN, D. J., P. R. PARRISH, J. I. LOWE, A. J. WILSON, JR. and P. D. WILSON: Bull. Environ. Contam. Toxicol. 6, 113 (1971).

HANSEN, D. J., E. MATTHEWS, S. L. NALL and D. P. DUMAS: Bull. Environ. Contam. Toxicol. 8 46 (1972).

HANSEN, D. J., S. C. SCHIMMEL and JAMES M. KELTNER, JR.: Bull. Environ. Contam. Toxicol. 9, 129 (1973).

LOWE, J. I., P. R. PARRISH, J. M. PATRICK, JR. and J. FORESTER: Mar. Biol. 17, 209 (1972).

NIMMO, D. R., R. R. BLACKMAN, A. J. WILSON, JR. and J. FORESTER: Mar. Biol. 11, 191 (1971).

NIMMO, D. R., J. FORESTER, P. T. HEITMULLER and GARY H. COOK: Bull. Environ. Contam. Toxicol. (In Press).

PORTMAN, J. E.: Marine Pollution and Sea Life, FAO 212 (1972).

SCHIMMEL, S. C.: Trans. Amer. Fish. Soc. (In Press).

SPRAGUE, J. B. and D. W. DRURY: Adv. Water Pollut. Res. Proc. Fourth Int. Conf., 169 (1969).

®Registered trademark, Monsanto Company, St. Louis, Mo. Mention of trade names does not constitute endorsement by the Environmental Protection Agency.

CONTRIBUTION NO. 183

BIOMETRICS 30, 547–551
SEPTEMBER 1974

Reprinted from
BIOMETRICS Copyright © 1974
THE BIOMETRIC SOCIETY, Vol. 30, No. 3, September 1974

365: LESLIE MATRIX MODELS FOR FISHERIES STUDIES

A. L. JENSEN[1]

Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory,[2] Sabine Island, Gulf Breeze, Florida 32561, U.S.A.

SUMMARY

Two modifications of the Leslie matrix model are developed. In the first modification the egg stage as well as the age groups of a fish population are included in the vector of state. In the second modification only the recruited members of the population are included in the vector of state.

1. INTRODUCTION

In the Leslie Matrix Model (Leslie [1945; 1948]) the vector of the number of individuals of each age at time t, $\mathbf{N}_t$, is related to the vector of the initial number of individuals of each age, $\mathbf{N}_0$, by the equation,

$$\mathbf{N}_t = \mathbf{M}^t\mathbf{N}_0 , \tag{1}$$

where $\mathbf{M}$ is the population projection matrix,

$$\mathbf{M} = \begin{bmatrix} B_0 & B_1 & B_2 & \cdots & \cdot & B_{v-1} & B_v \\ P_0 & 0 & 0 & \cdots & \cdot & 0 & 0 \\ 0 & P_1 & 0 & \cdot\cdot & \cdot & 0 & 0 \\ 0 & 0 & & & & 0 & 0 \\ \cdot & \cdot & & & & \cdot & \cdot \\ \cdot & \cdot & & & & \cdot & \cdot \\ 0 & 0 & 0 & \cdot\cdot & 0 & P_{v-1} & 0 \end{bmatrix} \tag{2}$$

In matrix $\mathbf{M}$, B_x equals the number of females born to females of age x in one unit of time that survive to the next unit of time, P_x equals the proportion of females of age x at time t that survive to time $t + 1$, and v is the greatest age attainable. Equation (1) has frequently been applied in demographic and animal population studies (Keyfitz [1968], Pielou [1969], Usher [1971]). Jensen [1971] has applied equation (1) to a fish population.

Equation (1) is similar to the simple exponential model for population growth. Leslie [1959] proposed a modified matrix model to allow for the effect of population density on population growth. He divided each element in the population projection matrix by a quantity that depended on the size of the current population and the size of the population when the individuals were born. Several other modifications of the matrix model have been proposed. Williamson [1959] and Goodman [1968; 1969] modified the matrix model to include both sexes, and Lefkovitch [1965] developed a modification for organisms grouped

[1] Present address: School of Natural Resources, University of Michigan, Ann Arbor, Michigan 48104
[2] Associate laboratory of the National Environmental Research Center, Corvallis, Oregon

by life stages rather than age. Goodman [1969] developed a general class of models that can be applied to organisms grouped by life stages. Usher [1969] modified the model for study of forest trees which are classified by size rather than by age.

In this note two modifications of the matrix model are proposed: (1) a model for fish populations in which individuals are grouped by a life stage as well as by age, and (2) a model for the recruited members of a fish population. Goodman's [1969] general class of models can also include both life stages and age. A fish is recruited when it becomes large enough to be vulnerable to fishing gear. This group of fish is of importance because often data are available only for the age groups captured by a fishery.

A detailed analysis of fisheries data requires separation of males and females as in actuarial science and demography, but this is not often practiced in fisheries science because the limited data do not generally allow such a detailed analysis. It will be assumed that the number of females equals the number of males and that growth and mortality rates of males and females are equal, but the models also apply to either sex alone.

2. ONE LIFE STAGE AND AGE GROUPS

It is assumed that the eggs hatch in the time period after the adults spawn. For example, if the time period is one year and the fish spawn in the fall, the eggs hatch the following spring. A similar model can be constructed for species in which the adults spawn and the eggs hatch during the same year.

The number of eggs produced at time $t - 1$ is proportional to the sizes of the age groups at time $t - 1$, i.e.,

$$E(t-1) = (h_0, h_1, \cdots, h_v)\begin{bmatrix} N(0, t-1) \\ N(1, t-1) \\ \\ \\ N(v, t-1) \end{bmatrix}, \tag{3}$$

where some of the constants h_i, $i = 0, 1, 2, \cdots, v$, may be zero. The constant h_i is the number of eggs produced by the population per individual of age i. The size of the zero age group at time t is a function of the number of eggs produced at time $t - 1$, and the size of each nonzero age group at time t is a function of the size of the preceding age group at time $t - 1$, i.e.,

$$\begin{bmatrix} N(0, t) \\ N(1, t) \\ \\ \\ \\ N(v, t) \end{bmatrix} = \begin{bmatrix} S_0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & S_1 & & & & \cdot \\ 0 & 0 & & & & \\ \cdot & & & & & \\ & & & & \cdot & \\ 0 & 0 & & 0 & 0 & 0 \\ 0 & 0 & \cdots & 0 & S_v & 0 \end{bmatrix} \begin{bmatrix} E(t-1) \\ N(0, t-1) \\ N(1, t-1) \\ \\ \\ N(v, t-1) \end{bmatrix} \tag{4}$$

The survival function S_0 gives the proportion of the eggs that hatch, and the survival functions S_i, $i = 1, 2, \cdots, v$, give the proportion of individuals that survive from age

$i - 1$ to age i. The survival functions may be functions of time. Mathematical forms of these survival functions are discussed by Ricker [1954], Beverton and Holt [1957], Paulik and Greenough [1966], and Pennycuick *et al.* [1968].

Equations (3) and (4) can be combined into a single equation. Multiplication of the matrices,

$$\begin{bmatrix} S_0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & S_1 & & & 0 & 0 \\ 0 & 0 & & & 0 & 0 \\ \cdot & & & & & \\ & & & & \cdot & \cdot \\ & & & & 0 & 0 \\ 0 & 0 & \cdot & 0 & S_v & 0 \end{bmatrix} \cdot \begin{bmatrix} h_0 & h_1 & \cdots & & h_{v-1} & h_v \\ 1 & 0 & \cdots & \cdot & 0 & 0 \\ 0 & 1 & & & 0 & 0 \\ 0 & 0 & & & 0 & 0 \\ & & & & & \\ & \cdot & & & & \\ 0 & 0 & \cdots & 0 & 1 & 0 \\ 0 & 0 & \cdots & 0 & 0 & 0 \end{bmatrix} \tag{5}$$

gives the matrix,

$$\mathbf{A} = \begin{bmatrix} h_0S_0 & h_1S_0 & \cdots & h_{v-2}S_0 & h_{v-1}S_0 & h_vS_0 \\ S_1 & 0 & \cdots & 0 & 0 & 0 \\ 0 & S_2 & & 0 & 0 & 0 \\ 0 & 0 & & 0 & 0 & 0 \\ \cdot & \cdot & & \cdot & \cdot & \\ & \cdot & & & \cdot & \\ 0 & 0 & & 0 & 0 & 0 \\ 0 & 0 & & S_{v-1} & 0 & 0 \\ 0 & 0 & & 0 & S_v & 0 \end{bmatrix} . \tag{6}$$

Applying equation (6), the equation for population projection (equation 1) becomes,

$$\mathbf{N}_t = \mathbf{A}^t\mathbf{N}_0 \tag{7}$$

The above analysis shows that the matrix for population projection, **A**, is the product of: (1) a survival matrix which represents movement of individuals out of the life stage and among the age groups, and (2) a reproduction matrix which represents input of new individuals to the population.

Applying equation (7), the discrete age-discrete time equation for annual yield from a fishery (Jensen [1971]) becomes,

$$Y_t = \sum_{x=0}^{v} y_t(x), \tag{8}$$

where x is age in years, and $y_t(x)$ is the xth element in the vector $\mathbf{Y}_t$,

$$\mathbf{Y}_t = \mathbf{FWA}^t\mathbf{N}_0 \tag{9}$$

In equation (9), **F** is a diagonal matrix with the age specific mortality rates $F(x)$ on the diagonal and **W** is a diagonal matrix with the age specific weights $W(x)$ on the diagonal.

3. THE RECRUITED POPULATION

Equation (8) is of more practical value in fisheries studies if only age groups in the recruited population are considered. The Leslie Matrix Model cannot be directly applied to only the recruited population. The number of recruits at time t depends on the number of eggs produced by the population at some previous time $t - r$, where r is the age of the recruits. The sizes of all other age groups in the recruited population depend on the sizes of the preceding age group in the previous unit of time. Hence, recruitment and mortality of the recruited population must be separated.

Both Usher [1966] and Goodman [1969] have shown that the population projection matrix is the sum of two matrices,

$$\mathbf{M} = \mathbf{R} + \mathbf{D}. \tag{10}$$

The first matrix represents input of new members to the population and the second matrix represents transition of members between the age groups. For application to a recruited fish population, the square matrices $\mathbf{R}$ and $\mathbf{D}$ can be defined as:

$$\mathbf{R} = \begin{bmatrix} h_r S_r & h_{r+1} S_r & \cdots & h_v S_r \\ 0 & 0 & \cdots & 0 \\ \cdot & & & \cdot \\ \cdot & & & \\ 0 & 0 & \cdots & 0 \end{bmatrix} \tag{11}$$

$$\mathbf{D} = \begin{bmatrix} 0 & 0 & \cdots & 0 & 0 & 0 \\ S_{r+1} & 0 & \cdots & 0 & 0 & 0 \\ 0 & S_{r+2} & & & 0 & 0 \\ 0 & 0 & & & 0 & 0 \\ & & & & \cdot & \\ & & & & & \\ 0 & 0 & & & 0 & 0 \\ 0 & 0 & & 0 & S_v & 0 \end{bmatrix} \tag{12}$$

The function S_r gives survival from the egg to recruitment, and the functions S_{r+i}, $i = 1, 2, \cdots, v$, give survival from age $r + i - 1$ to age $r + i$. Mathematical forms of the survival function S_r are discussed by Ricker [1954], Beverton and Holt [1957], and Paulik and Greenough [1966].

Applying equations (10), (11), and (12) the vector of the number of individuals of each age at time t for the recruited population becomes,

$$\mathbf{N}_t = \mathbf{R}\mathbf{N}_{t-r} + \mathbf{D}\mathbf{N}_{t-1} \tag{13}$$

Applying equation (13), the discrete age-discrete time equation for annual yield from a fishery becomes,

$$Y_t' = \sum_{x=r}^{v} y_t'(x) \tag{14}$$

where $y_t'(x)$ is the xth element in the vector $\mathbf{Y}_t'$,

$$\mathbf{Y}_t' = \mathbf{F}\mathbf{W}(\mathbf{R}\mathbf{N}_{t-r} + \mathbf{D}\mathbf{N}_{t-1}). \tag{15}$$

LES MODÈLES DE MATRICE DE LESLIE POUR LES ÉTUDES DE PÊCHERIES

RESUME

On décrit deux modifications au modèle matriciel de Leslie. Dans la première on inclut dans le vecteur d'état le stade des oeufs aussi bien que les groupes d'âge de la population de poisson. Dans la seconde on n'inclut dans le vecteur d'état que les nouveaux membres de la population.

REFERENCES

Beverton, R. J. H., and Holt, S. J. [1957]. *On the Dynamics of Exploited Fish Populations*. H. M. S. O., London.

Goodman, L. A. [1968]. Stochastic models for the population growth of the sexes. *Biometrika 55*, 469–87.

Goodman, L. A. [1969]. The analysis of population growth when the birth and death rates depend on several factors. *Biometrics 25*, 659–81.

Jensen, A. L. [1971]. The effect of increased mortality on the young in a population of brook trout, a theoretical analysis. *Trans. Amer. Fish. Soc. 100*, 456–9.

Keyfitz, N. [1968]. *Introduction to the Mathematics of Population*. Addison-Wesley, Reading, Massachusetts.

Lefkovitch, L. P. [1965]. The study of population growth in organisms grouped by stages. *Biometrics 21*, 1–18.

Leslie, P. H. [1945]. On the use of matrices in certain population mathematics. *Biometrika 33*, 183–222.

Leslie, P. H. [1948]. Some further notes on the use of matrices in population mathematics. *Biometrika 35*, 213–45.

Leslie, P. H. [1949]. The properties of a certain lag type of population growth and the influence of an external random factor on a number of such populations. *Physiological Zool. 32*, 151–9.

Paulik, G. J., and Greenough, J. W. [1966]. Management analysis for a salmon resource system. In *Systems Analysis in Ecology*. Watt, K. E. F. (Ed.), Academic Press, New York.

Pielou, E. C. [1969]. *An Introduction to Mathematical Ecology*, Wiley-Interscience, New York.

Pennycuick, C. J., Compton, R. M., and Beckingham, L. [1968]. A computer model for simulating the growth of a population, or of two interacting populations. *J. Theoret. Biol. 18*, 316–29.

Ricker, W. E. [1954]. Stock and recruitment. *J. Fish. Res. Bd.* Canada. 11, 559–623.

Usher, M. B. [1969]. A matrix model for forest management. *Biometrics 25*, 309–15.

Usher, M. B. [1971]. Developments in the Leslie Matrix Model. In: *Mathematical Models in Ecology*. Jeffers, J. N. R. (Ed.), Blackwell Scientific, London.

Williamson, M. H. [1959]. Some extensions of the use of matrices in population theory. *Bull. Math. Biophys. 27*, 13–7.

Received February 1973, *Revised October* 1973

Key Words: Population dynamics; Leslie matrix models; Deterministic models; Recruited fish populations.

Predator–Prey and Competition Models with State Variables: Biomass, Number of Individuals, and Average Individual Weight

A. L. JENSEN[1]

Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Fla. 32561, USA[2]

JENSEN, A. L. 1974. Predator–prey and competition models with state variables: biomass, number of individuals, and average individual weight. J. Fish. Res. Board Can. 31: 1669–1674.

Applying the identity that biomass equals number of individuals multiplied by average individual weight, simultaneous equations for change with respect to time in biomass, number of individuals, and average individual weight are obtained for Kostitzin's predator–prey equations and for the Lotka–Volterra competition equations. By the same procedure applied here, simultaneous equations for these three variables can be obtained for other predator–prey and competition equations These equations can be applied to determine the biomass, number of individuals, and average individual weight of interacting fish populations under different rates of exploitation.

JENSEN, A. L. 1974. Predator–prey and competition models with state variables: biomass, number of individuals, and average individual weight. J. Fish. Res. Board Can. 31: 1669–1674.

A partir du postulat que la biomasse est égale au nombre d'individus multiplié par leur poids moyen, on obtient des équations simultanées représentant les changements dans le temps de la biomasse, du nombre d'individus et du poids moyen des individus pour les équations prédateur–proie de Kostitzin et les équations de Lotka–Volterra relatives à la concurrence. Une procédure semblable, appliquée ici, permet de formuler des équations simultanées pour ces trois variables applicables à d'autres modèles prédateur–proie et concurrence. Ces équations peuvent servir à déterminer la biomasse, le nombre d'individus et le poids individuel moyen de populations de poissons agissant les unes sur les autres, lorsque celles-ci sont soumises à des taux d'exploitation différents.

Received January 29, 1973
Accepted July 10, 1974

Reçu le 29 janvier, 1974
Accepté le 10 juillet, 1974

ALL species that are fished, live in close association with other species, and studies of interactions among fish species are important to fisheries management (Larkin 1963, 1966). Larkin (1956, 1963, and 1966) has applied predator–prey and competition models to determine the theoretical result of exploiting either or both of a pair of competing species and either or both of a predator–prey pair.

Application of the classical predator–prey and competition models to fisheries studies results in surplus production models for yield in which growth, reproduction, and natural mortality of the interacting species are all combined in a common expression. Larkin's studies were done using the number of individuals of each species as the vector of state. The decision to construct models for fish population interactions in terms of number of individuals rather than in terms of biomass is an important decision. Jensen (1972) has shown that because biomass, B, number of individuals, N, and average individual weight, $\bar{W}$, are related by the identity,

$$B(t) = N(t)\ \bar{W}(t), \qquad (1)$$

where t is time; independent models cannot be simultaneously constructed for these three variables. Jensen (1972) has derived simultaneous equations for biomass, number of individuals, and average individual weight implied by Schaefer's surplus production equation. He showed that if change with respect to time in biomass of a population is

[1]Present address: School of Natural Resources, Natural Resources Building, University of Michigan, Ann Arbor, Mich. 48104, USA.

[2]Associate Laboratory of the National Environmental Research Center, Corvallis, Oreg.

Printed in Canada (J2819)
Imprimé au Canada (J2819)

described by the logistic equation, then change in number of individuals with respect to time can be described by the logistic equation only if there is no change with respect to time in average individual weight or if change in number of individuals is independent of change in average individual weight. These are not realistic assumptions for fish populations.

In this note simultaneous equations for biomass, number of individuals, and average individual weight are developed for the Lotka (1956) and Volterra (1928) competition equations, and for Kostitzin's (1939) predator–prey equations. Larkin (1963) applied the Lotka–Volterra equations to study interspecific competition and exploitation, and Larkin (1966) applied Kostitzin's predator–prey equations to study exploitation in a predator–prey situation. Larkin (1966) concluded that Kostitzin's predator–prey equations are more realistic for fish populations than the Lotka–Volterra predator–prey equations. Fish prey on a variety of organisms and extinction of a single prey species may not lead to extinction of the fish population. In the Lotka–Volterra predator–prey model extinction of the predator results in exponential growth of the prey and extinction of the prey results in extinction of the predator. In Kostitzin's predator–prey equations the population density of the prey has an upper limit in the absence of the predator, and the population density of the predator has a lower limit in the absence of the prey. Kostitzin's predator-prey equations are nearly identical in mathematical form to the Lotka–Volterra competition equations. The method illustrated here to obtain equations for biomass, number of individuals, and average individual weight can be applied to obtain similar equations for other predator–prey and competition models.

Mathematical symbols are listed below; the first species is the prey species or the first of two competing species, and the second species is a predator species or the second of two competing species.

b_1, b_2 = coefficients of interaction between first and second species

B_1, B_2 = biomass of first and second species

F_1, F_2 = fishing mortality coefficients for biomass of first and second species

F_3, F_4 = fishing mortality coefficients for number of individuals of first and second species

F_5, F_6 = fishing mortality coefficients for average individual weight of first and second species

g_1, g_2 = coefficient of growth in average individual weight of first and second species

h_1, h_2 = coefficient of intraspecific interaction for first and second species

k_1 = coefficient of increase in number of individuals of first species

k_2 = coefficient of increase in number of individuals of second species in competition model and coefficient of mortality of predator in predator–prey model

N_1, N_2 = number of individuals of first and second species

p_1, p_2 = coefficients of intraspecific interaction for first and second species

q_1, q_2 = coefficients of interaction between first and second species

r_1 = coefficient of biomass increase of first species

r_2 = coefficient of biomass increase of second species in competition model and coefficient of negative feedback for biomass of predator in predator–prey model

u_1, u_2 = coefficient of interaction between first and second species

$\overline{W}_1$, $\overline{W}_2$ = average individual weight of first and second species

Relation among biomass, number of individuals, and average individual weight — Differentiation of equation (1) with respect to time gives the equation,

$$\frac{dB}{dt} = N\frac{d\overline{W}}{dt} + \overline{W}\frac{dN}{dt}. \qquad (2)$$

If equations are specified for $d\overline{W}/dt$ and dN/dt, the equation for dB/dt is implicitly specified by equation (2). If equations are constructed independently for both $d\overline{W}/dt$ and dN/dt, changes in average individual weight and number of individuals are independent. Such equations imply two independent upper limits — one that determines the maximum number of individuals and one that determines the maximum average individual weight (Jensen 1972). If an equation relating growth to environmental carrying capacity is constructed for biomass there is a single upper limit, termed the environmental carrying capacity. An equation with

a single upper limit and mutual dependence of number of individuals and average individual weight provides a more realistic description of fish populations than a model with two upper limits and mutual independence of average individual weight and number of individuals. Therefore, the Lotka–Volterra competition equations and Kostitzin's predator–prey equations are developed in terms of biomass; then mutually dependent equations, corresponding to the biomass equations, are developed for number of individuals and average individual weight.

The equations for change in number of individuals and the equations for change in average individual weight are not uniquely determined by equation (2) and the equation for biomass change. To develop equations for change in number of individuals and equations for change in average individual weight it is necessary to make additional assumptions. These assumptions concern: 1) the form of the equations for number of individuals, 2) the mutual dependence of average individual weight and number of individuals, and 3) the patterns of algebraic signs in the equations for change in average individual weight. Many different systems of simultaneous equations, which correspond to different assumptions, can be derived for biomass, number of individuals, and average individual weight. The systems of equations derived in this note are simple systems in which the mathematical form of the equations for dB/dt, dN/dt, and $d\overline{W}/dt$ are similar, $d\overline{W}/dt$ does not equal zero, and N_1, N_2, $\overline{W}_1$, and $\overline{W}_2$ are all interrelated.

It is noteworthy that the equations for dN/dt and dB/dt have been tested with empirical observations. The equations for $d\overline{W}/dt$ have not previously appeared in the literature, and these equations have not been tested with empirical observations. Although the form of the equations for $d\overline{W}/dt$ result from the form of the equations for dN/dt and dB/dt, the equations for $d\overline{W}/dt$ must be considered as untested hypotheses until they are tested with empirical observations.

Predator–prey and competition models have been applied interchangeably to biomass and number of individuals. Therefore, it appears reasonable when developing simultaneous equations for biomass, number of individuals, and average individual weight, to assume that the equations for change in number of individuals are of the same mathematical form as the equations for change in biomass. For change in average individual weight and change in number of individuals to be mutually dependent both the equation for change in number of individuals and the equation for change in average individual weight must contain terms for average individual weight and number of individuals. The above assumptions, together with equation (2), uniquely determine the equations for change in number of individuals. For example, if biomass is defined by the logistic equation, $dB/dt = a_1B - a_2B^2$, then, to be of the same mathematical form as the biomass equation, contain both number of individuals and average individual weight, and satisfy equation (2), the equation for change in number of individuals must be $dN/dt = b_1N - b_2N^2\overline{W}$.

When the equations for dB/dt and dN/dt are specified, the form of the equation for $d\overline{W}/dt$ is implicitly specified to within a narrow degree, and only a small amount of biological information is necessary to complete its specification. For example, if for the logistic equation biomass and number of individuals are described by the equations given above, to satisfy equation (2) average individual weight must be given by $d\overline{W}/dt = 0$, $d\overline{W}/dt = \pm c\overline{W}$, or $d\overline{W}/dt = \pm c_1\overline{W} \pm c_2\overline{W}^2N$. Only the last equation for dW/dt satisfies the condition that N and $\overline{W}$ are mutually dependent. It is biologically realistic to assume that input to average individual weight is proportional to average individual weight and that an increase in number of individuals results in a more intense intraspecific competition and a lower rate of change in average individual weight. Therefore, the equation for change in average individual weight is $d\overline{W}/dt = c_1\overline{W} - c_2\overline{W}^2N$.

Kostitzin's predator–prey equations — Under the assumptions: 1) the equations for change in number of individuals are of the same form as the equations for change in biomass, 2) the variables N_1, N_2, $\overline{W}_1$, and $\overline{W}_2$ are interrelated, and 3) selection of algebraic signs in the equations for change in average individual weight is correct, application of equation (2) shows that Kostitzin's predator–prey equations for change in biomass, number of individuals, and average individual weight are:

$$\begin{aligned}
dB_1/dt &= r_1B_1 - a_1B_1^2 - b_1B_1B_2 - F_1B_1\\
dB_2/dt &= -r_2B_2 - a_2B_2^2 + b_2B_1B_2 - F_2B_2\\
dN_1/dt &= k_1N_1 - p_1B_1N_1 - q_1N_1B_2 - F_3N_1\\
dN_2/dt &= -k_2N_2 - p_2N_2B_2 + q_2N_2B_1 - F_4N_2\\
d\overline{W}_1/dt &= g_1\overline{W}_1 - h_1\overline{W}_1B_1 \pm u_1\overline{W}_1B_2 - F_5\overline{W}_1\\
d\overline{W}_2/dt &= g_2\overline{W}_2 + u_2\overline{W}_2B_1 - h_2\overline{W}_2B_2 - F_6\overline{W}_2.
\end{aligned} \qquad (3)$$

The constants are related by the equations: $r_1 = k_1 + g_1$, $r_2 = k_2 - g_2$, $a_1 = p_1 + h_1$, $a_2 = p_2 + h_2$, $b_1 = q_1 \pm u_1$, $b_2 = q_2 + u_2$, $F_1 = F_3 + F_5$, and $F_2 = F_4 + F_6$. Under equilibrium conditions, where all of the derivatives are zero, solution of

equations (3) for B_1 shows the following additional constraints on the constants,

$$\frac{F_1 - r_1 + b_1B_2}{-a_1} = \frac{F_2 + r_2 + a_2B_2}{b_2} = \frac{F_3 - k_1 + q_1B_2}{-p_1} =$$

$$\frac{F_4 + k_2 + p_2B_2}{-q_2} = \frac{F_5 - q_1 \pm u_1B_2}{-h_1} = \frac{F_6 - g_2 + h_2B_2}{u_2},$$

where $B_2 = (-r_2a_1 - b_2F_1 + r_1b_2 - F_2a_1)/(a_1a_2 + b_1b_2)$.

In equations (3), the biomass equations are identical to the equations obtained when Kostitzin's predator–prey equations are applied to biomass alone. But the equations for change in number of individuals are different from the equations that would be obtained if Kostitzin's equations had been applied to number of individuals alone. Both the equation for change in number of prey and the equation for change in number of predators contain terms for prey biomass and predator biomass.

In the equation for change in number of prey, the birth rate of the prey is proportional to the number of prey. Mortality of the prey depends on both the product of the number of prey with prey biomass and the product of the number of prey with predator biomass.

In the equation for change in number of predators, the birth rate of the predator depends on the product of the number of predators with prey biomass. Mortality of the predator depends both on the number of predators and the product of the number of predators with predator biomass.

These equations for change in number of prey and for change in number of predators are biologically realistic. Birth rate of the prey is proportional to the number of prey. Mortality of the prey increases with either an increase in the number of prey or with an increase in prey biomass. Higher prey biomass and a larger prey population density both result in more intense intraspecific competition. Mortality of the prey also increases with predator biomass. A larger predator biomass requires a larger amount of food.

For both natural and experimental fish populations it has been established that average individual weight is related to the number of individuals (Nikolskii 1969; Swingle and Smith 1941). And the carrying capacity of an environment for fish appears to be related to the biomass of the fish population rather than to the number of individuals (Swingle and Smith 1941). Equations for change in number of individuals that are a function of biomass as well as a function of number of individuals are, therefore, more complete and realistic than equations that do not contain terms for biomass.

If Kostitzin's equations had been applied to number of individuals alone, mortality of the prey would have been a function of neither predator biomass nor prey biomass. But if average individual weight of the predator and prey are not constant, the number of prey does not accurately determine the amount of food the prey supply the predator and the number of predators does not accurately determine the food requirements of the predators.

In equations (3), the equations for both biomass and number of individuals are similar to the equations obtained when Kostitzin's equations are applied to each of these variables alone, and the equations for each variable alone have been thoroughly investigated both biologically (Gause 1964) and mathematically (Goel et al. 1971). Equations for change in average individual weight of a predator and its prey have not been previously proposed, and they must be considered more carefully than the equations for change in biomass and number of individuals.

The equations for change in average individual weight cannot be developed independently of the equations for biomass and number of individuals. The equations for change in average individual weight are developed by: 1) specifying the equations for change in biomass and the equations for number of individuals, 2) the requirement that the variables be mutually dependent, and 3) selection of algebraic signs based on biological assumptions.

The mathematical form of the equations for change in average individual weight is the same as the mathematical form of the equations for biomass and number of individuals, but the algebraic signs are different. Equations (3) indicate that an increase in the prey biomass results in more intense intraspecific competition and a slower change in average individual weight of prey. The equation for change in average individual weight of the prey also contains the term $\pm u_1\bar{W}_1B_2$. The sign of this term is positive if predators select larger prey, the sign is negative if predators select smaller prey, and this term is zero if on the average predators select all size-groups of prey equally often.

Change in average individual weight of the predator depends on both average individual weight of the predator and the product of average individual weight of the predator with biomass of prey; this second term realistically indicates that change in average individual weight of the predator depends on the biomass of food available. Loss in average individual weight of the predator depends on the product of the average individual weight of the predator with predator biomass. This term realistically indicates that as predator biomass increases,

intraspecific competition increases and change in average individual weight decreases.

Lotka–Volterra competition equations — Under the assumptions applied to obtain equations (3), application of equation (2) shows that the Lotka–Volterra competition equations for change in biomass, number of individuals, and average individual weight are:

$$\begin{aligned}
dB_1/dt &= r_1B_1 - a_1B_1^2 - b_1B_1B_2 - F_1B_1 \\
dB_2/dt &= r_2B_2 - a_2B_2^2 - b_2B_1B_2 - F_2B_2 \\
dN_1/dt &= k_1N_1 - p_1B_1N_1 - q_1N_1B_2 - F_3N_1 \\
dN_2/dt &= k_2N_2 - p_2N_2B_2 - q_2N_2B_1 - F_4N_2 \\
d\bar{W}_1/dt &= g_1\bar{W}_1 - h_1\bar{W}_1B_1 - u_1\bar{W}_1B_2 - F_5\bar{W}_1 \\
d\bar{W}_2/dt &= g_2\bar{W}_2 - h_2\bar{W}_2B_2 - u_2\bar{W}_2B_1 - F_6\bar{W}_2.
\end{aligned} \tag{4}$$

The constants are related by the equations: $r_1 = k_1 + g_1$, $r_2 = k_2 + g_2$, $a_1 = p_1 + h_1$, $a_2 = p_2 + h_2$, $b_1 = q_1 + u_1$, $b_2 = q_2 + u_2$, $F_1 = F_3 + F_5$, and $F_2 = F_4 + F_6$. Under equilibrium conditions, where all of the derivatives are zero, solution of equations (4) for B_1 shows restrictions on the constants of equations (4) similar to those obtained for equations (3).

In equations (4), the equations for change in biomass are identical to the equations obtained when the Lotka–Volterra competition equations are applied to biomass alone. The equations for change in number of individuals and the equations for change in average individual weight are different from the equations that would be obtained by application of the Lotka–Volterra competition equations to either variable alone.

The equation for change in population density of the first species contains the intraspecific competition term $p_1B_1N_1$ and the equation for change in population density of the second species contains the intraspecific competition term $p_2B_2N_2$. These terms indicate that the intensity of intraspecific competition depends on both the number of individuals and the biomass of the species. The equations for change in population density of the first species contains the interspecific competition term $q_1N_1B_2$ and the equation for change in population density of the second species contains the interspecific competition term $q_2N_2B_1$. These terms indicate that the intensity of interspecific competition depends on the number of individuals of the species and on the biomass of the competing species.

In equations (4), the change in average individual weight of both competing species is proportional to their average individual weight. Loss in average individual weight of each species depends on the biomass of both species; as the biomass of both species increases, both intraspecific and interspecific competition increase. Equations (4) are more realistic for fish populations than the equations obtained when the Lotka–Volterra equations are applied to number of individuals alone. Changes in number of individuals are related to population biomass. Equations (4) contain terms for biomass, whereas equations obtained when the Lotka–Volterra model is applied to number of individuals alone do not contain terms for biomass.

Discussion — Relations among biomass, number of individuals, and average individual weight are important in fisheries biology. For fish populations the environmental carrying capacity is the maximum biomass that the environment can sustain, and the same resources can support large populations of small fish or small populations of large fish of the same species (Nikolskii 1969; Swingle and Smith 1941). Fishing a population decreases the number of fish and, by selection of older and larger individuals, decreases the average individual weight. Expanding surplus production models to include number of individuals and average individual weight as well as biomass may, therefore, increase the usefulness of these models for fisheries management.

The expanded models can be applied to determine number of individuals, biomass, and average individual weight for predator–prey and competing species that result from different exploitation rates. For example, for Kostitzin's predator–prey model at equilibrium under average environmental conditions,

$$B_1 = \frac{F_1a_1a_2 - r_1a_1a_2 - r_2a_1b_1 - a_1b_1F_2}{-a_1(a_1a_2 + b_1b_2)}$$

$$B_2 = \frac{b_2r_1 - r_2a_1 - b_2F_1 - a_1F_2}{a_1a_2 + b_1b_2}$$

$$N_1 = \frac{F_1a_1a_2 - r_1a_1a_2 - r_2a_1b_1 - a_1b_1F_2}{-a_1\bar{W}_1(a_1a_2 + b_1b_2)}$$

$$N_2 = \frac{b_2r_1 - r_2a_1 - b_2F_1 - a_1F_2}{\bar{W}_2(a_1a_2 + b_1b_2)}$$

$$\bar{W}_1 = \frac{F_1a_1a_2 - r_1a_1a_2 - r_2a_1b_1 - a_1b_1F_2}{-a_1N_1(a_1a_2 + b_1b_2)}$$

$$\bar{W}_2 = \frac{b_2r_1 - r_2a_1 - b_2F_1 - a_1F_2}{N_2(a_1a_2 + b_1b_2)}$$

The above equations are as easily applied as the surplus production equations for biomass alone, but data are needed for number of individuals and average individual weight as well as for biomass.

Accurate estimation of the constants in equations (3) and (4) is difficult. A linearization and curve fitting procedure similar to the one applied by Schaefer (1957) to estimate the constants in the Schaefer surplus-production model can be applied to estimate the constants in equations (3) and (4). The equations are linearized by estimation of the derivatives with the two point formula (Schaefer 1957; Hamming 1962). Schaefer's method of data partition does not produce unique estimates of the constants (Pella and Tomlinson 1969). Least squares can be applied to the linearized equations to estimate the constants as illustrated by Pella and Tomlinson (1969). The two point formula for linearization results in underestimation of the derivative (Hamming 1962; Pella and Tomlinson 1969) and as a general rule numerical integration should be applied instead of numerical differentiation whenever possible (Hamming 1962). Pella and Tomlinson (1969) have developed a method for approximation of the general surplus-production model in which numerical integration is applied instead of numerical differentiation. Equations (2) and (3) cannot, however, be solved in closed form and the method of Pella and Tomlinson cannot be applied.

In summary, biomass, number of individuals, and average individual weight are mutually dependent variables. For most fish populations the environmental carrying capacity is the maximum biomass sustainable by the environment rather than the maximum number of individuals. Population models for fish should, therefore, be constructed in terms of biomass or in terms of biomass and number of individuals. Surplus production models that are constructed for number of individuals alone are not more easily constructed than models for biomass alone, and they ignore the interrelations among average individual weight, biomass, and number of individuals.

Data for application of competition and predator–prey equations to natural or experimental fish populations do not appear to exist. This lack of data limits application of models for interactions of exploited fish populations to theoretical considerations such as those of Larkin (1956, 1963, and 1966). Continued development of models for interaction among species is necessary for theoretical studies, and the models can also serve as guides for the design of studies on experimental and natural fish populations.

Acknowledgments — I thank J. J. Pella for useful criticism of an earlier version of this note, for indicating several errors, for indicating the need to discuss the development of the equations in more detail, and for indicating the restrictions on the parameters. W. W. Fox Jr. gave helpful criticism of a manuscript closely related to this one.

GAUSE, G. F. 1964. The struggle for existence. Hafner, New York, N.Y. (*Originally published 1934*)

GOEL, N. S., S. C. MAITRA, AND E. W. MONTROLL. 1971. On the Volterra and other nonlinear models of interacting populations. Academic Press, New York, N.Y. 145 p.

HAMMING, R. W. 1962. Numerical methods for scientists and engineers. McGraw-Hill, New York, N.Y.

JENSEN, A. L. 1972. Population biomass, number of individuals, average individual weight, and the linear surplus-production model. J. Fish. Res. Board Can. 29: 1651–1655.

KOSTITZIN, V. A. 1939. Mathematical biology. Harrap, London. 411 p.

LARKIN, P. A. 1956. Interspecific competition and population control in freshwater fish. J. Fish. Res. Board Can. 13: 327–342.

1963. Interspecific competition and exploitation. J. Fish. Res. Board Can. 20: 647–678.

1966. Exploitation in a type of predator–prey relationship. J. Fish. Res. Board Can. 23: 349–356.

LOTKA, A. J. 1956. Elements of mathematical biology. Dover Publications, New York, N.Y. (*Originally published 1925*)

NIKOLSKII, G. V. 1969. Theory of fish population dynamics. Translated by J. E. S. Bradley. Oliver and Boyd Ltd., Edinburgh, Scotland. 323 p.

PELLA, J. J., AND P. K. TOMLINSON. 1969. A generalized stock production model. Inter-Amer. Trop. Tuna Comm. Bull. 13: 421–496.

RICKER, W. E. 1958. Handbook of computations for biological statistics of fish populations. Bull. Fish. Res. Board Can. 119: 300 p.

SCHAEFER, M. B. 1957. A study of the dynamics of the fishery for yellowfin tuna in the eastern tropical Pacific Ocean. Inter-Amer. Trop. Tuna. Comm. Bull. 2: 245–285.

SWINGLE, H. S., AND E. V. SMITH. 1941. Experiments on the stocking of fish ponds. Trans. N. Am. Wildl. Nat. Resour. Conf. 5: 267–276.

VOLTERRA, V. 1928. Variations and fluctuations of the number of individuals in animal species living together. J. Cons. Int. Explor. Mer 3: 1–51.

CONTRIBUTION NO. 190

Accumulation of Mirex-^{14}C in the Adult Blue Crab (*Callinectes sapidus*)

by WILHELM P. SCHOOR

U.S. Environmental Protection Agency
Gulf Breeze Environment Research Laboratory
Sabine Island, Gulf Breeze, Fla. 32561
(Associate Laboratory of the National Environmental Research Center, Corvallis, Ore.)

Carrier-solubilized mirex is absorbed from a disperse aqueous system by juvenile (Lowe et al. 1971), and larval stages (Bookhout et al. 1972) of the blue crab. Since in both cases only whole-body residues were determined, it was thought to be of interest to establish the actual distribution of mirex in the tissues.

Experimental

Adult blue crabs were exposed to mirex-^{14}C (Mallinckrodt Inc.)[1] having a specific activity of 6.34 mCi/mM in a final concentration of 0.05-0.25 ppb and 0.3% polyethylene glycol 200 in filtered sea water that was diluted with distilled water to give 10 ppt salinity. All tests were conducted in battery jars containing 3ℓ of solution at 25°C. Exposure time ranged from 15 minutes to 16 hours.

Tissue samples were counted in the following manner. Hemolymph serum was obtained by centrifuging the clotted hemolymph. About 0.5 g of hepatopancreas, 0.2 g brain and thoracic ganglion, 0.5 g muscle, and 1.0 ml of hemolymph serum were each added to 2.0 ml Soluene 100 (Packard Instrument Co.) and digested at 40°C overnight. Ten ml of scintillation fluid (5.5 g PPO, 0.1 g dimethyl POPOP, 667 ml toluene, and 333 ml triton X-100) were added with 1.0 ml hexane for clarification, and the amount of mirex-^{14}C determined with a Packard Tri-Carb Scintillation Spectrometer. Any quenching was compensated for by means of an internal standard.

Results and Discussion

Uptake of mirex-^{14}C by organs from solutions that contained 0.22 ppb (measured) of mirex-^{14}C was as follows:

Hemolymph serum (10 crabs)	0.24 - 0.69 µg/ℓ
Muscle (2 crabs)	0.65 - 1.1 µg/kg
Brain and thoracic ganglion (5 crabs)	0.75 - 19 µg/kg
Hepatopancreas (6 crabs)	1.6 - 31 µg/kg

[1] Mention of commercial products or trade names does not constitute endorsement by the U. S. Environmental Protection Agency.

Bulletin of Environmental Contamination & Toxicology,
Vol. 12, No. 2 © 1974 by Springer-Verlag New York Inc.

Mirex-^{14}C is, I believe, absorbed through the gills because the hemolymph serum showed traces of mirex-^{14}C after 5 minutes of exposure, the hepatopancreas after 15 minutes. No difference was noted between male and female crabs. Response to the toxicant usually progressed through increased aggressiveness to decreased aggressiveness; followed by loss of equilibrium and death, although some crabs recovered. Crabs in 1% Carbowax 200 solutions showed no behavioral differences from those in solutions without it.

The rate of uptake and the distribution of mirex-^{14}C in the blue crab is similar to that observed for pink shrimp (Penaeus duorarum) exposed to Aroclor® 1254. (Nimmo et al. 1971).

References

BOOKHOUT, C. G., WILSON, A. J. JR., DUKE, T. W. and J. I. LOWE: Water and Soil Pollution 1, 165 (1972).

LOWE, J. I., WILSON, A. J. JR. and J. M. PATRICK, JR. Unpublished results, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida 32561 (1971).

NIMMO, D. R., BLACKMAN, R. R., WILSON, A. J. JR. and J. FORESTER: Marine Biology 11, 191 (1971).

® Registered trademark, Monsanto Company, St. Louis, Mo.

EPA-600/4-74-004
October 1974

PROCEEDINGS

OF

SEMINAR ON METHODOLOGY

FOR

MONITORING THE MARINE ENVIRONMENT

SEATTLE WASHINGTON

OCTOBER 1973

Program Element No. 1HA326
ROAP/TASK - PEMP/2

SPONSORED BY

OFFICE OF MONITORING SYSTEMS

U.S. ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

BIOLOGICAL PROBLEMS IN ESTUARINE MONITORING

P. A. Butler *

INTRODUCTION

Awareness of the extent of persistent organochlorine pollution both in the physical environment and the biota has made apparent the need for continuing surveillance programs to assess the problems. During the period 1965-72, samples collected in the National Estuarine Monitoring Program, as well as in other studies, revealed some of the difficulties involved in the interpretation of residue data. Bivalve molluscs are efficient bioassay tools for identifying the ebb and flow of pollutants in surrounding waters, and the monthly monitoring of molluscan populations made obvious the dynamic nature of organochlorine pollution in the estuary. The image of polluted versus unpolluted estuaries was soon modified by monitoring data that indicated instead the movement of relatively discrete masses of clean and polluted water through the estuary. Organochlorine residues in molluscs fluctuated from month to month in response to the sometimes transitory nature of the pollution and contrasted sharply at times with residues observed in other elements of the associated biota.

To gain increased understanding of the significance of residue levels, sample collections in problem areas were intensified in variety, frequency, and size; and additional work was undertaken under laboratory conditions. The experimental program demonstrated that the uptake and retention of persistent residues varied unpredictably with the environmental element sampled. It became clear that surveillance or monitoring systems had to be carefully designed if they were to provide answers to specific program objectives.

---

*Office of Pesticides Programs
Gulf Breeze Environmental Research Laboratory,
Gulf Breeze, Florida
Gulf Breeze Laboratory Contribution No. 191

A primary concern is the extent of environmental degradation - with the implied intention of reversing or at least halting harmful pollution trends. But individual agency goals are much more specific. For expedience and because of monetary restrictions, programs are usually designed to clarify rather narrowly defined sectors of the whole pollution picture. Often, the foremost question concerns only the existence of a human health problem, and if this is not likely to occur, then some environmental pollution problems may go uninvestigated. The idea that any changes harmful to the environment will eventually affect man is still not generally accepted.

The basic needs for environmental surveillance require programs designed to provide current data that will be adequate to identify later deleterious changes. Monitoring data should indicate existing problems as well as their extent in time and space. To provide information of this type, protocols must take into account the influence of various physiological and ecological factors affecting the substrate selected for study. Some of the anomalous residue data acquired so far are understandable, but reasons for others are less certain. The following discussion of factors affecting persistent organochlorine residues indicates some of the options available in selecting the most informative sample types.

FIELD AND LABORATORY OBSERVATIONS

Residue differentials resulting from kind of species monitored:

The necessity for utilizating different species to monitor pesticides in different coastal areas prompted the conduct of laboratory experiments to determine the relative sensitivity of molluscan species selected for their diversity in salinity tolerance. A number of controlled experiments have shown the relative uniformity of DDT residue formation in the eastern oyster (Crassostrea virginica) under varying estuarine conditions of salinity and temperature. Observations of four other molluscan species exposed simultaneously to a mixture of common organochlorine pesticides indicated considerable variation in the relative rates at which residues were acquired, then lost when clean water was restored to them (Table 1) (3).

Studies were directed toward evaluating residue flushing rates in the hard clam (Mercenaria mercenaria) because of

Bioassay animal	Magnification in Body after 5 days exposure	Percentage loss after 7 days in clean water
Soft clam (Mya arenaria)	3000	74
Eastern oyster (Crassostrea virginica)	1200	50
Marsh clam (Rangia cuneata)	700	50
Asiatic clam (Corbicula fluminea)	600	30
Hard clam (Mercenaria mercenaria)	500	75

Table 1. Average biomagnification and depuration rates of a mixture of seven common chlorinated pesticides by molluscs exposed in a flowing seawater system.

the prevalence of this species in the New England area and its observed poor performance in storing DDT residues. The biological half-life of pesticide residues in molluscs under the same conditions is of importance in determining the movement of pollutants in the estuary. Clams and oysters with DDT residues of about 40 ppm were placed in aquaria with uncontaminated flowing seawater. The clams flushed out 50 percent of their DDT residue within five days. At the end of 15 days less than 2% of the original DDT burden remained. In contrast, the oysters still contained 50% of the residue at the end of the 15 days and significant amounts of DDT were present after a month. Clearly, clams would have to be sampled more frequently than oysters to determine pollution inputs.

Species differentials in pesticide uptake are apparent in populations monitored in Conscience Bay, New York (6). During the first half of 1968, monthly mussel samples (Mytilus edulis) from this bay contained about 50 ppb of DDT and its metabolites. In the latter half of the year and until April 1969, hard clam was substituted because it was easier to collect. During this period no DDT residues were detected. In April and thereafter, the mussel was again utilized and DDT residues were found to be once more 50 ppb or higher. In this case, the convenience of collecting hard clam samples 'determined' the presence or absence of DDT pollution in the bay.

Similar discrepancies were observed in simultaneous collections of spot (Leiostomus xanthurus)and oysters in a South Carolina estuary in 1968. Residues of DDT occurred in all monthly samples of the fish and levels fluctuated between 100 and 300 ppb although the fish sampled were of uniform size. Oyster samples showed DDT pollution was present only during the first six months of the year. If DDT pollution was present after that, it was not stored by the oyster at levels chemically detectable. Numerous other studies have demonstrated the retention and gradual increase of organochlorine residues in fish, at least until their first spawning period. Consequently, it is not possible to determine from periodic fish analyses the seasonal occurrence of DDT pollution in an estuary.

Unexpected variations occur in the sensitivity or selectivity of the biota in the same ecosystem to organochlorine pollution even though the species monitored are presumed to occupy similar tropic levels. These differences are well-illustrated in a study of monitoring methods conducted in Virginia (12). Sample analyses demonstrated the existence of residues of three different polychlorinated biphenyl compounds (Aroclors 1242, 1254,

and 1260) in different types of samples. All samples were large enough to minimize individual variation. In March, Aroclor 1242 was detected only in anchovies (Anchoa sp.); Aroclor 1254 was present in silversides (Menidia sp.), oysters and two series of plankton tows. A third series of plankton samples contained only Aroclor 1260. Three months later, Aroclor 1242 was present in both anchovies and silversides while Aroclor 1254 was no longer found in silversides but was still present in oysters and plankton. Aroclor 1260 was not detected. These variations in residue accumulations in a short period in one river system are not easily explained (Table 2).

		March	June
Aroclor	1242	Fish A 1/	Fish A
			Fish S
Aroclor	1254	Fish S 2/	
		Oysters	Oysters
		Plankton	Plankton
		Sediment	Sediment
Aroclor	1260	Plankton	

1/ Anchovies
2/ Silversides

Table 2. Estuarine samples containing residues of PCB's at two sampling periods

Uptake and depuration rates in molluscs under laboratory conditions must be accepted as relative figures and extrapolated to field conditions with caution. In one study, for example, oysters exposed to mercury accumulated residues of about 28 ppm (8). Relatively small declines in residues took place in the following 160 days of depuration. The authors concluded from the data that mercury residues acquired by oysters in nature might require years to decline to acceptable levels. Their experimental conditions were described as a 'natural system' but the oysters were supplied only 2 ℓ clean water/animal per hour. This volume of water should be compared to their well-known utilization of up to 30 ℓ per hour even under experimental conditions (11). It is probable that in this experiment, the mercury was continuously recycled and the oysters never had opportunity to adequately flush their tissues.

The mercury exposure experiment is perhaps comparable to a situation monitored in Mecox Bay, Long Island (6). The waters of this bay are typically isolated from the ocean by sand bars, and only periodically do winter storms cut the bars and permit flushing (10). In the period 1966-72, the oysters in this bay were continuously contaminated with DDT and at a maximum level higher than that observed in any of the other 16 estuarine stations monitored in the New York area despite apparently low use records of DDT in the area. In my opinion, these oysters were continuously contaminated with recycled DDT because of the inadequate supply of clean water.

Past dependence on fortuitous samples (found dead, happened to be caught in a net, etc.) to assess pollution levels has resulted in an uneven if not confused picture of general environmental contamination. Broad community studies frequently show persistent organochlorine residues differing by an order of magnitude in species of similar habit (9,20). Consequently, valid assessment of persistent residues in any one species of a community requires not only an understanding of its position in the trophic structure but also its variability as compared to similar species.

Residue differentials resulting from age of individuals monitored.

In fish and other vertebrates, the localization of organochlorines in highly lipid tissues and their persistence, at least in part, for long periods is well-documented. For example, DDT tends to accumulate gradually in pinfish (Lagodon rhomboides) and an approximately tenfold increase from 0-to-1-year fish has been documented (14).

There is an approximate doubling of DDT residues in lake trout in the Great Lakes each year up to age 10 (17). Accumulation of dieldrin residues in these fish is proportional to age but of lower magnitude. Approximate doubling of mercury levels during the first few years has been reported in salmon (Salmo salar) from rivers in Sweden (18).

In contrast, organochlorine residues in molluscs do not persist from year to year in the absence of pollution despite their affinity for lipid tissues. Residue levels fluctuate widely depending on the input of pollutants to the estuarine system and not on the age of the mollusc. Residue levels can be correlated frequently with pesticide usage in the drainage system when oysters of similar size are monitored. However, oysters of different size do react differentially to similar pollutant levels and, in general, residues per gram of tissue are higher in small oysters than in oysters of signficantly larger size. Although the data are not at hand, I assume that this difference is a function of the larger amount of water circulated (filtered) per gram of tissue in small oysters compared to large ones. This higher biological magnification of residues in small oysters might be disastrous since, with a given amount of contaminated tissue consumed, predators would ingest much more DDT from small as opposed to large oysters.

This demonstrated increase in persistent residues with age complicates interpretation of data in many instances where the age of individuals making up the sample are unavailable and difficult to ascertain.

Residue differentials resulting from variations among individuals.

For obvious reasons, uniform populations or aggregates of individuals are selected as often as possible as indicators of environmental degradation. But both laboratory and field studies demonstrate that there can be much individual variation in levels of organochlorine residues. DDT residue levels in a presumably uniform sample of yearling pinfish in a Florida estuary ranged from 13.7 ppm to 0.5 ppm (14). The standard error in these data was more than 230% of the arithmetic mean. The average of two of the fish was 13.2 ppm while the average residue in the other eight fish was only 1.5 ppm DDT. Not all groups analyzed have shown this diversity but it must be anticipated in data interpretations. Diversity in residue levels of molluscan populations is less extreme and may be illustrated by one group of mature oysters exposed to DDT under controlled

conditions in flowing seawater for 96 hours. In the 10 individuals, total DDT residues ranged from about 4 to 25 ppm with a mean value of 11.6 ppm. In this case, the standard deviation was only about 50% of the mean.

Much larger samples must be collected to obtain more uniform data, but this is a costly and time-consuming process. We undertook in 1971 an exploratory project to examine the merits of larger sample size (12). Oysters, two species of fish, plankton and sediment cores were collected concurrently at two stations in two Virginia rivers at a two-month interval. Ten samples of 15 oysters and 10 samples of 25 fish were collected at each station. In general, PCB's were the principal residues found and the spread of values was reasonably small. Standard errors, for example, were about 15 to 30% of the arithmetic means. Data on sediment cores were less uniform even though samples were collected in a restricted area. In one series, for example, only 3 of 10 cores had measurable residues of PCB. This means that had only replicate cores been taken there would have been about a 50-50 chance that one of the cores would have contained a residue. These data are significant in light of the fact that sometimes only a single sediment core is collected to assess pollution in an entire estuary.

Numerous studies have shown that the magnitude of persistent residue levels in sediments is usually inversely proportional to grain size. In consequence, care must be exercised in selecting representative sample sites, as well as in analyzing an adequate number of samples.

Despite the mathematical pleasure in achieving uniformity in sampling results, it must be emphasized that averaging data, either in the electric blender or the calculator, may lead to serious management errors. Animals, in general, are not responsive to average pollution levels, they survive and flourish as a result of environmental extremes. A single high incidence of endrin in the environment can be satisfactorily averaged away on paper, but at the time of its occurrence all of the endemic animals may have been killed.

There is a further important consideration in assessing the importance of organochlorine residues in aquatic biota. It is axiomatic that, with a given level of environmental pollution, the sensitive species and the sensitive individuals of more tolerant species will be affected first. In one experiment, pinfish were fed a diet contaminated with about 4 ppm of DDT (4). At the end of the 14th day, the ten surviving fish were sacrificed and found to have average residues of about 4 ppm. The 25 fish dying during the 2-week period averaged about 0.6 ppm of DDT, less than 1/6 as

much as the resistant individuals. Obviously, the magnitude of residues occurring in apparently healthy populations is not necessarily an indication of tolerable pollution levels.

Residue differential showing seasonal variations.

Monitoring programs and laboratory studies in which periodic samples have been collected at sufficiently short intervals may show clearly defined seasonal variations in residue levels. Such cyclic changes in the presence of relatively constant pollution loading are indicative of basic physiological changes in the monitored species. For example, studies of speckled seatrout in Texas showed a 75% decrease in gonad DDT residues in mature fish in the late fall (7). Lowe reported a more than 50% decline in DDT and a 70% decline in toxaphene residues in oysters continuously exposed to these pollutants (15). Oysters exposed simultaneously to PCB, dieldrin and DDT in the laboratory lost from 45 to 80% of the residues within a short period (16). Whole body residues of mercury in oysters are also reported to decline seasonally in a manner similar to the organochlorine compounds (8).

In each case, the abrupt declines have been clearly identified with the normal spawning period of the animal in question. That there should be a significant percentage loss of such residues on a seasonal basis is entirely predictable in view of the localization, for example, of DDT in oyster gametes (1).

Seasonal declines in levels of organochlorine residues in oysters are also clearly associated with fluctuating levels in the input of pollutants into the aquatic system. In southwest Florida, the former agricultural use of DDT was intensified just prior to the harvest of sweet corn and sugar cane. Residue levels of DDT in oysters collected monthly in an associated river basin reflected this management practice; peak DDT residues in oysters were as much as 1000x higher than minimal residue levels in 1967-68 (6).

Fluctuating industrial discharges may also have significant seasonal effects on organochlorine residues in molluscs. Spring and fall manufacturing peaks in a pesticide-producing plant, for example, were closely followed by more than 10-fold increases in the pesticide residues in oysters collected about 10 miles downstream from the discharge pipes (5).

Organochlorine residue patterns in estuarine biota resulting from agricultural and industrial practices may be masked by the effects of marked changes in river discharge in the drainage basin because of rainfall variations.

Residue differentials affected by body region monitored.

Regardless of the mode of entry of organochlorine into living tissues, there is a partitioning and partial immobilization of these compounds in fatty tissues because of their lipophilic nature. Such segregation is clearly demonstrated in fish in which adipose tissues are highly localized. In coho salmon, for example, DDT residues in a mid-body fillet range from an average of about 65 ppm in fatty tissues to less than 6 ppm in the muscle. The whole body DDT residue of similar fish was about 12 ppm (17).

As discussed above, residue levels increase with age in fish. In aquatic mammals the localization of residues in older individuals may be even more striking. In a dead porpoise (Tursiops truncatus) found near Pensacola, Florida, total DDT residues ranged from about 1.5 ppm in blood, 7 ppm in the muscle, 9 ppm in the brain to 33 ppm in the liver, and more than 500 ppm in the blubber (13). Even in oysters, where total body fat is only about 4%, there is a marked localization of DDT residues in the digestive gland, and, seasonally, in the gonad which contains more fat than other organs.

In general monitoring or surveillance programs, the localization of persistent residues in particular body regions has little importance from the point of view of either human health or resource protection so long as the monitored species is small enough to be analyzed on a whole-body basis. Residues large enough to warrant further investigation will show up in such analyses regardless of their location in the body.

It is quite another matter, however, if as in market-basket surveillance programs, only the products, e.g., lobster and shrimp tails or tuna muscle, are examined. It is conceivable then the edible portions would contain negligible organochlorine residues while the discarded body parts could contain amounts detrimental to the productivity of the animal itself or to other animals preying on it in nature.

SUMMARY

Successful monitoring of the estuarine environment for persistent organochlorine pollutants is dependent in large measure on the collection of appropriately biased samples. Statistically randomized sample collections are unsatisfactory for the simple reason that pollution patterns are not random. The transport of persistent residues is dependent on a large number of biological factors which in turn are modified by physical and chemical parameters of the environment.

Monitoring programs have several basic functions and requirements. First, they should record existing residues of persistent pollutants that occur at significant trophic levels in aquatic ecosystems. Sample collection protocols, as well as analytical procedures, must be sufficiently standardized to ensure the comparability of data, not only from one area to another but also from year to year. It is essential that monitoring programs collect comparable data for sufficiently long periods of time so that pollution trends can be identified. Finally, it should be stressed that monitoring data must be transmitted on a timely basis to action agencies. Agencies mandated to identify and regulate pollution sources, agencies with resource protection responsibilities, and agencies concerned with human welfare must have clearly established communication channels with environmental monitoring programs.

ACKNOWLEDGEMENTS

I wish to thank Alfred J. Wilson and Jerrold Forestor of the Gulf Breeze Environmental Research Laboratory and Roy Schutzmann of the Pesticides Monitoring Laboratory of the Environmental Protection Agency for many of the chemical analyses reported.

LITERATURE CITED

1. Butler, Philip A., 1966. The problem of pesticides in estuaries. Trans. Amer. Fish. Soc. Spec. Publ. 3:110-115.

2. Butler, Philip A., 1966. Pesticides in the marine environment. J. Appl. Ecol. 3(Suppl):253-259.

3. Butler, Philip A., 1968. Pesticide residues in estuarine mollusks. In Proc. Natl. Symp. Estuarine Pollut., p. 107-121, Stanford University, Stanford, Calif., 1967.

4. Butler, Philip A., 1969. The significance of DDT residues in estuarine fauna. In Chemical Fallout, p. 205-220. C. C. Thomas, Springfield, Ill.

5. Butler, Philip A., 1969. Monitoring pesticide pollution. BioScience, 19(10):889-91.

6. Butler, Philip A., 1973. Organochlorine residues in estuarine molluscs, 1965-1972 - National Pesticide Monitoring Program. Pestic. Monit. J., 6(4):238-362.

7. Butler, Philip A., Ray Childress, and Alfred J. Wilson, Jr. 1970. The association of DDT residues with losses in marine productivity. In M. Ruivo (editor), Marine Pollution and Sea Life, p. 262-266. Fishing News (Books) Ltd. London.

8. Cunningham, P. A. and M. R. Tripp, 1973. Accumulation and depuration of mercury in the American oyster Crassostrea virginica. Mar. Biol. 20:14-19.

9. Duke, T. W., J. I. Lowe and A. J. Wilson, Jr., 1970. A polychlorinated biphenyl (Aroclor 1254) in the water, sediment, and biota of Escambia Bay, Florida. Bull. Environ. Contam. Toxicol. 5(2):171-180.

10. Foehrenbach, Jack, 1970. N. Y. State Dept. of Environmental Conservation, personal communication.

11. Galtsoff, Paul S., 1964. The American oyster, Crassostrea virginica Gmelin. U. S. Fish Wildl. Serv. Bull. 64:480 p.

12. Gillespie, J. R., Jr., 1972. Estuarine Monitoring Program - Sample Evaluation Project. NMFS Contract No. N-042-37-72(N). (unpublished)

13. Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida, unpublished data.

14. Hansen, David J. and Alfred J. Wilson, Jr., 1970. Significance of DDT residues from the estuary near Pensacola, Fla. Pestic. Monit. J. 4(2):51-56.

15. Lowe, Jack I., Paul D. Wilson, Alan J. Rick, and Alfred J. Wilson, Jr., 1971. Chronic exposure of oysters to DDT, toxaphene and parathion. Proc. Natl. Shellfish Assoc., 61:71-79.

16. Parrish, Patrick R., 1973. Chronic effects of three toxic organics on the American oyster, Crassostrea virginica. Proc. Natl. Shellfish Assoc. Meeting. June 1973. Abstract.

17. Reinert, Robert E., 1969. Insecticides and the Great Lakes. Limnos Magazine, Great Lakes Foundation, 2(3):4-9.

18. Reinert, Robert E., 1970. Pesticide Concentrations in Great Lakes Fish. Pestic. Monit. J. 3(4):233-240.

19. Westöö, G., 1973. Methylmercury as percentage of total mercury in flesh and viscera of salmon and seatrout of various ages. Science 181:567-568.

20. Woodwell, George M., Charles F. Wurster, Jr. and Peter A. Isaacson, 1967. DDT residues in an east coast estuary. Science, 156:821-824.

CONTRIBUTION NO. 192

Biological Sciences

OCCURRENCE OF SNOOK ON THE NORTH SHORE OF THE GULF OF MEXICO[1]

NELSON R. COOLEY

U. S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561

ABSTRACT: *The known range of snook is extended about 100 miles westward to Santa Rosa Sound.*

THE geographical range of *Centropomus undecimalis* (Bloch) has been reviewed by Marshall (1958) and Martin and Shipp (1971). Snook are found in tropical and subtropical estuarine waters of the eastern coast of the Americas. In the United States, snook have been reported as far north as Georgia (Dahlberg, 1972) and the Carolinas (Lunz, 1953; Martin and Shipp, 1971), but are abundant only in Texas and peninsular Florida. In Texas, the range rarely extends north of Port Aransas, although Jordan and Gilbert (1882) reported the species from the vicinity of Galveston. On the east coast of Florida, Marshall (1958) placed the

[1]Contribution No. 192 from Gulf Breeze Environmental Research Laboratory, Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon.

northern limit in the vicinity of Volusia County, noting that the species is occasionally taken in Duval County and in the St. John's River. On the west coast of Florida, he noted that the northern limit was in the vicinity of Hernando County, the species appearing to be absent from the north shore of the Gulf of Mexico. Subsequently, Yerger (1961) reported a single adult from the Gulf off Alligator Harbor, Franklin County, Florida, but the first record from a northern Gulf coast estuary appears to be a 351-mm snook caught in St. Andrew Bay, Bay County, Florida in August 1963 (Vick, 1964). Vick also noted that local commercial fishermen told him that two or three snook were caught in that bay each year, usually in August.

This report extends the known range of the species approximately 100 miles westward along the northern Gulf coast of Florida into a second estuary. I identified an adult snook, 395 mm standard length, that was caught on 3 November 1973 in a gill net in Santa Rosa Sound off Woodlawn Beach, Santa Rosa County, Florida by Mr. J. A. Briggs, a commercial fisherman. The specimen was deposited in the museum of the Gulf Breeze Environmental Research Laboratory as GBERL-1911. Water temperature and salinity were not taken at the collecting site, but should have been similar to those recorded that day in the Sound at Sabine Island, 10 miles west of Woodlawn Beach, namely, 21.0° to 22.0°C and 29.5 to 30.0‰.

The scarcity of snook along the northern Gulf coast is probably related to their known sensitivity to cold (see Marshall, 1958, for review of temperature tolerance). Nevertheless, during warm seasons, isolated specimens from endemic populations along the southwestern coast of Florida could move out of their nominal range into localities along the northwestern coast of the state.

LITERATURE CITED

DAHLBERG, M. D. 1972. An ecological study of Georgia coastal fishes. U. S. Dept. Commer., Natl. Mar. Fish. Serv., Fish. Bull. 70:323-353.

JORDAN, D. S., AND C. H. GILBERT. 1882. Notes on fishes observed about Pensacola, Florida, and Galveston, Texas, with descriptions of new species. Proc. U. S. Natl. Mus. 5:241-307.

LUNZ, G. R. 1953. First record of the marine fish *Centropomus undecimalis* in South Carolina. Copeia 1953:240.

MARSHALL, A. R. 1958. A survey of the snook fishery of Florida, with studies of the biology of the principal species, *Centropomus undecimalis* (Bloch). Florida State Bd. Conserv. Tech. Paper 22, 37 p.

MARTIN, J. R., AND R. L. SHIPP. 1971. Occurrence of juvenile snook, *Centropomus undecimalis*, in North Carolina waters. Trans. Amer. Fish. Soc. 100:131-132.

VICK, N. G. 1964. The marine ichthyofauna of St. Andrew Bay, Florida, and nearshore habitats of the northeastern Gulf of Mexico. Texas A. & M. Res. Found., A. & M. Proj. 286-D, 77 p.

YERGER, R. W. 1961. Additional records of marine fishes from Alligator Harbor, Florida, and vicinity. Quart. J. Florida Acad. Sci. 24:111-116.

CONTRIBUTION NO. 193

Translocation of Four Organochlorine Compounds by Red Mangrove (Rhizophora Mangle L.) Seedlings[1]

by GERALD E. WALSH, TERRENCE A. HOLLISTER, and JERROLD FORESTER

U.S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, Fla. 32561
(Associate Laboratory of the National Environmental Research Center, Corvallis, Ore.)

Mangrove vegetation is common in tropical estuaries and serves as a habitat and source of food for many animals (ODUM 1971). Because mangrove, both living and detrital, is eaten by a variety of animals, we conducted studies to learn translocational patterns of four organochlorine compounds in seedlings to determine if these persistent compounds could be introduced into estuarine food webs that receive contributions from mangrove.

Previous studies showed that mangroves and other plants translocated certain toxicants from the soil to leaves. Seedlings of the red mangrove (Rhizophora mangle L.) translocated the herbicides 2,4-D and picloram from soil to roots, hypocotyls, stems, and leaves (WALSH et al. 1973). Translocation of the insecticides dieldrin by alfalfa and hay (MUMMA et al. 1966), dieldrin, endrin, and heptachlor by soybeans (NASH and BEALL 1970), and mirex by peas and beans (MEHENDALE et al. 1972) has been demonstrated.

Insecticides have been found to be associated with mangrove from natural stands. Dieldrin (0.021 ppm - parts per million) and polychlorinated biphenyls (0.181 ppm) were found in red mangrove leaves from St. John and St. Croix in the Virgin Islands[2]. We found DDD in roots (0.022 ppm), hypocotyls (0.220 ppm), stems (0.032 ppm), and leaves (0.019 ppm) of red mangrove seedlings from Joyuda, Puerto Rico (unpublished data).

In the present study, we investigated translocation of the insecticides dieldrin, methoxychlor, and mirex and the polychlorinated biphenyl (PCB) Aroclor® 1242 by red mangrove seedlings in the laboratory.

METHODS

Seedlings 18.5 to 38.2 cm long were obtained from trees in the Loxahatchee River near Jupiter, Florida, and planted in plastic boxes that contained muddy sand and natural sea water from an estuary near Gulf Breeze, Florida. Fifteen seedlings were planted in each box. Salinity of the water that covered the sediment was 25 parts per thousand; pH of the sediment was between 6.3 and 6.7. Air temperature was 20 - 23° C. Light was provided by overhead Grow-Lux® flourescent tubes in a regime of alternate 12-hr periods of light and darkness.

---

[1] Contribution No. 193, Gulf Breeze Environmental Research Laboratory.

[2] Philip A. Butler (Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida). Personal communication: Unpublished data, EPA National Monitoring Program.

Bulletin of Environmental Contamination & Toxicology, Vol. 12, No. 2 © 1974 by Springer-Verlag New York Inc.

Technical grade dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonapthalene), methoxychlor (1,1,1-trichloro-2,2-bis (p-methoxyphenyl) ethane), mirex (dodecachlorooctahydro-1,3,4-metheno-1H-cylobuta (cd) pentalene), and Aroclor 1242 (a mixture of polychlorinated biphenyl isomers), dissolved together in 10 ml of acetone, were added to the surface of the water. The same amount of acetone was added to eight control boxes. Application rates were 0.06, 0.11, 0.28, 0.56, 1.12, 2.80, 5.60, and 11.20 kg/ha (0.05, 0.10, 0.25, 0.50, 1.00, 2.50, 5.00, and 10.00 lb/acre). These rates were equal to concentrations of 0.038, 0.075, 0.150, 0.300, 0.600, 1.50, 3.00, and 6.00 ppm in the muddy sand.

Three boxes of seedlings were treated at each application rate after one or two pairs of leaves had emerged from the stems. Two or three seedlings were collected from each box (total of 8 or 9 plants per sample) each week for six weeks after application. The seedlings were washed with tap water and then with acetone. Roots, hypocotyls, stems, and leaves of plants from each concentration and the controls were analyzed separately for organochlorine residues.

Samples were homogenized in a blender with four times their weight of anhydrous sodium sulfate, then extracted for four hours with 10% ethyl ether in petroleum ether in a Soxhlet apparatus. The extract was concentrated to approximately 15 ml and transferred to a florisil column (MILLS et al. 1965).

Methoxychlor, mirex, and Aroclor 1242 were eluted with 6% ethyl ether in petroleum ether; dieldrin with 15% ethyl ether in petroleum ether. Approximately 1 ml of metallic mercury was added to approximately 5 ml of the extracts of roots and hypocotyls to remove sulfur compounds that interfered with electron capture gas chromatography.

The samples were analyzed with a model 2100 Varian Aerograph gas chromatograph equipped with 182.8 cm X 2 mm I.D. glass columns and electron capture detectors. Two columns were packed with 2% OV - 101, one with 3% OV - 210, and one with a 1:1 ratio of 2% OV - 101 and 3% OV - 210, all on Gas Chrom Q. Quantitation was made on the OV - 101 columns. The other columns were used to confirm the analyses. Carrier gas was pre-purified nitrogen. Operating conditions were: injector, 210° C; columns, 195° C; detectors, 215° C; gas flow, 25 ml/min.

Aroclor 1242 was quantified by measurement of total peak height of the 12 major peaks, which were compared with heights of the same peaks in a standard solution of known concentration. The other compounds were quantitated by peak height.

® Aroclor, Registered Trademark, Monsanto Co., St. Louis, Mo.; Gro-Lux, Sylvania Electric Products Inc., Salem, Mass. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

Recovery rates were above 80% in quality-control samples to which known amounts of the compounds had been added. Residue data do not include a correction factor for recovery rate.

RESULTS AND DISCUSSION

Mangrove seedlings translocated the four organochlorine compounds tested, but no visible effects of the compounds on the seedlings were noted. None of the compounds was detected in control seedlings.

Dieldrin

Dieldrin was translocated to hypocotyls and leaves more rapidly than were the other compounds. It was detected in hypocotyls and leaves one week after exposure at all concentrations and these plant parts contained more of the chemical than did roots and stems (Table 1). Dieldrin was never detected in stems and was not found in roots at exposure concentrations less than 0.28 kg/ha. Accumulation of dieldrin in leaves was not related to length of exposure: concentrations were similar during the entire exposure. Average residues in leaves ranged from 0.072 to 0.113 ppm but were not related directly to exposure concentrations.

TABLE 1

Average concentrations of dieldrin in roots, hypocotyls, stems, and leaves of red mangrove seedlings during six weeks of exposure to eight application rates

Application Rate	Average Concentration, Parts Per Million			
kg/ha	Roots	Hypocotyl	Stem	Leaves
0.06	ND*	0.01	ND	0.07
0.11	ND	0.02	ND	0.11
0.28	Tr**	0.02	ND	0.11
0.56	Tr	0.04	ND	0.07
1.12	0.03	0.08	ND	0.11
2.80	0.04	0.08	ND	0.07
5.60	0.04	0.08	ND	0.09
11.20	0.06	0.16	ND	0.10

* Not detected; ** Trace, present but not quantifiable. Limit of detection = 0.01 ppm.

Concentrations of dieldrin in hypocotyls were often related directly to application rates and to length of exposure. At application rates of 1.12 kg/ha and above, concentrations in hypocotyls increased as exposure time increased (Figure 1). Concentrations of dieldrin detected in hypocotyls at the end of the six-week exposures were less than concentrations of methoxychlor, mirex, and Aroclor 1242 in hypocotyls of seedlings exposed at the same concentration rates.

Methoxychlor

Methoxychlor was present in roots at the three highest application rates and was translocated to hypocotyls of seedlings treated at rates of 0.28 kg/ha and above (Table 2). It was never detected in stems or leaves.

Residues in hypocotyls increased throughout the six-week exposure and were related directly to application rates above 0.56 kg/ha at the end of the exposure (Figure 2).

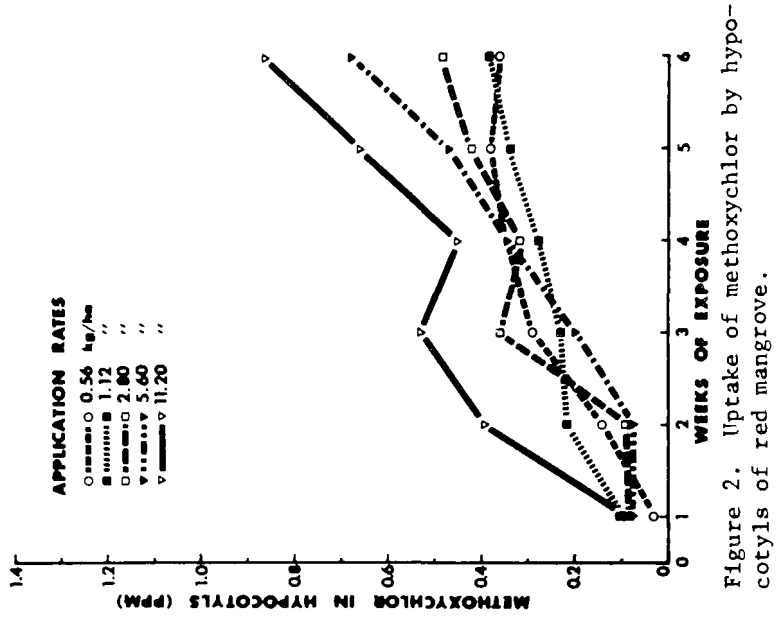


Figure 2. Uptake of methoxychlor by hypocotyls of red mangrove.

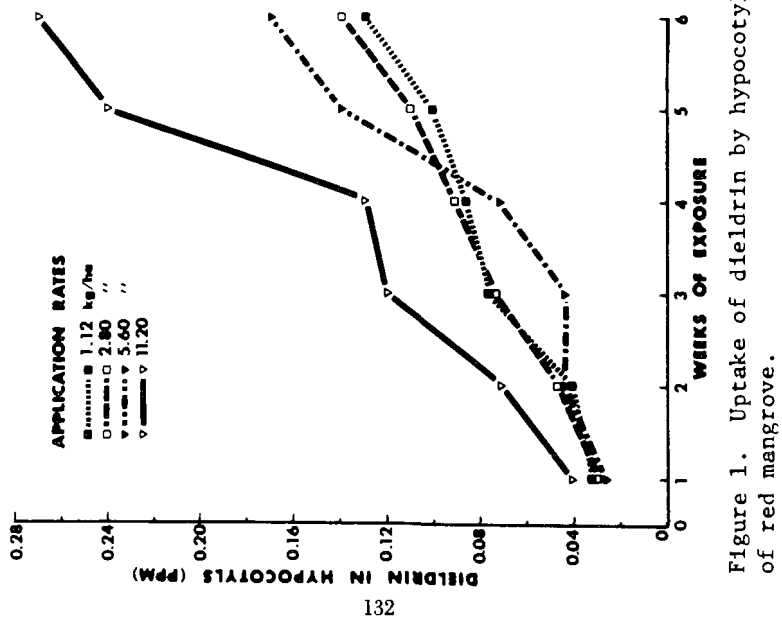


Figure 1. Uptake of dieldrin by hypocotyls of red mangrove.

TABLE 2

Average concentrations of methoxychlor in roots, hypocotyls, stems, and leaves of red mangrove seedlings during six weeks of exposure to eight application rates

Application Rate	Average Concentration, Parts Per Million			
kg/ha	Roots	Hypocotyl	Stem	Leaves
0.06	ND*	ND	ND	ND
0.11	ND	ND	ND	ND
0.28	ND	0.03	ND	ND
0.56	ND	0.26	ND	ND
1.12	ND	0.26	ND	ND
2.80	0.02	0.29	ND	ND
5.60	0.06	0.31	ND	ND
11.20	0.07	0.49	ND	ND

* Not detected. Limit of detection = 0.02 ppm.

Mirex

Mirex was detected in seedlings treated at 11.20 kg/ha but not at lower application rates (Figure 3). Residues were greatest in all plant parts after two weeks of exposure and decreased rapidly thereafter. It was not found in leaves after six weeks of exposure and only 0.03 ppm was detected in roots at that time.

Aroclor 1242

Aroclor 1242 was detected in roots of seedlings exposed at application rates of 5.60 and 11.20 kg/ha but not in stems. It

TABLE 3

Average concentrations of Aroclor 1242 in roots, hypocotyls, stems, and leaves of red mangrove seedlings during six weeks of exposure to eight application rates

Application Rate	Average Concentration, Parts Per Million			
kg/ha	Roots	Hypocotyl	Stem	Leaves
0.06	ND*	ND	ND	ND
0.11	ND	ND	ND	ND
0.28	ND	ND	ND	ND
0.56	ND	0.35	ND	0.46
1.12	ND	0.79	ND	1.11
2.80	ND	0.92	ND	0.92
5.60	0.10	0.53	ND	0.80
11.20	0.10	1.50	ND	0.90

* Not detected. Limit of detection = 0.1 ppm.

Fig. 3

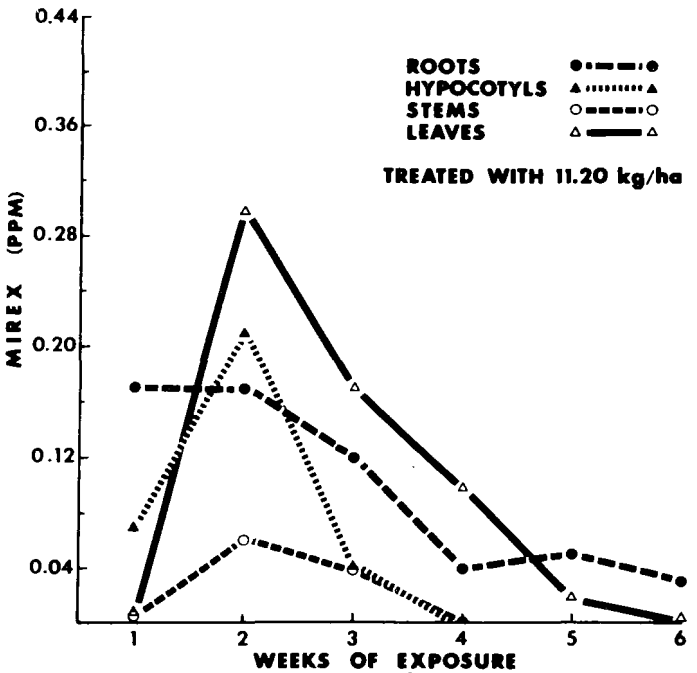


Figure 3. Mirex in roots, hypocotyls, stems and leaves of red mangrove treated with 11.20 kg/ha.

Fig. 4

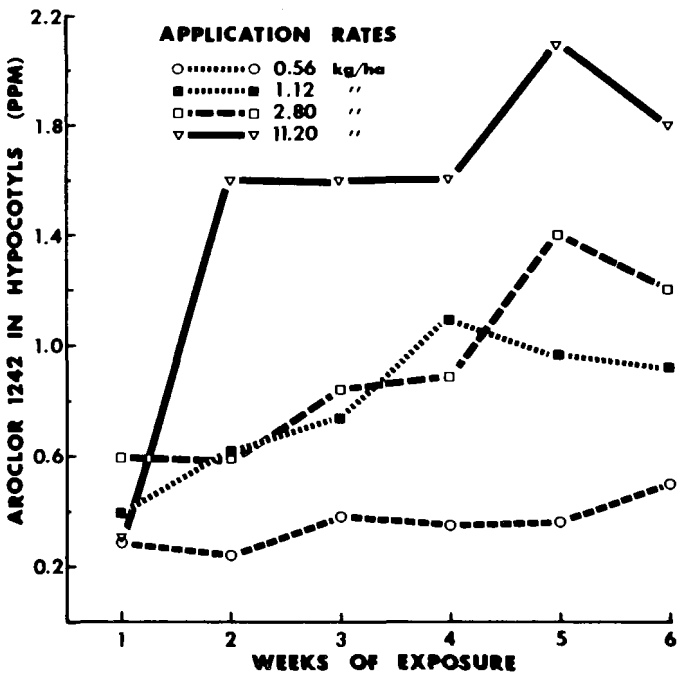


Figure 4. Uptake of Aroclor 1242 by hypocotyls of red mangrove.

was detected in hypocotyls and leaves at application rates of 0.56 kg/ha and greater (Table 3).

Concentrations of Aroclor 1242 in leaves did not change with time at any treatment rate, but concentrations in hypocotyls increased in relation to exposure time for at least five weeks (Figure 4). In hypocotyls, concentrations of PCB were greater than concentrations of each of the other three compounds.

SUMMARY

Mangrove seedlings from the field were found to contain DDD, dieldrin, and PCBs.

In the laboratory, mangrove seedlings translocated dieldrin, methoxychlor, mirex, and Aroclor 1242 (a PCB) from soil to various plant parts. Dieldrin was detected in hypocotyls and leaves of seedlings exposed to application rates of 0.06 kg/ha and above; methoxychlor in hypocotyls at rates of 0.28 kg/ha and above; Aroclor 1242 in hypocotyls and leaves at rates of 0.56 kg/ha and above; and mirex in roots, hypocotyls, stems, and leaves only at the highest treatment rate of 11.20 kg/ha.

The data show that these persistent organochlorine compounds can be translocated to seedlings. If the compounds are present in the natural mangrove environment, it is possible that they could enter seedlings and pass to higher trophic levels when seedlings are eaten by estuarine organisms.

ACKNOWLEDGEMENTS

We thank Mr. Robert L. Goodrick of the Central and Southern Florida Flood Control District for providing the seedlings used in these studies. We also thank Dr. Philip A. Butler, EPA, Gulf Breeze Environmental Research Laboratory, for redidue data on mangroves from the Virgin Islands, and Dr. Seppo E. Kolehmainen, Puerto Rico Nuclear Center, for sending seedlings from Puerto Rico.

REFERENCES

MEHENDALE, H. M., L. FISHBEIN, M. FIELDS, and H. B. MATTHEWS. Bull. Environ. Contam. Toxicol. 8, 200 (1972).

MILLS, P. A., J. F. ONLEY, and R. A. GAITHER. J. Assoc. Off. Agric. Chem. 46, 182 (1965).

MUMMA, R. O., W. B. WHEELER, D. E. H. FREAR, and R. H. HAMILTON. Science 152, 530 (1966).

NASH, R. G. and M. L. BEALL, JR. Science 168, 1109 (1970).

ODUM, W. E. Univ. Miami, Sea Grant Tech. Bull. No. 7, 162 p. (1971).

WALSH, G. E., R. BARRETT, G. H. COOK, and T. A. HOLLISTER. Bioscience 23, 361 (1973).

CONTRIBUTION NO. 195

Reprinted from:
POLLUTION AND PHYSIOLOGY OF MARINE ORGANISMS

IMPLICATIONS OF PESTICIDE RESIDUES IN THE COASTAL ENVIRONMENT

THOMAS W. DUKE and DAVID P. DUMAS

U. S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, Florida 32561

Residues of pesticides occur in biological and physical components of coastal and oceanic environments and some of the residues have been implicated in degradation of portions of these environments. The presence of many pesticides can be detected at the parts-per-trillion level, but the effects of such levels of pesticides on the organisms and systems in which they occur are not clear in many instances. Knowledge of these effects is especially important when the residues occur in the coastal environment—a dynamic, highly productive system where fresh water from rivers mingles with salt water from the sea. The coastal zone interfaces with man's activities on land and, therefore, is especially susceptible to exposure to acute doses of degradable pesticides, as well as chronic doses of persistent ones.

This paper briefly reports the state-of-the-art of research on the effects of pesticides on coastal aquatic organisms. For a comprehensive review of recent literature in this field, see Walsh (1972b);

for a compilation of data, see the EPA Report to the States (1973).

Patterns of pesticide usage are changing in this country and these changes are reflected in amounts of various pesticides produced annually. Smaller amounts of the organochlorine pesticides are being applied because of their persistence in the environment, the capability of organisms to concentrate them (bioconcentration) and their adverse effects on nontarget organisms. For many uses, organophosphates and carbamates have replaced organochlorines because organophosphates and carbamates hydrolyze rapidly in water and, therefore, are not accumulated to the same extent as organochlorines. Some of the organophosphates, however, are extremely toxic to aquatic organisms on a short-time basis (Coppage, 1972). Much effort is being devoted to developing biological control measures that will introduce viruses and juvenile insect hormones into the environment as part of an integrated pest control program. The integrated pest control approach combines biological and chemical methods to control pests in an effort to reduce the amount of synthetic chemicals being added to the environment. A list of several important pesticides that are used currently or appear as residues in marine organisms or both is presented in Table 1.

Samples collected in the National Estuarine Monitoring Program and in other programs show that a variety of pesticides occur in biota and nonliving components of the marine environment. Pesticide residues have been reported in whales from the Pacific Ocean (Wolman and Wilson, 1970), fish from southern California (Modin, 1969), invertebrates and fish from the Gulf of Mexico (Giam *et al.*, 1972), fish from estuaries along the Gulf of Mexico (Hansen and Wilson, 1970), fauna in an Atlantic coast estuary (Woodwell *et al.*, 1967), zooplankton from the Atlantic Ocean (Harvey *et al.*, 1972), and shellfish from all three coasts (Butler, 1973). These residues indicate that pesticides can reach nontarget organisms in the marine environment and give some indications of

TABLE 1
Toxic Organics Used as Pesticides or Appearing as Residues in Marine Organisms or Both

Organochlorines (Insecticides)	Organophosphates (Insecticides)	Carbamates (Insecticides)	Herbicides
Chlordane	Diazinon	Carbaryl	2,4-D
DDT	Guthion	Carbofuran	Picloram
Dieldrin	Malathion		Triazines
Endrin	Naled		Urea
Methoxychlor	Parathion		
Mirex	Phorate		
Toxaphene			

biological reservoirs of pesticides in this environment. The information obtained in these monitoring programs is invaluable to those interested in managing our natural resources, but care must be exercised in interpreting monitoring data.

Biological problems that affect the interpretation of monitoring data were discussed recently by Butler (1974). Factors affecting persistent organochlorine residues include kind of species sampled, age of individuals monitored, natural variations in individuals, seasonal variation, and selection of tissues to be analyzed. Laboratory experiments and observations in the field have shown that filter-feeding mollusks are good indicators of the presence of organochlorine pesticides in estuarine waters. These animals are sedentary, have the capacity to concentrate the chemicals in their soft tissues many times the concentration in the water and lose the chemicals rather quickly when exposed to clean water. Obviously, mollusks would be helpful in locating the source of a particular organochlorine. Conversely, pelagic fish might not be useful in locating a particular source because they could have accumulated a residue some distance from the point of collection.

As patterns of pesticide usage change, techniques for monitoring the occurrence of the pesticides also must change. Occurrences of organophosphates, carbamates and biological control agents cannot be monitored in the same manner as occurrences of organochlorine and other more persistent chemicals. To help identify the presence of a pesticide it may be necessary to utilize changes in biological systems, as opposed to routine chemical analyses of organisms or other components of the environment. Also required is a concomitant effort to understand the effect of residues on the organisms and systems in which they occur.

CONCEPT OF EFFECTS

The implications of pesticide residues in the marine and other environments depends upon the effect of the chemicals on the component in which they occur. A conceptual model of possible effects of pesticides and other toxic substances on biological systems is shown in Figure 1 (Dr. John Couch, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida, unpublished personal communication). The possible impact of a stressor on a biological system is described as the system changes from (1) a normal steady-state to (2) one of compensation to (3) decompensation to death. Accordingly, a pesticide could be considered to have an adverse effect if it temporarily or permanently altered the normal steady-state of a particular biological system to such a degree as to render the homeostatic (compensating) mechanism incapable of maintaining an acceptable altered steady-state.

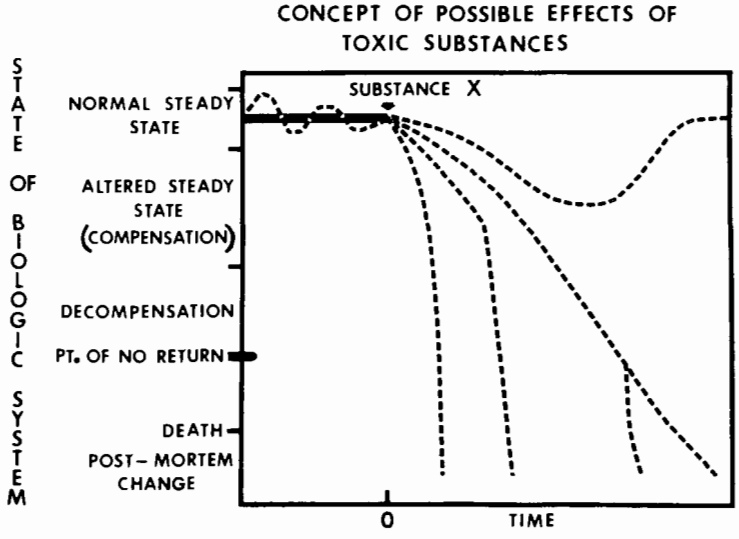


Fig. 1. Concept of possible effects of toxic substances.

NORMAL STEADY-STATE

It has been said that the most consistent trait of biological systems is their inconsistency. The normal steady-state of a particular biological system, therefore, is difficult to define. Each system, from an estuarine ecosystem to a system within individual organisms, has a natural range of variability in such factors as population density, species diversity, community metabolism, oxygen consumption, enzyme production, avoidance mechanisms, osmotic regulation, natural pathogens, and others. Obviously, much must be known about the normal or healthy system before an evaluation can be made of the effect of a pesticide on the system.

In relation to this, the impact of pesticides on ecosystems is poorly understood because often the "normal" system itself is poorly understood. An ecosystem can be considered a biological component that consists of all of the plants and animals interacting in a complex manner with their physical environment. The "normal" state of a dynamic coastal ecosystem no doubt depends upon the characteristics of a particular ecosystem, and changes as the system matures. The importance of symbiosis, nutrient conservation, and stability as a result of biological action in an estuarine ecosystem is pointed out by Odum (1969). According to Odum, in many instances, biological control of population and nutrient cycles prevents destructive oscillations within the system. Therefore, a pollutant that interferes with these biological actions could adversely affect the ecosystem.

ALTERED STEADY-STATE (COMPENSATION)

An acute dose of a pesticide could cause a biological system to oscillate outside its normal range of variation, yet with time, the system could return to the normal state without suffering lasting effects. An example of this phenomenon at the ecosystem level was demonstrated by Walsh, Miller, and Heitmuller

(1971), who introduced the herbicide dichlobenil into a small pond on Santa Rosa Island. Applied as a wettable powder at a concentration of one part per million, the herbicide eliminated the rooted plants in the pond. As the benthic plants died, blooms of phytoplankton and zooplankton occurred and a normal oxygen regime was maintained. As benthic plants returned, the number of plankters dropped. The pond returned to a "normal" state in reference to the primary producers approximately 3 months after treatment. A possible example of such compensation in an individual organism was shown recently when spot, *Leiostomus xanthurus*, were exposed to Aroclor®[a] 1254 under laboratory conditions (Couch, 1974). Even though in many fish no outward signs of stress were present, the livers of the fish accumulated excess fat during the tests. For a period of time, the liver evidently was able to contend with excessive fat accumulation, but eventually chronic damage leading to necrosis occurred; therefore, the fish entered another biological state.

DECOMPENSATION TO DEATH

The effect of a stress can eventually reach the point where the biological system can no longer compensate and death results. In the instance in which Aroclor 1254 was related to fat globules in the liver of fish, continued exposure to the chemical caused a necrotic liver. Eventually, the test organisms died as a result of the exposure. In the past, most of the data upon which criteria and standards were based used death as the criterion for effect. Much time and effort now are being devoted to developing other criteria, such as effects of relative concentrations of the chemicals on tissue and cell structure, enzyme

---

[a] ® Registered trademark: Aroclor, Monsanto Co. Mention of commercial products does not constitute endorsement by the U. S. Environmental Protection Agency.

reaction, osmotic regulation, behavioral patterns, growth and reproduction.

ASSESSMENT OF EFFECTS

The concept just presented is helpful in visualizing the manner in which pesticides can affect coastal organisms and systems. However, quantitative information must be developed in order to assess the effect of a particular pesticide on the environment or on a component of the environment. For example, it is not enough to know that a pesticide causes an altered steady-state in a fish and eventually causes death. The level of pesticide in the environment that causes the effect must be known and, perhaps even more important, the level at which no effect occurs must be known.

Much of the quantitative information available on effects of pesticides on marine organisms is in terms of acute mortality of individual organisms. In many instances, these data were obtained through routine bioassay tests in which known amounts of pesticides are administered to test organisms for a given period of time. In routine bioassays, the test organisms are examined periodically and compared with control organisms. If conducted for a short time in relation to the life span of the organisms, usually 96 hrs, the tests are considered acute. Longer tests over some developmental stage or reproductive cycles are termed chronic. (An excellent discussion of bioassays and their usefulness is presented by Sprague (1969, 1970).)

Often, it is necessary to estimate the effect of a pesticide on the coastal environment from only a minimum amount of data. Interim guidelines sometimes must be issued on the basis of a few acute bioassays while more meaningful data are being obtained. An application factor is helpful in these instances. This factor is a numerical ratio of a safe concentration of a pesticide to the acutely lethal concentration

(LC_{50}). An estimate can be made for an "acceptable" level of a pesticide in marine waters by multiplying the LC_{50} determined in acute bioassays by the appropriate application factor. In many instances, an arbitrary application factor of 0.01 is used when necessary scientific data have not yet been developed. For a discussion on obtaining the application factor experimentally, see Mount (1968) and Brungs (1969).

Information obtained by various bioassay tests on some toxic organics of current interest is shown in Table 2. These results were compiled from the literature and indicate the most sensitive organisms tested against these pesticides and organochlorines. The data give a general idea of the relative toxicity of the various pollutants.

During the past few years, the need for data on chronic or partial chronic exposures and on sublethal effects of pesticides on marine organisms has become evident. Chronic studies involve the exposure of organisms to a pesticide over an entire life cycle, and often are referred to as "egg-to-egg" studies. A subacute chronic is conducted over part of a life cycle. Sublethal studies are designed to determine if a pesticide has an effect at concentrations less than those that are lethal to the organisms and utilize such criteria as growth, function of enzyme systems, and behavior of populations of organisms.

EFFECT OF PESTICIDES ON GROWTH OF ORGANISMS

The effects of pesticides on marine phytoplankton are often related to growth of the organisms. The effects often vary according to the pesticide and to the species of phytoplankton. For example, Menzel *et al.* (1970) found that growth in cultures of marine phytoplankton was affected by DDT, dieldrin and endrin. *Dunaliella* apparently was not affected by concentrations up to 1000 parts per billion. In *Cyclotella*, cell division was completely inhibited by dieldrin and endrin and DDT slowed division of the cells. The authors suggested that estuarine species,

TABLE 2
Toxicity of Selected Pesticides to Marine Organisms

Substance Tested	Formulation	Organism Tested	Common Name	Conc. (ppb, Act. Ingred.) in Water	Method of Assessment	Test Procedure	Reference
Insecticides							
Organochlorines:							
Chlordane	100%	*Palaemon macrodactylus*	Korean shrimp	18 (10-38)	TL-50	96-hr static lab bioassay	Earnest, unpublished
DDT Compounds							
p,p'-DDT(1,1,1-Trichloro-2,2-bis (p-chlorophenyl) ethane	Technical 77%	*Penaeus duorarum*	Pink shrimp	0.12 0.17 (0.09-0.32)	TL-50 TL-50	28-day bioassay 96-hr intermittent flow lab bioassay	Nimmo *et al.*, 1971 Earnest, unpublished
p,p'-DDD(p,p'-TDE) (1,1-Dichloro-2,2-bis (p-chlorophenyl) ethane	99%	*Palaemon macrodactylus*	Korean shrimp	2.5 (1.6-4.0)	TL-50	96-hr intermittent flow lab bioassay	Earnest, unpublished
p,p'-DDE (1,1-Dichloro-2,2-bis (p-chlorophenyl) ethylene	---	*Falco peregrinus*	Peregrine falcon	---	eggshell thinning	DDE in eggs highly correlated with shell thinning	Cade *et al.*, 1971
Dieldrin	100%	*Anguilla rostrata*	American eel	0.9	LC-50	96-hr static lab bioassay	Eisler, 1970
Endrin	100%	*Mugil cephalus*	Striped mullet	0.3	LC-50	96-hr static lab bioassay	Eisler, 1970
	100%	*Menidia menidia*	Atlantic silverside	0.05	LC-50	96-hr static lab bioassay	Eisler, 1970
Methoxychlor	89.5%	*Palaemon macrodactylus*	Korean shrimp	0.44 (0.21-0.93)	TL-50	96-hr static lab bioassay	Earnest, unpublished
Mirex	Technical	*Penaeus duorarum*	Pink shrimp	1.0	100% paralysis/ death in 11 days TLM	Flowing water bioassay	Lowe *et al.*, 1971
Toxaphene	100%	*Gasterosteus aculeatus*	Threespine stickleback	7.8		96-hr static lab bioassay	Katz, 1961

TABLE 2—Continued
Toxicity of Selected Pesticides to Marine Organisms

Substance Tested	Formulation	Organism Tested	Common Name	Conc. (ppb Act. Ingred.) in Water	Method of Assessment	Test Procedure	Reference
Insecticides							
Organophosphates:							
Diazinon	Technical Grade	*Cyprinodon variegatus*	Sheepshead minnow	100	Mean inhibition of brain AChE (Result: >84%)	Static bioassay, 48-hr LC 40-60	Coppage, 1972
Guthion	93%	*Gasterosteus aculeatus*	Threespine stickleback	4.8	TLM	96-hr static lab bioassay	Katz, 1961
	Technical Grade	*Cyprinodon variegatus*	Sheepshead minnow	3	Mean inhibition of brain AChE (Result: 84%)	Static bioassay, 72-hr LC 40-60	Coppage, 1972
	Technical Grade	*Lagodon rhomboides*	Pinfish	10	Mean inhibition of brain AChE (Result: 80%)	Flowing seawater bioassay, 24-hr LC 40-60	Coppage and Matthews, 1974
	Technical Grade	*Leiostomus xanthurus*	Spot	20	Mean inhibition of brain AChE (Result: 96%)	Flowing seawater bioassay, 24-hr LC 40-60	Coppage and Matthews, 1974
Malathion	Technical Grade	*Cyprinodon variegatus*	Sheepshead minnow	190	Mean inhibition of brain AChE (Result: >84%)	Static bioassay, 24-hr LC 40-60	Coppage, 1972
	Technical Grade	*Lagodon rhomboides*	Pinfish	238	Mean inhibition of brain AChE (Result: 88%)	Flowing seawater bioassay, 24-hr LC 40-60	Coppage and Matthews, 1974
	Technical Grade	*Leiostomus xanthurus*	Spot	238	Mean inhibition of brain AChE (Result: 70%)	Flowing seawater bioassay, 24-hr LC 40-60	Coppage and Matthews, 1974
	100%	*Thalassoma bifasciatum*	Bluehead	27	LC-50	96-hr static lab bioassay	Eisler, 1970

TABLE 2—Continued
Toxicity of Selected Pesticides to Marine Organisms

Substance Tested	Formulation	Organism Tested	Common Name	Conc. (ppb Act. Ingred.) in Water	Method of Assessment	Test Procedure	Reference
Insecticides							
Organophosphates:							
Naled	Technical Grade	*Lagodon rhomboides*	Pinfish	23	Mean inhibition of brain AChE (Result: 89%)	Flowing seawater bioassay, 72-hr LC 40-60	Coppage and Matthews, 1974
	Technical Grade	*Leiostomus xanthurus*	Spot	70	Mean inhibition of brain AChE (Result: 85%)	Flowing seawater bioassay, 24-hr LC 40-60	Coppage and Matthews, 1974
Parathion	Technical Grade	*Cyprinodon variegatus*	Sheepshead minnow	10	Mean inhibition of brain AChE (Result: 84%)	Static bioassay, 72-hr LC 40-60	Coppage, 1972
	Technical Grade	*Lagodon rhomboides*	Pinfish	10	Mean inhibition of brain AChE	Flowing seawater bioassay, 24-hr LC 40-60	Coppage and Matthews, 1974
	Technical Grade	*Leiostomus xanthurus*	Spot	10	Mean inhibition of brain AChE (Result: 90%)	Flowing seawater bioassay, 24-hr LC 40-60	Coppage and Matthews, 1974
Methyl Parathion	100%	*Crangon septemspinosa*	Sand shrimp	2	LC-50	96-hr static lab bioassay	Eisler, 1969
Phorate	Technical Grade	*Cyprinodon variegatus*	Sheepshead minnow	5	Mean inhibition of brain AChE (Result: >84%)	Static bioassay 72-hr LC 40-60	Coppage, 1972
Carbamates:							
Carbaryl	100%	*Palaemon macrodactylus*	Korean shrimp	7.0 (1.5-28)	TL-50	96-hr intermittent flow lab bioassay	Earnest, unpublished
	Technical Grade	*Lagodon rhomboides*	Pinfish	1333	Mean inhibition of brain AChE (Result: 81%)	Flowing seawater bioassay, 24-hr LC 40-60	Coppage, unpublished

TABLE 2—Continued
Toxicity of Selected Pesticides to Marine Organisms

Substance Tested	Formulation	Organism Tested	Common Name	Conc. (ppb Act. Ingred.) in Water	Method of Assessment	Test Procedure	Reference
Insecticides							
Carbamates:							
Carbofuran	Acetone wash from sand-coated particle formulation	*Cyprinodon variegatus*	Sheepshead minnow	Unknown	Mean inhibition of brain AChE (Result: 84%)	Static bioassay 48-hr LC 40-60	Coppage, unpublished
Herbicides							
2,4-D and derivatives	Ester	*Crassostrea virginica*	American oyster	740	TLM	14-day static lab bioassay	Davis and Hidu, 1969
Picloram							
Tordon® 101 (39.6% 2,4-D 14.3% picloram)	---	*Isochrysis galbana*	---	5×10^5	50% decrease in O_2 evolution[a]	---	Walsh, 1972a
Triazines:							
Ametryne	Technical acid	*Chlorococcum* sp.	---	10	50% decrease in growth	Measured as ABS. (525mμ) after 10 days[b]	Walsh, 1972a
	Technical acid	*Isochrysis galbana*	---	10	50% decrease in O_2 evolution[a]	---	Walsh, 1972a
	Technical acid	*Monochrysis lutheri*	---	10	50% decrease in O_2 evolution[a]	---	Walsh, 1972a
	Technical acid	*Phaeodactylum tricornutum*	---	10	50% decrease in O_2 evolution[a]	---	Walsh, 1972a
Atrazine	Technical acid	*Chlorococcum* sp.	---	100	50% decrease in growth	Measured as ABS. (525mμ) after 10 days[b]	Walsh, 1972a
	Technical acid	*Chlamydomonas* sp.	---	60	50% decrease in O_2 evolution[a]	---	Hollister and Walsh, 1973
	Technical acid	*Monochrysis lutheri*	---	77	50% decrease in O_2 evolution[a]	---	Hollister and Walsh, 1973

TABLE 2—Continued
Toxicity of Selected Pesticides to Marine Organisms

Substance Tested	Formulation	Organisms Tested	Common Name	Conc. (ppb Act. Ingred.) in Water	Method of Assessment	Test Procedure	Reference
Herbicides							
Triazines:							
Atrazine	Technical acid	*Isochrysis galbana*	---	100	50% decrease in O_2 evolution[a]	---	Walsh, 1972a
	Technical acid	*Phaeodactylum tricornutum*	---	100	50% decrease in O_2 evolution[a]	---	Walsh, 1972a
Simazine	Technical acid	*Isochrysis galbana*	---	500	50% decrease in growth	Measured as ABS. (525mμ) after 10 days[b]	Walsh, 1972a
	Technical acid	*Phaeodactylum tricornutum*	---	500	50% decrease in growth	Measured as ABS. (525mμ) after 10 days[b]	Walsh, 1972a
Urea:							
Diuron	---	*Protococcus* sp.	---	0.02	0.52 OPT. DEN. expt/OPT. DEN. control[b]	10-day growth	Ukeles, 1962
	---	*Monochrysis lutheri*	---	0.02	0.00 OPT. DEN. expt/OPT. DEN. control[b]	10-day growth	Ukeles, 1962
	Technical	*Chlorococcum* sp.	---	10	50% decrease in growth	10-day growth	Walsh, 1972a
	Technical acid	*Isochrysis galbana*	---	10	50% decrease in growth	10-day growth	Walsh, 1972a
	---	*Monochrysis lutheri*	---	290	0.67 OPT. DEN. expt/OPT. DEN. control[b]	10-day growth	Ukeles, 1962

[a] O_2 evolution measured by Gilson differential respirometer on 4 mℓ of culture in log phase. Length of test 90 min.

[b] ABS. (525mμ) = Absorbance at 525 millimicrons wavelength. OPT. DEN. expt/OPT. DEN. control = Optical density of experimental culture/optical density of control culture.

such as *Dunaliella*, are perhaps less susceptible than are open ocean forms, such as *Cyclotella*. Similar studies on phytoplankton and PCBs by Fisher *et al.* (1972) also suggested that coastal phytoplankton may be more resistant to organochlorines than are those found in open ocean. Isolates of diatoms from the Sargasso Sea were more sensitive than clones from estuaries and the continental shelf. Herbicides applied to four species of marine unicellular algae adversely affected their growth (Walsh, 1972a). Urea and triazine herbicides were the most toxic of the formulations tested. In some instances, smaller amounts of herbicides were required to inhibit growth than to inhibit oxygen evolution. Interestingly, *Dunaliella* was most resistant of the four species tested, as occurred in Menzel's *et al.* studies (1970).

The effect of mirex and a PCB, Aroclor 1254, on growth of ciliate, *Tetrahymena pyriformis*, was studied by Cooley *et al.* (1972). Both chemicals caused significant reductions in growth rate and population density and the ciliate accumulated both toxicants from the culture media, concentrating mirex up to 193 times and Aroclor to approximately 60 times the nominal concentration in the media. The authors postulate that if this ciliate encountered similar concentrations of these materials in nature, the results would be a reduction of their availability as food organisms and nutrient regenerators. Also, the capacity of the organisms to concentrate mirex and Aroclor could provide a pathway for entry of these chemicals into the food web.

Growth rates of young oysters, *Crassostrea virginica*, as indicated by height and in-water weight, was significantly reduced in individuals exposed to 5 micrograms of Aroclor 1254 per liter (ppb) for 24 weeks, but growth rate was not affected in individuals exposed to 1 part per billion for 30 weeks (Lowe *et al.*, 1972). Oysters exposed to 1 part per billion concentrated the chemical 101,000 times, but less than 0.2 part per million remained after 12 weeks of depuration. The growth rate of the oyster

was a much more sensitive indicator, since no significant mortality occurred in oysters exposed to 5 ppb.

The effects of mirex on growth of crabs, as measured by the duration of developmental stages of crabs as an indicator of their growth, is illustrated by the work of Bookhout *et al.* (1972). The duration of developmental stages of zoea and the total time of development was generally lengthened with an increase in concentration of mirex from 0.01 to 10.0 parts per billion. *Menippe* did not demonstrate this effect, but the percentage of the extra 6th zoeal stage increased as concentrations of mirex increased. This method of determining the effect of mirex on crabs appears to be more sensitive than previous tests with juvenile blue crabs reported by McKenzie (1970) and Lowe *et al.* (1971).

EFFECTS OF PESTICIDES ON BEHAVIOR OF ORGANISMS

The behavioral activity of organisms is a sensitive criterion for determining the effect of pesticides on marine organisms. Dr. H. G. Kleerekoper has successfully studied the interactions of temperature and a heavy metal on the locomotor behavior of fish in the laboratory (Kleerekoper and Waxman, 1973) and will present data on the effect of pesticides on marine fish later in this volume. Hansen (1969) showed that the estuarine fish, *Cyprinodon variegatus*, avoided water containing DDT, endrin, Dursban®[b] or 2,4-D in controlled laboratory experiments, but the fish did not avoid test concentrations of malathion or Sevin®.[c] Likewise, grass shrimp, *Palaemonetes pugio*, an important forage food for estuarine organisms, avoided 1.0 and 10.0 ppm of 2,4-D by seeking

---

[b] ® Registered trademark: Dursban, Dow Chemical Company.

[c] ® Registered trademark: Sevin, Union Carbide Company.

water free of this herbicide, but did not avoid the five insecticides tested (Hansen *et al.*, 1973). The capacity of coastal organisms to avoid water containing pesticides may enhance their survival by causing them to move to an area free of pesticides. Avoidance could be disastrous to a population if, by avoiding the pesticides, the population is unable to reach an area where spawning normally occurs.

EFFECTS OF PESTICIDES ON ENZYME SYSTEMS

Inhibition of the hydrolyzing enzyme, acetylcholinesterase (AChE), by organophosphate and carbamate pesticides can be used as an indication of the effect of these chemicals on estuarine fish (Coppage, 1972). Evidently, esterase-inhibiting pesticides bind active sites of the enzyme and block the breakdown of acetylcholine, which causes toxic accumulation of acetylcholine. As a result, nerve impulse transfers can be disrupted. Laboratory bioassays with estuarine fish spot, *Leiostomus xanthurus*, showed that lethal exposures of this fish to malathion reduced the AChE activity level by 81%. Such information developed in the laboratory is useful in evaluating effects of pesticides applied in the field.

EFFECTS OF PESTICIDES ON ECOSYSTEMS AND COMMUNITIES

Few data are available concerning the effects of pesticides at the ecosystem or community level of organization. This is not surprising considering the complexities of ecosystems and our lack of knowledge of the structure and function of coastal zones. Effects of pesticides could be masked by variations in population densities and it would require several years to evaluate such variations. However, it is possible to design laboratory and field experiments to yield information on this complex system.

An experimental community that received 10 micrograms per liter of a polychlorinated biphenyl, Aroclor 1254, did not recover to a "normal" state in

terms of numbers of phyla and species after 4 months (Hansen, 1974). Communities of planktonic larvae were allowed to develop in "control" aquaria and aquaria that received the Aroclor 1254. Communities that received 10 micrograms per liter of the chemical were dominated by tunicates, whereas controls were dominated by arthropods. The Shannon-Weaver species diversity index was not altered by Aroclor 1254, but numbers of phyla, species and individuals decreased.

The capacity of a fish population to compensate for the effect of a pesticide was suggested in a recent study made in Louisiana, where malathion was applied aerially to control mosquito vectors of Venezuelan equine encephalomyelitis (Coppage and Duke, 1972). Fish were collected from the coastal area before, during and after the application of malathion. Acetylcholinesterase (AChE) activity in the brains of fish were used as an indicator of the effect of malathion on the community of fish. Levels of inhibition during and soon after spraying in one lake approached levels that were associated with death of fish in laboratory bioassay studies. The AChE activity of the fish population returned to normal within 40 days after application of the chemical.

CONCENTRATION FACTORS

The capacity of organisms to concentrate a pesticide is another factor that must be considered when evaluating the impact of these chemicals on a coastal system. Many of the persistent pesticides are passed through the food web through accumulation and bioconcentration. Some question exists about the mechanisms involved in trophic accumulation of fat-soluble hydrocarbons from water by aquatic organisms (Hamelink *et al.*, 1971). Whatever the mechanisms for accumulation, many coastal organisms have the capacity to concentrate pesticides many times more than the concentration occurring in the water around them. Concentration factors, the ratio of the amount of

pesticide in the animal to that in the water, for some specific organisms and pesticides determined by investigators at the Gulf Breeze Environmental Research Laboratory are shown in Table 3.

STATE OF THE ART

Concern about the occurrence of pesticides in the marine environment is continually emphasized because surveillance and research on these chemicals are given high priority by knowledgeable scientists. The analytical capability for determining residues of some pesticides in the parts per trillion range is available, but we often do not understand the biological or ecological significance of these residues. We need more information on chronic exposures of sensitive marine organisms during complete reproductive cycles and on effects of sublethal levels of exposure. Also, information is required on the structure and function of coastal ecosystems and criteria for evaluating the stress of pesticides on these systems. Laboratory microcosms and other kinds of experimental environments no doubt will be useful in this evaluation.

As mentioned previously, use-patterns of pesticides in this country are changing. We must be prepared to evaluate possible effects, on the environment, of integrated pest control procedures, whereby biological control may be just important as chemical control of pests. Viruses and juvenile-hormone mimics are being tested for use as pesticides and could inadvertently reach the coastal zone. The research effort to evaluate the impact of these new agents must take into account that the coastal environment already contains residues of pesticides, persistent organochlorines, and other pollutants.

TABLE 3
Accumulation of Pesticides from Water by Marine Organisms[a]

Substance Tested	Organism Tested	Common Name	Exp. Conc.	Conc. Factor	Time	Special Details	Reference
Insecticides							
Organochlorines:							
Chlordane	*Pseudomonas* spp.	---	10 ppm	0.83	10 days	Mixed culture of four species	Bourquin, unpublished
DDT	*Brachidontes recurvus*	Hooked mussel	1 ppb	24,000	1 week	Whole body residues (Meats)	Butler, 1966
	Mercenaria mercenaria	Hard-shell clam	1 ppb	6,000	1 week	Whole body residues (Meats)	Butler, 1966
	Mya arenaria	Soft-shell clam	0.1 ppb	8,800	5 days	Whole body residues (Meats)	Butler, 1971
	Crassostrea gigas	Pacific oyster	1.0 ppb	20,000	7 days	Whole body residues (Meats)	Butler, 1966
	Penaeus duorarum	Pink shrimp	0.14 ppb	1,500	3 weeks	Whole body residues	Nimmo *et al.*, 1970
	Lagodon rhomboides	Pinfish	0.1, 1.0 ppb	10,600 38,000	2 weeks	Whole body residues	Hansen and Wilson, 1970
Dieldrin	*Mercenaria mercenaria*	Hard-shell clam	0.5 ppb	760	5 days	Whole body residues (Meats)	Butler, 1971
Endrin	*Mercenaria mercenaria*	Hard-shell clam	0.5 ppb	480	5 days	Whole body residues (Meats)	Butler, 1971
Methoxychlor	*Mercenaria mercenaria*	Hard-shell clam	1.0 ppb	470	5 days	Whole body residues (Meats)	Butler, 1971
Mirex	*Tetrahymena pyriformis* W	---	0.9 ppb	193	1 week	Axenic cultures incubated at 26°C; concentration factor on dry weight basis	Cooley *et al.*, 1972
	Penaeus duorarum	Pink shrimp	0.1 ppb	2,600	3 weeks	Whole body residues	Lowe *et al.*, 1971

TABLE 3—Continued
Accumulation of Pesticides from Water by Marine Organisms[a]

Substance Tested	Organism Tested	Common Name	Exp. Conc.	Conc. Factor	Time	Special Details	Reference
Insecticides							
Organochlorines:							
Mirex	*Rhithropanopeus harrisii*	Mud crab (larvae)	0.1 ppb	1,000	7 weeks	Static culture bowl method with a change to fresh medium + chemical each day	Bookhout *et al.*, 1972
	Callinectes sapidus	Blue crab (juveniles)	0.1 ppb	1,100-5,200	3 weeks	Whole body residues	Lowe, unpublished
	Thalassia testudinum	Turtle grass	0.1 ppb	0 leaves 0.36 rhizomes	10 days	Plants exposed to chemical through rhizomes; concentration factor on wet weight basis	Walsh and Hollister, unpublished
Halogenated Hydrocarbon:							
Polychlorinated biphenyl (PCB)							
Aroclor® 1254	*Tetrahymena pyriformis* W	---	1 ppm	60	1 week	Axenic cultures incubated at 26°C; concentration factor on dry weight basis	Cooley *et al.*, 1972
	Palaemonetes pugio	Grass shrimp	0.62 ppb	2,069	1 week	Whole body residues (Meats)	Nimmo and Heitmuller, unpublished
				26,580	5 weeks	Whole body residues (Meats)	Nimmo and Heitmuller, unpublished

TABLE 3—Continued
Accumulation of Pesticides from Water by Marine Organisms[a]

Substance Tested	Organism Tested	Common Name	Exp. Conc.	Conc. Factor	Time	Special Details	Reference
Insecticides Halogenated Hydrocarbon: Polychlorinated biphenyl (PCB) Aroclor ® 1254	*Penaeus duorarum*	Pink shrimp	2.5 ppb	1,800 7,600	2 days 9 days	Whole body residues Whole body residues	Nimmo *et al.*, 1971 Nimmo *et al.*, 1971
	Lagodon rhomboides	Pinfish	5 ppb	2,800-21,800	2-15 weeks	Whole body residues	Hansen *et al.*, 1971
	Leiostomus xanthurus	Spot	1 ppb 5 ppb	17,000-27,000 9,200-30,400	4-8 weeks 3-6 weeks	Whole body residues Whole body residues	Hansen *et al.*, 1971 Hansen *et al.*, 1971
	Thalassia testudinum	Turtle grass	5820 ppb	0 leaves 0 rhizomes	10 days	Plants exposed to chemical through *rhizomes*; concentration factor on wet weight basis	Walsh and Hollister, unpublished
Herbicide Tordon ® 101 (39.6% 2,4-D; 14.3% picloram)	*Rhizophora mangle*	Red mangrove	14.4 ppb	Stems 1.28 (2,4-D) 0.64 (picolinic acid)	20 days	Seedlings treated when two pairs of leaves were present; concentration factor on wet weight basis	Walsh *et al.*, 1973
	Thalassia testudinum	Turtle grass	5 ppm	Leaves 0 (2,4-D) 0 (picolinic acid)	10 days	Plants exposed to chemical through rhizomes; concentration factor on wet weight basis	Walsh and Hollister, unpublished

[a]Information developed at the Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory.

ACKNOWLEDGMENTS

We thank C. M. Herndon for assistance in preparing the manuscript; N. R. Cooley, D. J. Hansen and M. E. Tagatz for their constructive criticism of the manuscript; and the investigators at this laboratory for freely supplying data (some unpublished) for the tables.

LITERATURE CITED

Bookhout, C. G., A. J. Wilson, Jr., T. W. Duke, and J. I. Lowe. 1972. Effects of mirex on the larval development of two crabs. *Water, Air and Soil Pollution 1*:165-80.

Bourquin, A. W. Unpublished data. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida.

Brungs, W. A. 1969. Chronic toxicity of zinc to the fathead minnow, *Pimephales promelas* Rafinesque. *Trans. Am. Fish. Soc. 98*:272-79.

Butler, P. A. 1966. Pesticides in the marine environment. *J. Appl. Ecol. 3*:253-59.

_____. 1971. Influence of pesticides on marine ecosystems. *Proc. Roy. Soc. London B 177*:321-29.

_____. 1973. Organochlorine residues in estuarine mollusks, 1965-1972—National Pesticide Monitoring Program. *Pestic. Monitor. J. 6*:238-62.

_____. 1974. Biological problems in estuarine monitoring. Proceedings of Seminar on Standardization of Methodology in Monitoring the Marine Environment held in Seattle, Washington, October 16-18, 1973. In press.

Cade, T. J., J. L. Lincer, C. M. White, D. G. Roseneau, and L. G. Swartz. 1971. DDE residues and egg-shell changes in Alaskan falcons and hawks. *Science 172*:955-57.

Cooley, N. R., J. M. Keltner, Jr., and J. Forester. 1972. Mirex and Aroclor ® 1254: effect and accumulation by *Tetrahymena pyriformis* Strain W. *J. Protozool. 19*:636-38.

Coppage, D. L. Unpublished data. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida.

_____. 1972. Organophosphate pesticides: specific level of brain AChE inhibition related to death in sheepshead minnows. *Trans. Am. Fish. Soc. 101*:534-36.

_____ and T. W. Duke. 1972. Effect of pesticides in estuaries along the Gulf and southeast Atlantic Coasts. In: *Proc. Second Gulf Conf. on Mosquito Suppression and Wildl. Manage.*, pp. 24-31.

_____ and E. Matthews. 1974. Short-term effects of organophosphate pesticides on cholinesterases of estuarine fishes and pink shrimp. In press.

Couch, J. 1974. Histopathologic effects of pesticides and related chemicals on the livers of fishes. Proc. of Symposium on Research of Fishes sponsored by the Registry of Comparative Pathology. University of Wisconsin Press. In press.

Davis, H. C. and H. Hidu. 1969. Effects of pesticides development of clams and oysters and on survival and growth of the larvae. *Fish. Bull. 67*:393-404.

Earnest, R. Unpublished data. Effects of pesticides on aquatic animals in the estuarine and marine environment. In: *Annual Progress Report 1970*. Columbia, Mo.: Fish-Pesticide Research Laboratory, Bur. Sport Fish. Wildl., U. S. Dept. Interior.

Eisler, R. 1969. Acute toxicities of insecticides to marine decapod crustaceans. *Crustaceana 16*: 302-10.

_____. 1970. Acute toxicities of organochlorine and organophosphorus insecticides to estuarine fishes. *Bur. Sport Fish. Wildl. Tech. Paper* No. 46.

Environmental Protection Agency. 1973. Effects of pesticides in water—a report to the states.

Fisher, N. S., L. B. Graham, E. J. Carpenter, and C. F. Wurster. 1973. Geographic differences in phytoplankton sensitivity to PCBs. *Nature 241*: 548-49.

Giam, C. S., A. R. Hanks, R. L. Richardson, W. M. Sackett, and M. K. Wong. 1972. DDT, DDE, and polychlorinated biphenyls in biota from the Gulf of Mexico and the Caribbean Sea. *Pestic. Monitor. J. 6*:139-43.

Hamelink, J. L., R. C. Waybrant, and R. C. Ball. 1971. A proposal: exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. *Trans. Am. Fish. Soc. 100*:207-14.

Hansen, D. J. 1969. Avoidance of pesticides by untrained sheepshead minnows. *Trans. Am. Fish. Soc. 98*:426-29.

_____. 1974. Aroclor ® 1254: effect on composition of developing estuarine animal communities in the laboratory. Contributions in Marine Science, University of Texas. In press.

_____, P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr., and P. D. Wilson. 1971. Chronic toxicity, uptake, and retention of Aroclor 1254 in two estuarine fishes. *Bull. Environ. Contam. and Toxicol. 6*:113-19.

_____, S. C. Schimmel, and J. M. Keltner, Jr. 1973. Avoidance of pesticides by grass shrimp *(Palaemonetes pugio). Bull. Environ. Contam. and Toxicol. 9*:129-33.

_____ and A. J. Wilson, Jr. 1970. Significant of DDT residues in the estuary near Pensacola, Florida. *Pestic. Monitor. J. 4*:51-56.

Harvey, G. R., V. T. Bowen, R. H. Backus, and G. D. Grice. 1972. Chlorinated hydrocarbons in open-ocean Atlantic organisms. In: *The Changing Chemistry of the Oceans*, pp. 177-88, ed. by D. Dyrssen and D. Jagner. Stockholm: Almquist and Wiksell.

Hollister, T. A. and G. E. Walsh. 1973. Differential responses of marine phytoplankton to herbicides: oxygen evolution. *Bull. Environ. Contam. and Toxicol.* 9:291-95.

Katz, M. 1961. Acute toxicity of some organic insecticides to three species of salmonids and to the threespine stickleback. *Trans. Am. Fish. Soc. 90*:264-68.

Kleerekoper, H. and J. B. Waxman. 1973. Interaction of temperature and copper ions as orienting stimuli in the locomotion behavior of the gold-fish *(Carassius auratus)*. *J. Res. Bd. Canada 30*:725-28.

Lowe, J. I. Unpublished data. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida.

_____, P. R. Parrish, J. M. Patrick, Jr., and J. Forester. 1972. Effects of the polychlorinated biphenyl Aroclor ® on the American oyster *Crassostrea virginica*. *Mar. Biol. 17*: 209-14.

_____, _____, A. J. Wilson, Jr., P. D. Wilson, and T. W. Duke. 1971. Effects of mirex on selected estuarine organisms. *Trans. 36th N. Am. Wildl. and Natural Resources Conf.*, March 7-10, 1971, Portland, Oregon, 171-86.

McKenzie, M. D. 1970. Fluctuations in abundance of the blue crab and factors affecting mortalities. *South Carolina Wildlife Resources Dept., Marine Resources Division Technical Report 1*.

Menzel, D. W., J. Anderson, and A. Randike. 1970. Marine phytoplankton vary in their response to chlorinated hydrocarbons. *Science 167*:1724-26.

Modin, J. C. 1969. Chlorinated hydrocarbon pesticides in California bays and estuaries. *Pestic. Monitor. J. 3*:1-7.

Mount, D. I. 1968. Chronic toxicity of copper to fathead minnows (*Pimephales promelas*, Rafinesque). *Water Research 2*:215-23.

Nimmo, D. R., R. R. Blackman, A. J. Wilson, Jr., and J. Forester. 1971. Toxicity and distribution of Aroclor ® 1254 in the pink shrimp, *Penaeus duorarum*. *Mar. Biol. 11*:191-97.

_____ and P. T. Heitmuller. Unpublished data. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida.

_____, A. J. Wilson, Jr., and R. R. Blackman. 1970. Localization of DDT in the body organs of pink and white shrimp. *Bull. Environ. Contam. and Toxicol. 5*:333-41.

Odum, E. P. 1969. The strategy of ecosystem development. *Science 164*:262-70.

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Water Research 3*:793-821.

_____. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. *Water Research 4*:3-32.

Ukeles, R. 1962. Growth of pure cultures of marine phytoplankton in the presence of toxicants. *Appl. Microbiol. 10*:532-37.

Walsh, G. E. 1972a. Effects of herbicides on photosynthesis and growth of marine unicellular algae. *Hyacinth Control J. 10*:45-48.

_____. 1972b. Insecticides, herbicides and polychlorinated biphenyls in estuaries. *J. Wash. Acad. Sci. 62*:122-39.

_____, R. Barrett, G. H. Cook, and T. A. Hollister. 1973. Effects of herbicides on seedlings of the red mangrove, *Rhizophora mangle* L. *BioScience 23*:361-64.

_____ and T. A. Hollister. Unpublished data. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida.

_____, C. W. Miller, and P. T. Heitmuller. 1971. Uptake and effects of dichlobenil in a small pond. *Bull. Environ. Contam. and Toxicol. 6*: 279-88.

Wolman, A. A. and A. J. Wilson, Jr. 1970. Occurrence of pesticides in whales. *Pestic. Monitor. J. 4*: 8-10.

Woodwell, G. M., C. F. Wurster, and P. A. Isaacson. 1967. DDT residues in an east coast estuary: a case of biological concentration of a persistent insecticide. *Science 156*:821-24.

CONTRIBUTION NO. 196

(17)

Effects of the Polychlorinated Biphenyl, Aroclor®1016, on Estuarine Animals

PATRICK R. PARRISH, DAVID J. HANSEN, JOHN N. COUCH, JAMES M. PATRICK, JR., AND GARY H. COOK
U.S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory

Acute toxicity and rate of uptake and depuration of the polychlorinated biphenyl (PCB), Aroclor® 1016, were determined for certain estuarine animals in flowing sea water bioassays. Ninety-six hour EC50's were: American oyster (*Crassostrea virginica*), 10.2 μg/l; brown shrimp (*Penaeus aztecus*), 10.0 μg/l; and grass shrimp (*Palaemonetes pugio*), 9.1 μg/l. Pinfish (*Lagodon rhomboides*) did not die when exposed to 100 μg/l for 96 hours, but significant mortality occurred when pinfish were exposed to 32 μg/l for 42 days. Further, alterations in the pancreatic exocrine tissue surrounding the portal veins occurred in pinfish from the 42-day exposure. Maximum whole-body residue (wet-weight) in pinfish was 17,000× the nominal concentration in test water and whole-body residue after a 56-day depuration period in PCB-free water decreased 61%. Oysters exposed to 10 μg/l for 84 days accumulated the chemical 13,000× the concentration in test water and no PCB residue was detectable after a 56-day depuration period. — *® Registered trademark, Monsanto Company, St. Louis, MO. Mention of commercial products or trade names does not constitute endorsement by the Environmental Protection Agency. — Contribution No. 196, Gulf Breeze Environmental Research Laboratory.*

Reprinted from *The ASB Bulletin,*
Vol. 21, No. 2, April 1974, p. 74.

CONTRIBUTION NO. 198

Reprinted from:
POLLUTION AND PHYSIOLOGY OF MARINE ORGANISMS

New York San Francisco London

SOME PHYSIOLOGICAL CONSEQUENCES OF POLYCHLORINATED BIPHENYL- AND SALINITY-STRESS IN PENAEID SHRIMP

D. R. NIMMO and L. H. BAHNER

Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Gulf Breeze, Florida 32561

Estuaries are dynamic environments where there are many factors that fluctuate, such as temperature, salinity, currents, hydrostatic pressure, and oxygen or carbon dioxide concentrations. Unfortunately, domestic sewage (nutrients), oils, industrial chemicals, pesticides, metals, or altered temperatures are an influence in estuaries. The combined effects of the natural and man-introduced factors are largely unknown. In contrast, these interactions could adversely affect the biota of an estuary before such a trend was recognized. Therefore, one of the major problems facing us today is understanding and predicting the interactions of pollutants and natural stresses.

It is common knowledge that the commercial shrimps along the Gulf Coast undertake distinct euryhaline migrations. After adult shrimp spawn in the open Gulf from spring to fall, the postmysids and juveniles migrate into the fresher waters of bays where they grow rapidly to adulthood before returning to the Gulf. Obviously, these stages of

shrimp must be able to adjust to the changing salinities encountered in the estuary, and any factor diminishing the ability of the shrimp to adjust physiologically to these changes would have a detrimental effect on them.

One group of chemicals introduced by man that has recently been of concern to many ecologists is the PCBs, or polychlorinated biphenyls. In 1969, a PCB, identified as Aroclor® 1254[a] was discovered as a contaminant in water, sediment, and fauna of Escambia Bay, Florida (Duke *et al.*, 1970). An early survey indicated that whole body residues of the chemical in feral shrimp were as high as 14 mg/kg whole body (Nimmo *et al.*, 1971a). Subsequent toxicity tests on juvenile pink shrimp *(Penaeus duorarum)* revealed that about 1.0 µg/ℓ in the water would kill 50% of the experimental animals within 15 days (Nimmo *et al.*, 1971b).

While conducting bioassays at our laboratory we noted on several occasions that salinity appeared to affect toxicity. In one instance, adult pink shrimp were exposed chronically to a sublethal concentration of the chemical (about 1.0 µg/ℓ). The purpose of the test was to determine whether structural damage might occur in gill tissue. On day 27 of exposure at which time we had recorded no previous deaths from the PCB, the salinity of the incoming water decreased from 20 o/oo to 11 o/oo within 4 hrs due to rain, tides and wind. As a result, ten experimental shrimp died before the salinity had returned to 20 o/oo. During the next 2 days, the salinity was lowered again by aberrant tides and climatic conditions and more experimental, but not control, shrimp died. We, therefore, became interested in the possible interaction of Aroclor® 1254 and environmental stress, particularly the effect of PCB on the ability of shrimp to regulate osmotically and ionically at reduced salinities.

---

[a]Mention of commercial products does not constitute endorsement by the U. S. Environmental Protection Agency.

MATERIALS AND METHODS

Adult brown shrimp *(Penaeus aztecus)*, 11.5 to 13.7 cm, rostrum to telson, were captured near Gulf Shores, Alabama, and used in our studies. Approximately equal numbers of both sexes were used and the methods of exposure to the Aroclor ® 1254 were similar to those reported previously (Nimmo *et al.*, 1971a) except that the shrimp were maintained at 30 ± 1 o/oo S, 25 ± 2°C, and the exposures to the chemical were "sublethal" and lasted but 7 days. Three µg/ℓ were chosen as the test concentration because previous tests with adult brown shrimp, as well as adult pink shrimp *(P. duorarum)*, demonstrated that this concentration would cause 50 o/oo mortality within 30 days.

Following exposure to PCB, equal numbers of PCB-exposed and control shrimp were transferred to separate aquaria. The experimental procedure is shown in Figure 1. Since the possibility existed of physiological stress from handling or inherent in the experimental design, both PCB-exposed and control shrimp were analyzed for osmotic and ionic concentrations after being subjected to the procedure without external salinity change (30 o/oo). While temperature was kept constant, the salinity in each aquarium was gradually lowered during 8 hrs to a predetermined level. For the first group, the salinity was maintained at 30 o/oo for 8 hrs; the second, salinity was lowered from 30 o/oo to 22 o/oo; the third, from 30 o/oo to 10 o/oo; and the fourth, from 30 o/oo to 7 o/oo. Although there was a time differential between groups of shrimp, and, therefore, a possible difference in test animals due to a slight loss of PCB, analyses for the chemical revealed no significant difference in whole body concentrations among groups (Table 1). As in earlier studies, there was a wide range in individual concentrations of PCB (Nimmo *et al.*, 1971b).

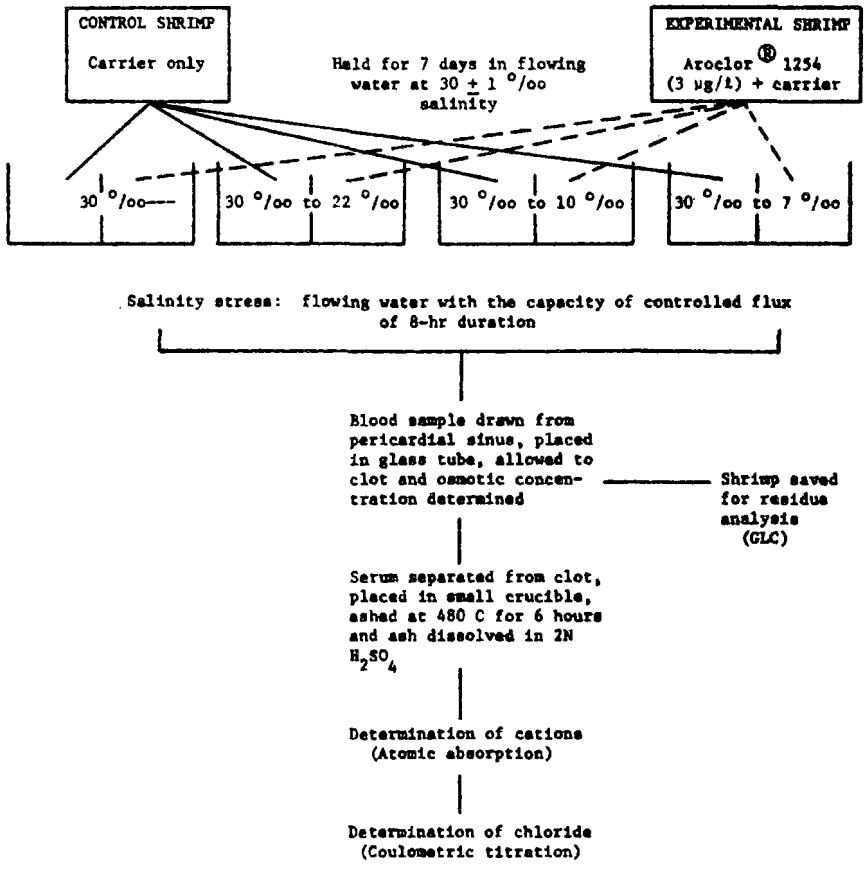


Fig. 1. Flow sheet for experimental procedure.

Samples of whole blood (hemolymph) for osmotic concentration were removed from each shrimp by pericardial puncture. A ground-glass syringe (1 ml), fitted with a #22 gauge stainless steel needle, was inserted into the animal at an oblique angle to obtain at least 0.3 ml of blood from each animal. The blood was immediately transferred to an osmometer tube and the osmotic pressure determined with a Fisk ® Model G-66 osmometer. Since the blood clotted quickly, it was difficult to determine whether actual

TABLE 1
*Whole Body Concentrations of Aroclor® 1254 in PCB-Exposed Shrimp**

Salinity o/oo	mg/kg Average	Range
30	9.6	3.0-13.7
22	7.8	1.9-18.8
10	7.1	3.9-14.0
7	8.5	2.8-15.0

*Concentration of Aroclor® 1254 in the test water was 3 ppb; length of exposure, 7 days. Control shrimp had less than 0.1 ppm of Aroclor® 1254.

osmotic pressure was measured on whole blood or on serum, but an individual analysis showed no significant difference in osmotic concentrations. Replicate determinations of 10 separate aliquots of pooled sera from several shrimp yielded a standard error of 1.2 (mean concentration ∿ 629 mOs).

Analyses of ions were performed on ashed sera. To prepare the sample, the clot contained in the osmometer tube was squeezed with a small glass rod, the clot was removed and 0.2 ml of the serum was transferred to a small crucible. The crucible was placed in an oven and the contents ashed at 480°C for 6 hrs, cooled and the ash was dissolved in 2N H_2SO_4. Analysis of chloride was performed with a Buchler-Cotlove Chloridometer® and cations were determined on a Model 403 Perkin-Elmer® atomic absorption spectrophotometer equipped with a deuterium arc-background corrector and an HGA-70 heated graphite atomizer. Cations in standard solutions were in the same proportions as those in the sera. As a check on our methods, an analysis of a single aliquot of serum

by emission and atomic absorption yielded identical results for potassium. Five replicates of pooled sera from several shrimp yielded a standard error of 0.24 mEq/l for Cl (mean = 257 mEq/l). Replicate analyses on serum aliquots yielded standard errors in mEq/l of 8.26 for Na (mean = 324.9), of 0.04 for Mg (mean = 16.0), of 0.07 for K (mean = 8.1), of 0.19 for Ca (mean = 4.74), and of 0.10 for Cu (mean = 2.84).

The 95% confidence interval was used to evaluate significance of differences in the data. The 95% intervals are indicated in the graphs by vertical bars on each datum and are listed in each table. Since it is sometimes difficult to relate "osmolality" or "osmotic concentration" to the environment, we expressed the concentration of the environment as salinity. The relationship between mOs and salinity is indicated along the X axis in Figure 2.

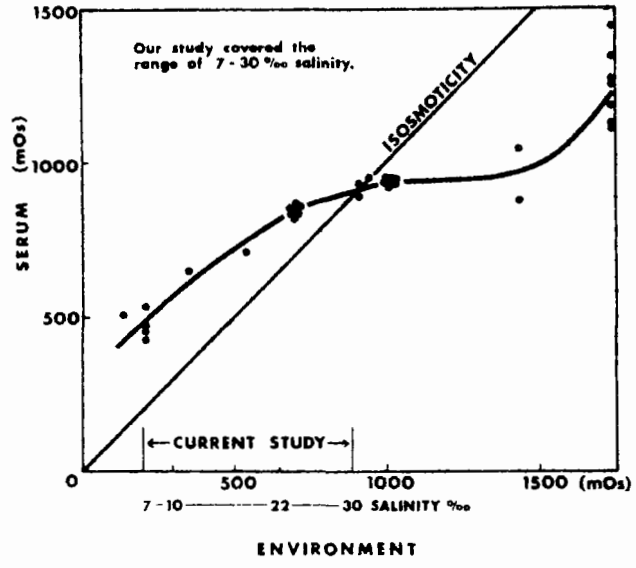


Fig. 2. Osmotic concentration: Serum-environment in Penaeus aztecus *(after McFarland and Lee, 1963). Our study covered the range of 7 o/oo to 30 o/oo salinity.*

Concentrations of Aroclor ® 1254 were determined on individual shrimp by gas chromatography, using procedures summarized earlier (Nimmo *et al.*, 1971b).

RESULTS

The most significant result of this study was the discovery that a sublethal concentration of Aroclor 1254 at constant salinity for 7 days became lethal when the species was subjected to a gradual decrease in salinity over an 8-hr period. Usually the shrimp exhibited increased swimming activity while the salinity changed from 30 o/oo to 20 o/oo; this was the only observed behavioral aberration. Experimental shrimp began to die at a salinity of about 12 to 13 o/oo. After 8 hrs of exposure to 10 o/oo and 7 o/oo salinity, mortality of experimental shrimp was nearly 50%. When 50% of the experimental shrimp had become moribund or had died, living PCB-exposed shrimp were taken for analyses of osmotic concentrations and ion determinations on sera.

The results of these analyses indicated that concentrations of most major ions in the sera of PCB-exposed shrimp became significantly less as the ambient salinity decreased, the sum of major ions (*e.g.*, Na, Ca, Mg, K, Cu, and Cl) was 18% less after ambient salinity reached 10 o/oo or 7 o/oo (Fig. 3). Of this total, sodium was 16% less (Fig. 4), chloride, 19% (Fig. 5) and calcium, 25% (Fig. 6). There was some indication that magnesium decreased, although the loss was not statistically significant (Table 2). No apparent differences in potassium (Table 3), or copper (Table 4) were noted.

Data for iron (Table 5) are not included in the totals in Figure 3 because we could not distinguish the divalent from the trivalent form.

Despite significant alterations in the major ion complement or in some major ions, osmotic concentration was not significantly affected by PCB and salinity stress (Table 6). Seemingly, osmotic pressure was less in PCB-exposed shrimp at 10 o/oo or 7 o/oo

salinity, but individual variation was too great to show a significant difference from controls.

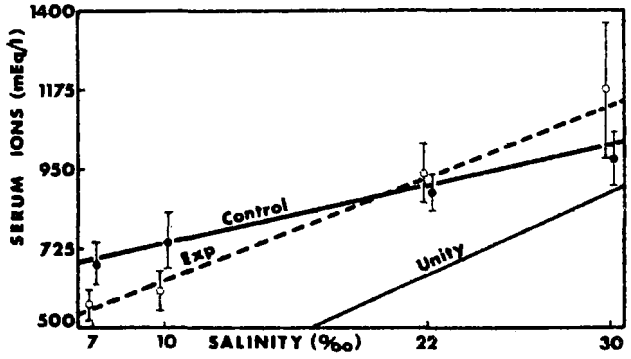


Fig. 3. Total ions in brown shrimp serum in relation to salinity.

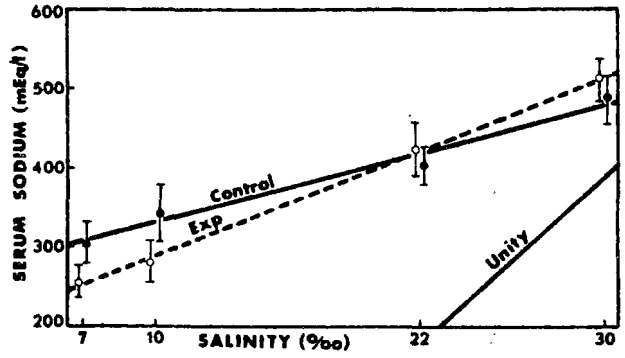


Fig. 4. Serum sodium in brown shrimp in relation to salinity.

DISCUSSION

Knowledge of interactions between toxic compounds and environmental factors is essential for predicting their effects on ecosystems or species. Examples of this need have been demonstrated in both fresh and marine investigations. In fresh water,

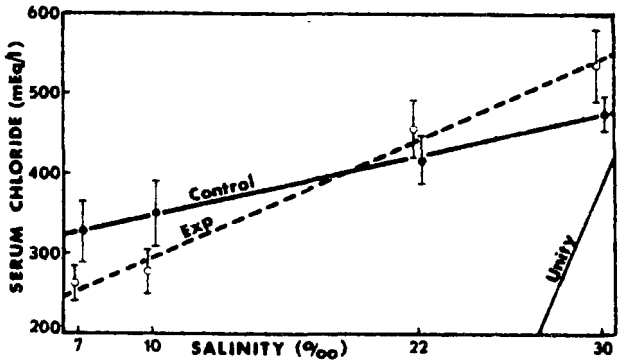


Fig. 5. Serum chloride in brown shrimp in relation to salinity.

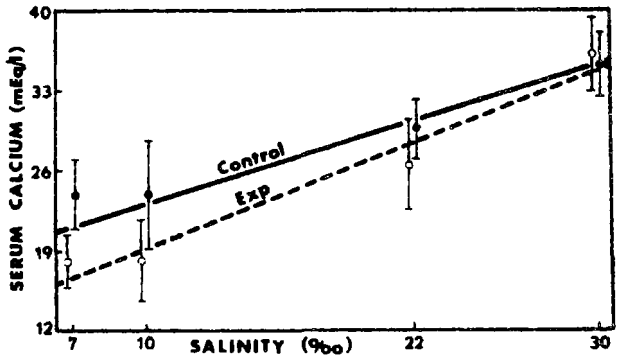


Fig. 6. Serum calcium in brown shrimp in relation to salinity.

low-level chronic exposure of the darter *(Etheostoma nigrum)* to dieldrin greatly affected its ability to survive thermal stress (Silbergeld, 1973). The sublethal effects of mercury on fiddler crabs *(Uca pugilator)* reduced survival times when crabs were placed under temperature and salinity stress (Vernberg and Vernberg, 1972). Mortality of fiddler crabs that were previously exposed to cadmium was greatest at high temperatures and low salinities (O'Hara, 1973).

TABLE 2
Average Serum Concentrations of Magnesium in Brown Shrimp in Relation to Salinity

Salinity o/oo	mEq/l Control	95% Conf. Interval	Experi-mental	95% Conf. Interval
30	20.1	16.7-23.5	17.3	12.7-21.8
22	16.3	14.2-18.4	14.6	12.3-16.9
10	14.8	12.4-17.2	13.0	11.0-15.0
7	15.0	11.7-18.3	11.5	8.9-14.2

TABLE 3
Average Serum Concentrations of Potassium in Brown Shrimp in Relation to Salinity

Salinity o/oo	mEq/l Control	95% Conf. Interval	Experi-mental	95% Conf. Interval
30	14.2	13.5-14.9	14.7	13.6-15.7
22	11.7	11.1-12.3	12.1	11.2-12.9
10	10.4	9.8-11.2	10.1	8.7-11.4
7	9.6	8.4-10.7	10.0	8.4-11.6

In our studies Aroclor ® 1254 possibly interfered with the adenosine triphosphatase (ATPase) activity in gills of shrimp. ATPase activity is associated with active ion transport (Tanaka, Sakamoto, and Sakamoto, 1971). Polychlorinated insecticides and the related polychlorinated biphenyls

have been shown by several "in vitro" assays to inhibit ATPase in the tissues of fishes (Davis and Wedemeyer, 1971a, 1971b; Davis, Friedhoff and Wedemeyer, 1972; Cutkomp *et al.*, 1972; Yap *et al.*, 1971) and in the nerves of lobsters (Matsumura and Narahashi, 1971). The need for greater efficiency

TABLE 4
Average Serum Concentrations of Copper in Brown Shrimp in Relation to Salinity

Salinity	mEq/l			
o/oo	Control	95% Conf. Interval	Experi-mental	95% Conf. Interval
30	4.7	3.6-5.9	5.3	4.4-6.3
22	4.0	3.4-4.6	5.9	4.8-7.0
10	5.2	4.0-6.4	4.6	3.8-5.3
7	4.8	3.9-5.7	5.7	5.0-6.4

TABLE 5
Average Serum Concentrations of Iron in Brown Shrimp in Relation to Salinity

Salinity	mM/l			
o/oo	Control	95% Conf. Interval	Experi-mental	95% Conf. Interval
30	.23	.17-.29	.31	.15-.48
22	.28	.15-.35	.18	.14-.22
10	.20	.15-.25	.26	.17-.35
7	.24	.14-.33	.33	.24-.41

TABLE 6
Average Serum Osmotic Concentrations in Brown Shrimp in Relation to Salinity

Salinity o/oo	Milliosmoles Control	95% Conf. Interval	Experimental	95% Conf. Interval
30	749	728-771	752	726-779
22	687	665-708	688	658-718
10	551	508-593	523	500-546
7	547	521-572	516	495-537

or capacity of ATPase in marine organisms can be inferred from the results of Pfeiler and Kirschner (1972), who showed that gill ATPase activity of rainbow trout adapted to salt water, was greater than in fish adapted to fresh water.

Polychlorinated hydrocarbons have interfered with either osmo- or ionic-regulation in aquatic animals (Eisler and Edmunds, 1966; Grant and Mehrle, 1970; Kinter *et al.*, 1972; Nimmo and Blackman, 1972). The physiological relationship of ionic effects to that of ATPase activity was first reported by Kinter *et al.* (1972), who postulated that lipophilic agents such as DDT and PCBs, might interact with the phospholipid-activating components of the lipoprotein enzyme. The effect of dieldrin on ion movement in the nervous system of cockroaches showed that dieldrin inhibited binding of calcium to the phospholipid moiety of the enzyme, thus inhibiting the movement of calcium across the nerve membrane (Hayashi and Matsumura, 1967). Calcium salts in fresh water greatly increased the ability of marine and euryhaline animals to survive in that medium (Black, 1957). Toxic symptoms of DDT poisoning in freshwater fish could be alleviated by the addition of calcium salts (Keffler, 1972). In

our studies calcium was lowered 25% in the sera of PCB-exposed shrimp at 7 o/oo salinity, and it may be that this reduction was responsible in part for the observed decrease in sodium, chloride, and other ions.

Field observations of juvenile and subadult brown shrimp by several investigators indicate that those shrimp tolerate a wide range of salinities:

Salinity	Location	Observer
0.22-0.36 o/oo	St. Lucie estuary, Florida	Gunter and Hall (1963)
0-1.0 o/oo	Mobile Bay, Ala.	Loesch (In Gunter *et al.*, 1964)
0-2.0 o/oo	Choctawhatchee Bay, Florida	Nimmo (unpubl. data)
69 o/oo	Laguna Madre, Texas	Simmons (1957)

Gunter, Christmas, and Killebrew (1964) found that in Texas bays young brown shrimp were most abundant at 10 to 30 o/oo, with greater abundance above 20 o/oo. Zein-Eldin and Aldrich (1965) found that postlarval brown shrimp withstood a wide range of salinity-temperature combinations.

It is evident that adult shrimp are osmoregulators at all but extremes of salinity (Fig. 2). Williams (1960) gives the isosmotic point of shrimp hemolymph at 26.5 o/oo (788 mOs), as compared to our calculation of 23.4 o/oo (694 mOs) in control shrimp. Nevertheless, there was no significant difference between PCB-exposed and control shrimp (Table 6). Obviously, a slight change in environmental osmotic pressure would not be critical to the osmoregulatory ability of the animals at isosmoticity, but was critical in the dilute environment. Therefore, the salinities where the PCB exerted its greatest effect in the laboratory (as judged from mortality of shrimp) were well within the range in which brown shrimp occur in nature.

Although there was no appreciable difference in osmotic concentration between control and PCB-exposed shrimp, there was significant difference in major ions in the sera of PCB-exposed shrimp (Fig. 3).

McFarland and Lee (1963) found that the point of convergence of the total ions in the hemolymph of feral shrimp to that of the environment was 27 o/oo (800 mOs). In our study, by extrapolation, convergence occurred at 34 o/oo (1000 mOs) in controls, whereas in PCB-exposed shrimp showed no convergence and total ions paralleled unity.

In surveys conducted soon after the PCB was first discovered in the Pensacola estuary, the distribution of shrimp in relation to salinity seemed to be related to the amount of the chemical in the animals (Nimmo *et al.*, 1971b). Of three species captured, brown shrimp had the highest whole-body residues (14 ppm) although most samples were lower. Seemingly, a concentration of 14 ppm PCB in feral shrimp would have been lethal if the animals were subjected to salinity stress such as imposed by our experimental procedure. We have unpublished data that suggest existence of a threshold in average whole-body concentration of PCB (5.6 to 7.8 mg/kg) in pink shrimp *(P. duorarum)* that would be lethal when superimposed on salinity stress caused by our procedure. However, a recent survey of feral shrimp from the Penacola estuary showed that young adult shrimp now have only a fraction of PCB concentrations found in 1969/70 periods. For example, a sample taken from Escambia Bay in August 1973 had a whole-body concentration of only 0.1 mg/kg.

Future studies should include research on interaction of PCB and salinity on juvenile and postlarval shrimp since chronic toxicity tests have shown that these stages were more susceptible to the chemical (Nimmo *et al.*, 1971a). In addition, studies by Dana Beth Tyler-Schroeder, of the Gulf Breeze Laboratory, have shown the susceptibility of larvae of grass shrimp *(Palaemonetes pugio)* to the PCBs, Aroclors ® 1016 and 1242, decreases with age (personal communication). Also, the "in vivo" effect of PCBs on ATPase activity in shrimp should be fully investigated.

ACKNOWLEDGMENT

This work was supported by Contribution No. 198 of the Gulf Breeze Environmental Research Laboratory.

LITERATURE CITED

Black, V. S. 1957. Excretion and osmoregulation. In: *Physiology of Fishes*, pp. 163-99, ed. by M. E. Brown. New York: Academic Press.

Cutkomp, L. K., H. H. Yap, D. Desaiah, and R. B. Koch. 1972. The sensitivity of fish ATPases to polychlorinated biphenyls. *Environ. Health Perspect. 1*:165-68.

Davis, P. W., J. M. Friedhoff, and G. A. Wedemeyer. 1972. Organochlorine insecticide, herbicide and polychlorinated biphenyl (PCB) inhibition of Na, K—ATPase in rainbow trout. *Bull. Environ. Contam. Toxicol. 8*:69-72.

_____ and G. A. Wedemeyer. 1971a. Inhibition by organochlorine pesticides of Na^+, K^+—activated adenosine triphosphatase activity in the brain of rainbow trout. *Proc. West. Pharmacol. Soc. 14*:47.

_____ and _____. 1971b. Na^+, K^+—activated ATPase inhibition in rainbow trout: a site for organochlorine pesticide toxicity. *Comp. Biochem. Physiol. 40B*:823-27.

Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor 1254 ®) in the water, sediment, and biota of Escambia Bay, Florida. *Bull. Environ. Contam. and Toxicol. 5*:171-80.

Eisler, R. and P. H. Edmunds. 1966. Effects of endrin on blood and tissue chemistry of a marine fish. *Trans. Am. Fish. Soc. 95*:153-59.

Grant, B. F. and P. M. Mehrle. 1970. Chronic endrin poisoning in goldfish, *Carassius auratus*. *J. Fish. Res. Bd. Canada 27*:2225-32.

Gunter, G., J. Y. Christmas, and R. Killebrew. 1964. Some relations of salinity to population distributions of motile estuarine organisms, with special reference to penaeid shrimp. *Ecology 45*: 181-85.

_____ and G. E. Hall. 1963. Biological investigations of the St. Lucie estuary (Florida) in connection with Lake Okeechobee discharges through the St. Lucie canal. *Gulf Res. Rep. 1*: 189-307.

Hayashi, M. and M. Matsumura. 1967. Insecticide mode of action: effect of dieldrin on ion movement in the nervous system of *Periplaneta americana* and *Blatella germanica* cockroaches. *J. Agric. Food Chem. 15*:622-27.

Keffler, L. R. 1972. A study of the influence of calcium on the effects of DDT on fishes. Ph.D. dissertation, The University of Mississippi, University, Mississippi.

Kinter, W. B., L. S. Merkens, R. H. Janicki, and A. M. Guarino. 1972. Studies on the mechanism of toxicity of DDT and polychlorinated biphenyls (PCBs): disruption of osmoregulation in marine fish. *Environ. Health Perspect. 1*:169-73.

Matsumura, F. and T. Narahashi. 1971. ATPase inhibition and electrophysiological change caused by DDT and related neuroactive agents in lobster nerve. *Biochem. Pharmacol. 20*:825-37.

McFarland, W. N. and B. D. Lee. 1963. Osmotic and ionic concentrations of penaeidean shrimps of the Texas coast. *Bull. Mar. Sci. Gulf Caribb. 13*:391-417.

Nimmo, D. R. and R. R. Blackman. 1972. Effects of DDT on cations in the hepatopancreas of penaeid shrimp. *Trans. Am. Fish. Soc. 101*:547-49.

_____, _____, A. J. Wilson, Jr., and J. Forester. 1971a. Toxicity and distribution of Aroclor ® 1254 in the pink shrimp, *Penaeus duorarum*. *Mar. Biol. 11*:191-97.

_____, P. D. Wilson, R. Blackman, and A. J. Wilson, Jr. 1971b. Polychlorinated biphenyl absorbed from sediments by fiddler crabs and pink shrimp. *Nature (London) 231*:50-52.

O'Hara, J. 1973. The influence of temperature and salinity on the toxicity of cadmium to the fiddler crab, *Uca pugilator*. *U. S. Fish. Wildl. Serv. Fish. Bull. 71*:149-53.

Pfeiler, E. and L. B. Kirschner. 1972. Studies on gill ATPase of rainbow trout *(Salmo gairdneri)*. *Biochim. Biophys. Acta 282*:301-10.

Silbergeld, E. K. 1973. Dieldrin: effects of chronic sublethal exposure on adaptation to thermal stress in freshwater fish. *Environ. Sci. Technol. 7*:846-49.

Simmons, E. G. 1957. An ecological survey of the upper Laguna Madre of Texas. *Publ. Inst. Mar. Sci., Univ. Tex. 4*:156-200.

Tanaka, R., T. Sakamoto, and Y. Sakamoto. 1971. Mechanism of lipid activation of Na, K, Mg—activated adenosine triphosphatase and K, Mg—activated phosphatase of bovine cerebral cortex. *J. Membrane. Biol. 4*:42-51.

Vernberg, W. B. and J. Vernberg. 1972. The synergestic effects of temperature, salinity and mercury on survival and metabolism of the adult fiddler crab, *Uca pugilator*. *U. S. Fish. Wildl. Serv. Fish. Bull. 70*:415-20.

Williams, A. B. 1960. The influence of temperature on osmotic regulation in two species of estuarin shrimps *(Penaeus)*. *Biol. Bull. (Woods Hole) 117* 560-71.

Yap, H. H., D. Desaiah, L. K. Cutkomp, and R. B. Koch 1971. Sensitivity of fish ATPases to polychlorinated biphenyls. *Nature (London) 233*: 61-62.

Zein-Eldrin, Z. P. and D. V. Aldrich. 1965. Growth and survival of postlarval *Penaeus aztecus* under controlled conditions of temperature and salinity. *Biol. Bull. (Woods Hole) 129*:199-216.

CONTRIBUTION NO. 200

(22)

A Salinity Controller for Flowing-Water Bioassays [1]

LOWELL H. BAHNER
U. S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory

Salinity and temperature are rate determining factors for many physiological functions and these variables affect the toxicity of several pesticides to marine organisms. Because some compounds that alter or interfere with osmoregulatory mechanisms in estuarine organisms appear more toxic as salinity changes, it is often desirable in estuarine bioassays (flow-through) to adjust salinity to a constant level.

A salinity controller consisting of a sea-water hydrometer, photocell detector, and a relay controlled by an electronic amplifier has been developed that monitors and adjusts salinity continuously in flow-through systems. The controller regulates electrical pumps or valves to adjust salinity within $\pm$ 1% of the desired level, and with minor modifications, can control temperature, water height, or light intensity.

[1] *Contribution No. 200, Gulf Breeze Environmental Research Laboratory.*

Reprinted from *The ASB Bulletin*,
Vol. 21, No. 2, April 1974, p. 37.

From: Abstracts of the Annual Meeting American Society for Microbiology. 1973

P256 Degradation of Malathion by Estuarine Microbes. Al W. Bourquin* and Gary H. Cook. U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida 32561

Pathways for the biological degradation of malathion were characterized using estuarine bacteria. Some correlation with microbial marshland ecosystems was attempted. Bacteria were isolated from estuarine muds previously untreated with malathion. Three strains were selected which metabolized malathion in Zobell's 2216 marine medium. None of the organisms used malathion as a sole carbon source. Early enzymatic hydrolysis products were identified as the mono- and di-carboxylic acid derivatives of malathion. Further microbial degradation products were characterized (including $^{14}CO_2$ liberated from the methoxy side chain) using ^{14}C-labelled malathion. Investigations into the possible effects of light, temperature, pH, and salinity on degradation of malathion were analyzed as a check on the biological system. Degradation products were characterized in the same manner as the biological samples. Chemical degradation of malathion increased rapidly with salinity with an accumulation of the mono-carboxylic acid derivative.

Correlation of the microbial, chemical, and physical degradations of malathion as it occurs in the environment was attempted using artificial microcosms.

Gulf Breeze Contribution No.201

Effects of Pesticides on Protozoa
Effets des Pesticides sur Protistes

NELSON R. COOLEY (U. S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561, U. S. A [Associate Laboratory, National Environmental Research Center, Corvallis, Oregon])

Little is known about effects of pesticides and related compounds on protozoa. Most studies have been performed on phytoflagellates, only a few on ciliates. Ciliates are the most numerous animals in the estuarine benthos and function as food for higher organisms and are important as regenerators of nitrogen and phosphorus. Because of the importance of ciliates in estuarine ecosystems and because estuaries often act as sinks for pesticides, my coworkers and I have performed studies on effects of pesticides and polychlorinated biphenyls on these animals.

Tetrahymena pyriformis W is sensitive to toxicants we have tested. The ciliates accumulated and concentrated the toxicants from the medium. Significant reduction in population growth rate and 96-hour population density occurred at low toxicant concentrations:

Toxicant	Growth Rate reduction	96-hr. population density reduction	Accumulation (X initial concentration)
Mirex	33% at 0.9 µg/ℓ	12% at 0.9 µg/ℓ	193 X
Aroclor 1248	18.9% at 1 mg/ℓ	9.6% at 1 mg/ℓ	48 X
Aroclor 1254	8% at 1 µg/ℓ	10% at 1 µg/ℓ	60 X
Aroclor 1260	19.1 to 25% at 1 mg/ℓ	13.6 to 22.4% at 1 mg/ℓ	79 X

However, exploratory experiments suggest that T. pyriformis may be less sensitive to malathion than to these chemicals.

The data suggest that pesticides and related toxicants that enter aquatic ecosystems could reduce the availability of ciliates as food and as nutrient regenerators, thereby disrupting nutrient cycles and perhaps altering species composition of ciliate communities. The ability of responsive ciliates to accumulate persistent compounds could permit translocation of the chemicals through food chains. In this manner, effects of the chemicals could be exerted at higher trophic levels.

Reprinted from Progress in Protozoology (Abstracts of papers read at the Fourth International Congress of Protozoology, Clermont-Ferrand, France, 2-9 September 1973), p. 91.

In: Proceedings of the First Microbiology
Seminar on Standardization of Methods.
March, 1974, EPA-R4-73-022, Environmental Monitoring Series. Washington, D. C.: U. S. Govt. Printing Off

IMPACT OF MICROBIAL SEED CULTURES ON THE AQUATIC ENVIRONMENT*

Al W. Bourquin**

The tremendous use of oil for energy in the United States has caused rapid increase in oil imports on large cargo carriers. These large tankers, with capacities equal to or greater than 100,000 dead-weight tons capacity, and increased shipping, has enhanced greatly the danger of major oil spills. With the impending danger of catastrophic spills, technology of clean-up is extremely limited. Present clean-up methods include adsorption and recovery, chemical dispersion, and physical removal. Each technique has limitations due to quantity and type of oil spilled, extent of the slick, and nature of the environment where the spill occurred or where the slick floated. Some authors believe no efficient and safe method exists for clean-up of a spill in shallow estuaries (1,2).

Extensive research is being conducted for the purpose of increasing microbial oil degradation by seeding oil slicks with hydrocarbonoclastic microorganisms. It may be possible that large quantities of selected microorganisms, under proper environmental conditions, could hasten degradation and ultimate removal of pollutant hydrocarbons (1).

The need for standardization of testing procedures for commercially available microbial formulations was pointed out at a recent international workshop held in Atlanta, Georgia. Papers were presented to show that at least two commercial products are completely ineffective or have very little hydrocarbonoclastic activity--below that of natural seawater (3). Other evidence, presented by EPA representatives, demonstrated that at least one commercial formulation contained at least four species of pathogenic microorganisms (4).

A panel, "Environmental Considerations in Microbial Degradation of Oil", at the Atlanta workshop recommended that a committee be formed to study the problems of effective and safe use of microbial seed cultures in the environment. The committee should be composed of members of a governmental agency, members of API--representing the petroleum industry, and members of the academic community who are active in oil pollution research (5).

---

* Submitted in writing for the record.

** Gulf Breeze Environmental Research Laboratory, Associate Laboratory of NERC-Corvallis

Microbial seed cultures are currently being studied for application to the environment as microbiological pesticides. Viruses have been isolated which attack selectively the cabbage boll; a bacterium has been isolated as a specific pathogen of mosquitoes; and chitinoclastic bacteria have been proposed as agents against plant predators in estuarine areas. The range of impact on the aquatic environment by seed cultures must be investigated adequately before they are used on a large scale.

CONTRIBUTION NO. 204

PROCEEDINGS

OF

SEMINAR ON METHODOLOGY

FOR

MONITORING THE MARINE ENVIRONMENT

SEATTLE WASHINGTON

OCTOBER 1973

Program Element No. 1HA326
ROAP/Task - PEMP/2

SPONSORED BY

OFFICE OF MONITORING SYSTEMS

U.S. ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

METHODS AND PROBLEMS IN ANALYSIS OF PESTICIDES IN THE ESTUARINE ENVIRONMENT

A. J. Wilson, Jr. and J. Forester
Gulf Breeze Environmental Research Laboratory,
Gulf Breeze, Florida

The presence of pesticides in the marine environment has been well documented. Cox (4) reported DDT concentrations in sea water along the Pacific Coast to range from 0.0023 parts per billion (microgram/liter) off Oregon and Washington to 0.0056 parts per billion off Southern California. Residues of DDT and dieldrin were detected in livers of fishes by Duke and Wilson (6) from the Northeastern Pacific Ocean and in gray whales by Wolman and Wilson (16). Other documentation of chlorinated hydrocarbons in the marine environment is presented by Goldberg et al. (7).

The Gulf Breeze Environmental Research Laboratory at Gulf Breeze, Florida, an associate laboratory of the National Environmental Research Center, Corvallis, Oregon, has been conducting research on the effects of pesticides in the marine environment since 1958. Since that time the laboratory has analyzed over twenty thousand samples for these pollutants in water, sediment, oysters, crabs, fish, birds and mammals. From 1965 until 1972 this facility analyzed over eight thousand samples for the National Pesticide Monitoring Program as reported by Butler (3). This report describes analytical methods employed by this Program, some recent studies in water analysis, and the need for adequate analytical quality control in marine monitoring.

NATIONAL PESTICIDE MONITORING PROGRAM

From studies at Gulf Breeze, bivalve mollusks appeared to be suitable animals to use as indicators of estuarine pollution. Adult bivalve mollusks are sessile, permitting repeated sampling of the same population. In addition, experiments by Butler (2) indicated that bivalve mollusks readily accumulate chlorinated hydrocarbons. Consequently, oysters, mussels and clams were the primary indicator organisms in this program.

Mollusks were collected at about 30-day intervals at 183 estuarine sites in 15 coastal states. Approximately 15 individuals were taken from each station by other agencies, prepared in their laboratories, and shipped to Gulf Breeze for analyses. The rationale for all analyses being conducted at Gulf Breeze was based on the premise that these methods of analyses would be consistent from station to station and month to month. This would eliminate variation in methodology and permit a more reliable interpretation of seasonal and geographic trends. In addition, it was not economically feasible at the outset to equip several satellite laboratories and conduct a suitable inter-laboratory quality control program.

Prior to the start of the program several preservatives were evaluated to find a method that would allow shipment of samples without dry ice. When samples were dehydrated by mixing them with a 9:1 mixture of anhydrous sodium sulfate and Quso, a micro fine silica, they could be held at room temperature for up to 15 days without loss or degradation of the chlorinated hydrocarbon. This procedure allowed shipment of samples in aluminum foil by surface mail from the collecting laboratory to the Gulf Breeze Laboratory.

Analytical Procedures

Mollusks were analyzed for aldrin, chlordane, o,p' and p,p' isomers of DDT and its metabolites, dieldrin, endrin, heptachlor, heptachlor epoxide, lindane, methoxychlor, mirex, and toxaphene.

Sample Preparation

The tissues of 15 individuals were shucked into a one-pint Mason jar and thoroughly homogenized with an Osterizer blender. Approximately 30 g of the homogenate was added to a second Mason jar and blended with a 9:1 mixture of sodium sulfate and Quso. By alternately chilling and blending, a free-flowing powder was obtained. The blended sample was wrapped in aluminum foil and shipped to Gulf Breeze. Upon receipt of the sample, it was weighed and extracted in a Soxhlet apparatus for 4 hours with petroleum ether.

Sample Clean-Up

The extracts were then purified by concentrating and transferring the extract to 250 ml separatory funnels. The extracts were diluted to 25 ml with petroleum ether and partitioned with two 50 ml portions

PERCENTAGE RECOVERY OF PESTICIDES FROM FORTIFIED OYSTER SAMPLES

PESTICIDE	ACTUAL (PPM)	FOUND (PPM)	% RECOVERY
DDE	0.033	0.026	79
	0.033	0.026	79
	0.34	0.30	88
	0.34	0.29	85
DDD	0.067	0.061	91
	0.067	0.064	96
	0.70	0.67	96
	0.70	0.63	90
DDT	0.10	0.087	87
	0.10	0.094	94
	1.0	0.95	95
	1.0	0.92	92

of acetonitrile previously saturated with petroleum ether. The acetonitrile was evaporated to dryness and the residue eluted from a Florisil column, Mills et al. (11). In this technique, increasing proportions of ethyl ether to petroleum ether were used to elute fractions containing increasingly polar insecticides.

Quantitation and Qualitation

The extracts were analyzed with Varian Aerograph electron capture gas chromatographs. Extracts were injected into at least two 180 cm x 2 mm (ID) columns of different liquid phases. The following columns have been used: DC-200, QF-1, DEGS, OV-101, mixed DC-200/QF-1, and mixed OV-101/OV-17. Liquid phases and gas chromatographic parameters were adjusted so that p,p' DDT would elute in approximately 12 minutes. The lower limit of detection for a 30 g mollusc sample was 0.010 parts per million (milligrams per kilogram). Residues were reported on a wet weight basis without adjustment for recovery rates. Thin layer chromatography and "p" values after Bowman and Beroza (1) were used for additional confirmation of compound identity.

Extraction efficiencies were determined by re-extracting samples for longer periods of time and with different solvent systems. Recovery rates were determined by fortification of samples with known levels of pesticides. Table 1 shows typical recovery rates of DDT and its metabolites from fortified oyster samples. The values were adjusted to account for naturally-occurring DDT residues.

SEA WATER ANALYSIS

Prior to 1971, the Gulf Breeze Laboratory belonged to the Bureau of Commercial Fisheries, United States Department of Interior. During those early days, there was little known regarding the effects and kinetics of pesticides in the marine environment. Consequently, in addition to scientific publications, the laboratory frequently published quarterly and annual progress reports containing provisional or preliminary data.

In 1968 the author submitted the following report for inclusion in a Progress Report of the Bureau of Commercial Fisheries (15).

STABILITY OF PESTICIDES IN SEA WATER

> We began preliminary studies to determine the stability of pesticides in sea water. Three p.p.b. of aldrin, p,p'-DDT, malathion, and parathion in acetone were added separately to four clear glass,

one-gallon bottles containing sea water (salinity 29.8 p.p.t.; pH 8.1). One chemical per bottle. After an initial sample of the water was analyzed, the bottles were sealed and completely immersed in an outdoor flowing sea-water tank. Table 5 shows the concentration of the chemical at the indicated time interval.

Although we used natural sea water in these preliminary experiments, the tests will be repeated with sterile artificial sea water so that the relative stability of the pesticides can be evaluated under standardized experimental conditions.

Because these studies showed a rapid loss of DDT in sea water, the report received a great deal of attention. As stated, these were preliminary studies but, unfortunately, many readers carried the data beyond the scope of the experiment. Obviously, additional studies were required to account for the rapid decline of these pesticides before any conclusions could be made.

Since these studies were conducted, several investigators have reported on the transport of pesticides in marine waters. Cox (4) reported that adsorption of DDT is implicated in the uptake mechanism for algal cells. His experiments also indicate that particles less than 1-2μ diameter carry most of the DDT residues in whole water. Working in the laboratory with six species of marine algae, Rice and Sikka (13) found that all species concentrated DDT to levels many times higher than the original concentration of the medium. Transformation of DDT and cyclodiene insecticide took place in surface films, plankton, and algae but not in water from the open ocean according to Patil et al. (12).

Recently, experiments have been conducted to determine the cause of loss of DDT in the 1968 studies at Gulf Breeze. The experiments were repeated under similar conditions with the exception that duplicate samples were analyzed. In 1968 the salinity was 29.8 ppt (parts per thousand) and the incubation temperature averaged 29 C; in 1973, the salinity was 24.0 ppt and the incubation temperature averaged 12 C. Figure 1 shows the percentage recovery of DDT (including DDE and DDD) during the two experiments. The results are similar except for the 17 day 1968 analysis.

Table 5.--Stability of pesticides in natural sea water (salinity 29.8 p.p.t.; pH 8.1)

Pesticide	Days after start of experiment					
	0	6	17	24	31	38
	P.p.b.	P.p.b.	P.p.b.	P.p.b.	P.p.b.	P.p.b.
p,p'-DDT.....	2.9	.75	1.0	.27	.18	.16
p,p'-DDE*..		.096	.95	.065	.034	.037
p,p'-DDD*..			.081	.041	.038	.037
Aldrin**.....	2.6	.58	.096	<0.01	<0.01	<0.01
Dieldrin*..		.74	1.0	1.0	.75	.56
Malathion....	3.0	<0.2	<0.2	---	---	---
Parathion....	2.9	1.9	1.25	1.0	.71	.37

*Metabolites of parent compound.

**From the seventeenth day onward, 2 unidentified peaks appeared on the chromatographic charts after aldrin had eluted.

Experiments were then performed to determine if DDT was adsorbed to the walls of the test containers. Additional experiments were designed to determine if DDT was converted to DDA, a water soluble metabolite of DDT, which would not have been detected by the method of analyses used in the initial study. These experiments showed that less than 1% of the DDT was adsorbed to the walls of the glass bottles and furthermore that there was no conversion to DDA.

Since petroleum ether was the solvent used for extracting the DDT from sea water, the following studies were initiated to evaluate the extraction efficiencies of other solvent systems. Duplicate one-gallon bottles of clear glass, containing 3.5 liters of sea water or distilled water, were fortified with 10.5 µg of p,p' DDT in 350 µℓ of acetone to yield a concentration of 3.0 ppb. Duplicate 500 ml samples were taken from each bottle and extracted with one of the following solvents: three 50 ml portions of petroleum ether, two 50 ml portions of 15% ethyl ether in hexane followed by one 50 ml portion of hexane, or three 50 ml portions of methylene chloride. All solvents were dried with sodium sulfate, concentrated to an appropriate volume and analyzed by electron capture gas chromatography. Just prior to extraction, all samples were fortified with o,p'DDE to evaluate the integrity of the analyses. The recovery rate of o,p'DDE in all tests was greater than 89%, indicating no significant loss during analyses.

After initial sampling, the bottles were sealed and incubated at 20 C under controlled light conditions (12 hours light, 12 hours dark). Duplicate samples of 500 ml were extracted at various time intervals.

Tables 2-5 show the average percentage recovery of p,p'DDT extracted from duplicate sea water or distilled water samples up to 14 days after initiation of the experiment. p,p'DDE was the only metabolite measured, and since it never exceeded 2% of the parent compound it is not included in the percentage recoveries. The sea water was collected adjacent to the Gulf Breeze Laboratory in Santa Rosa Sound and the salinity ranged from 16 ppt to 21 ppt.

Table 2 shows that immediately after the sea water (21 ppt) was fortified with 3.0 ppb of DDT all solvent systems removed 93% of the DDT. After six days of incubation this level of recovery was not observed with any of the solvents tested. However, methylene chloride was more efficient than petroleum ether or 15% ethyl ether in hexane. Part of this experiment was repeated with sea water (16 ppt) and incubated for 4 days with similar results (Table 3).

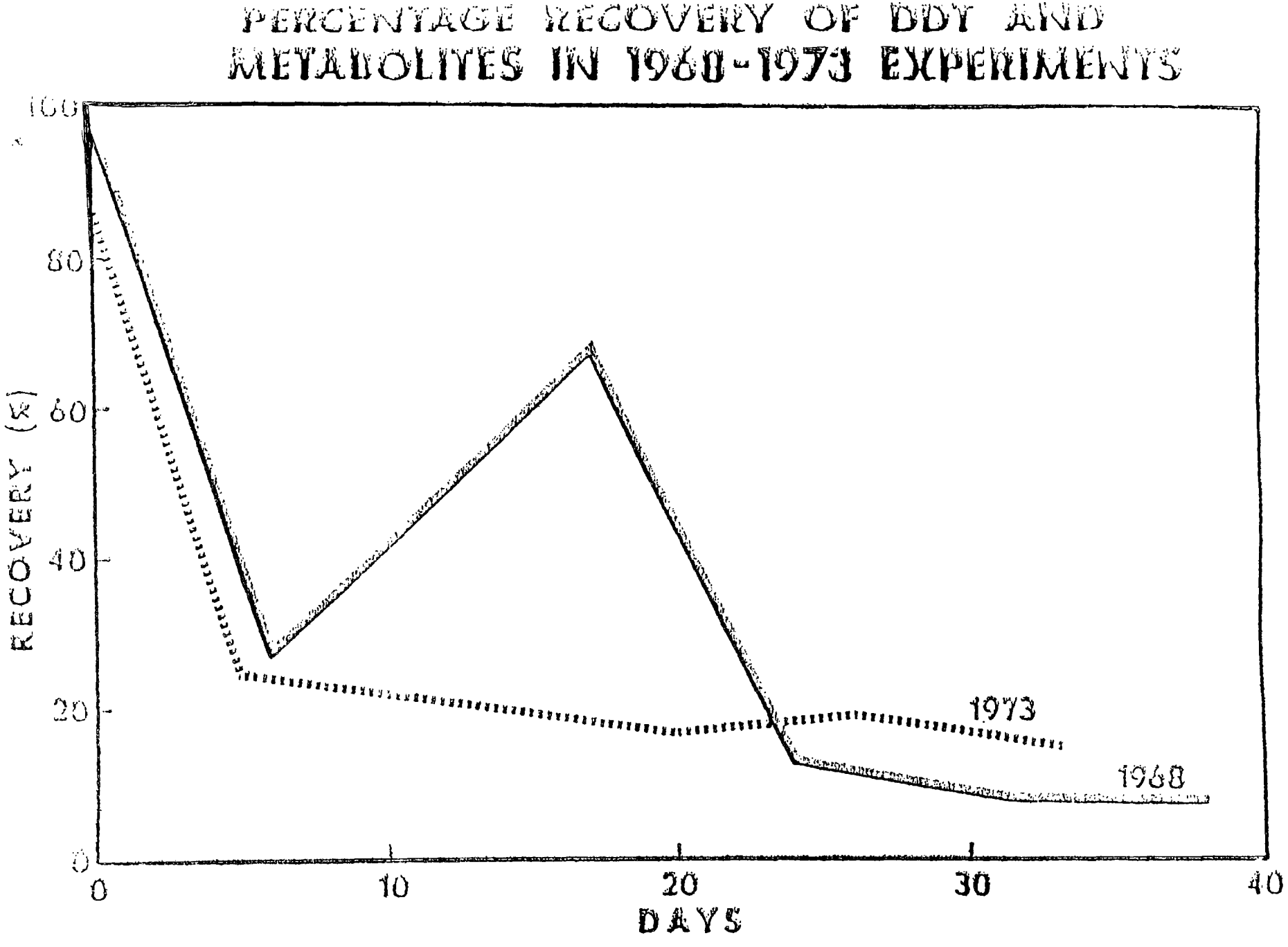


Figure 1. Percentage Recovery of DDT and Metabolites in 1968 and 1973 Experiments

TABLE 2

PERCENTAGE RECOVERY OF P,P' DDT FROM SEA WATER BY DIFFERENT EXTRACTION SOLVENTS

DAY	EXTRACTION SOLVENT		
	PETROLEUM ETHER	15% ETHYL ETHER IN HEXANE	METHYLENE CHLORIDE
0	93	93	93
6	67	66	76

TABLE 3

PERCENTAGE RECOVERY OF P,P' DDT FROM SEA WATER BY PETROLEUM ETHER AND METHYLENE CHLORIDE

DAY	PETROLEUM ETHER	METHYLENE CHLORIDE
0	90	95
4	67	85

TABLE 4

PERCENTAGE RECOVERY OF P,P' DDT FROM SEA WATER AND DISTILLED WATER BY PETROLEUM ETHER AND METHYLENE CHLORIDE

DAY	SEA WATER		DISTILLED WATER	
	PETROLEUM ETHER	METHYLENE CHLORIDE	PETROLEUM ETHER	METHYLENE CHLORIDE
0	90	94	90	91
7	58	78	90	91
14	46	68	94	92

TABLE 5

PERCENTAGE RECOVERY OF P,P'DDT FROM SEA WATER INCUBATED UNDER DIFFERENT LIGHT AND TEMPERATURE CONDITIONS

DAY	12 HOUR LIGHT AND 12 HOUR DARK AT 20 C	DARK AT 5 C
0	87	88
7	69	81
14	68	86

An experiment was performed with sea water (21 ppt) and distilled water using petroleum ether and methylene chloride. Table 4 shows that immediately after fortification recoveries were greater than 90% for water and solvents.

After 14 days, similar recoveries were observed only in distilled water. In sea water however, there was 49% and 28% reduction in recovery with petroleum ether and methylene chloride respectively. Since distilled water is devoid of particulate matter, this study suggests that DDT may be absorbed or adsorbed to plankton or particulate matter in sea water and the sorbed material was not removed resulting in low recoveries of DDT. This would explain the initially high extraction efficiency of DDT followed by the decline in recovery as DDT was associated with the particulate phase. Since methylene chloride was the most polar solvent used, it would have a greater affinity for removing the sorbed DDT.

In another test, duplicate bottles containing sea water (20 ppt) and DDT were incubated under controlled lighting conditions at 20 C and another set incubated at 5 C without light. Both were extracted with methylene chloride at various time intervals. Table 5 shows low recovery at 14 days under controlled lighting condition. However, those samples incubated at 5 C in total darkness did not show a significant decrease in recovery rate. Since the metabolic activity of plankton was probably inhibited under these temperatures and lighting conditions, these results suggest that DDT may be absorbed rather than adsorbed by plankton. However, Rice and Sikka (13) comparing the uptake of DDT by living and dead algae found that cells accumulated equal amounts of the pesticide.

Interaction of pesticides between water and particulate matter are complex. Not only do light and temperature appear to alter equilibria, but other physical and chemical factors have effects. Evaluating liquid-liquid extraction techniques of herbicides from river water, Suffet (14) observed that the isopropyl ester of 2,4-D was adsorbed to particulate matter in river water and that the amount changed by alteration of the pH of the water. Huang and Liao (10) found that adsorption of DDT to clays was rapid but the amount differed with the type of clay. A mixed culture of algae consisting mainly of Vauchenia had a greater adsorption for DDT than bentonite according to Hill and McCarty (9). Cox (5) reported that in natural marine populations virtually all of the DDT available for uptake was incorporated onto phytoplankton, but this may only account for 10% of the DDT residues recoverable from whole sea water.

These experiments support the work of other investigators in that DDT and other pesticides are extremely hydrophobic and can easily be adsorbed or absorbed by suspended matter from liquid solutions. The 1968 experiments at Gulf Breeze supports the concept that physical or chemical transformations of pesticides altered the extraction efficiences of the solvent and prevented complete recovery of the compounds. Obviously, additional work needs to be done to account for all of the chemical added to the test system.

It is difficult to relate laboratory findings directly to that of the estuary or open oceans. However, the laboratory data illustrate clearly some problems that could be encountered in monitoring sea water for pesticide pollution. The conventional analyses of water samples by liquid-liquid extraction techniques may provide invalid data if suspended matter is not considered. Standardized methods are needed to analyze the water column and suspended material separately.

Recently, a synthetic resin, Amberlite XAD-2 was evaluated as an adsorption medium for chlorinated hydrocarbons dissolved in sea water by Harvey (8). This technique utilizes large volumes of sea water and therefore permits greater sensitivity in analysis. In addition, the method eliminates the problems encountered in transport of large samples of water. Pollutants could be adsorbed on the resin at the sampling site and shipped to the appropriate analytical laboratory for desorption. Since the resin only removes the dissolved portion, additional samplings would be required to determine the levels absorbed or adsorbed to particulate matter.

ANALYTICAL QUALITY CONTROL

All pesticide residue laboratories should maintain an adequate analytical quality control program. The program should include both an intra-laboratory performance evaluation of personnel and methodology and an inter-laboratory sample exchange program. These programs are time consuming but are essential to the generation of valid analytical data.

Table 1 shows the recovery rates of oyster samples fortified with known concentrations of pesticides. Fortification techniques provide data only on the recovery efficiency of the total analytical procedure and not on the extraction step. Field residues may be subject to physical and chemical transformation and therefore may not be in the same physical or chemical state as the fortified sample. Table 2 illustrates the errors that can result from fortified samples. Extraction of water samples immediately after fortification yields

relatively high recovery efficiency with all solvent systems in sea water. Analyses several days later show the relative inefficiencies of the solvents systems used in sea water. Regardless of the analytical method used or the substrate being extracted, recovery data must be obtained on the extraction efficiency and the total analytical procedure.

There are several other factors in residue laboratories which, if ignored, may lead to inaccurate data. To name a few: (a) all glassware must be clean and free of residues; (b) the purity of all reagents used during analyses must be determined; (c) the accuracy of analytical standards must be maintained; (d) the condition of all components of the gas chromatograph must be optimized; and (e) laboratory personnel should be thoroughly trained.

An area that needs further study is the use of internal standards in marine pesticide monitoring. Currently, the Gulf Breeze Laboratory is no longer affiliated with a large monitoring program. Almost all samples submitted for analysis are of known identity. Most of these samples are fortified with an internal standard, just prior to analysis. The standard is usually a compound which behaves in an analytically similar way to the compound of interest. This technique is valuable in assessing the validity of the analysis. This same technique could also be applied to marine monitoring samples if an appropriate compound could be found that would not interfere with the monitored pesticides. It would be extremely valuable in laboratories with large sample volumes where close supervision of laboratory operations is not possible.

The analysis of marine samples for chlorinated hydrocarbon pesticides is at times complicated by the presence of polychlorinated biphenyl compounds (PCB). These compounds are industrial pollutants and are produced in the United States under the trade name Aroclor. They have chromatograph retention times similar to the organochlorine pesticides and therefore complicate the analysis when both are present in a sample. Several techniques have been described for the separation of PCB from organochlorine pesticides. A review of these methods were presented by Zitko and Choi (17). These techniques are time consuming and, in general, semiquantitative. In addition, differential absorption or metabolism of the Aroclor isomers in marine biota prevent accurate analysis of the PCB's. In view of these facts and the large number of samples that could result from a global monitoring program, this area of analysis required further study and/or standardization.

REFERENCES

1. Bowman, M. C. and M. Beroza, 1965. Extraction p-Values of Pesticides and Related Compounds in Six Binary Solvent Systems. J. Assoc. Agr. Chem. 48: 943-952.

2. Butler, P. A., 1966. Pesticides in the Marine Environment. J. Appl. Ecol. 3 (Suppl): 253-259.

3. Butler, P. A., 1973. Organochlorine Residues in Estuarine Mollusks 1965-1972 National Pesticide Monitoring Program. Pestic. Monit. J. 6: 238-362.

4. Cox, J. L., 1971. DDT Residues in Seawater and Particulate Matter in the California Current System. U. S. Fish. Wildl. Serv. Fish. Bull. 69: 443-450.

5. Cox, J. L., 1972. DDT Residues in Marine Plankton. Residue Reviews 44: 23-38.

6. Duke, T. W. and A. J. Wilson, 1971. Chlorinated Hydrocarbons in Livers of Fishes from the Northeastern Pacific Ocean. Pestic. Monit. J. 5: 228-32.

7. Goldberg, E. D., P. Butler, P. Miller, D. Menzel, R. Risebrough and L. Stickel, 1971. Chlorinated Hydrocarbons in the Marine Environment. National Academy of Sciences, Washington, D. C.

8. Harvey, G. R., 1972. Absorption of Chlorinated Hydrocarbons from Sea Water by a Crosslinked Polymer. Woods Hole Oceanographic Institute, Woods Hole, Massachusetts. Unpublished manuscript.

9. Hill, D. W. and P. L. McCarty, 1967. Anaerobic Degradation of Selected Chlorinated Hydrocarbon Pesticides. J. Water Pollut. Fed. 39: 1259-1277.

10. Huang, J. C. and C. S. Liao, 1969. Absorption of Pesticides on Clay Minerals. Presented at the 1969 Missouri Academy of Science Meeting, St. Louis, Missouri. Cited in Proc. of 25th Ind. Waste Conf., Purdue Univ., Lafayette, Ind., 1970.

11. Mills, P. A., J. F. Onley and R. A. Gaither, 1963. Rapid Method for Chlorinated Pesticide Residue in Non-Fatty Foods. J. Assoc. Agr. Chem. 46: 186-191.

12. Patil, K. C., F. Matsumura and G. M. Boush, 1972. Metabolic Transformation of DDT, Dieldrin, Aldrin and Endrin by Marine Microorganisms. Environ. Sci. Technol. 6: 629-632.

13. Rice, C. P. and H. Sikka, 1973. Uptake and Metabolism of DDT by Six Species of Marine Algae. J. Agr. Food Chem. 21: 148-152.

14. Suffet, I. H., 1973. The p-Value Approach to Quantitative Liquid-Liquid Extraction of Pesticides and Herbicides from Water. 3. Liquid-Liquid Extraction of Phenoxy Acid Herbicides from Water. J. Agr. Food Chem. 21: 591-598.

15. Wilson, A. J., J. Forester and J. Knight, 1970. Chemical Assays. 1969 Prog. Rep., Center for Estuarine and Menhaden Research, Gulf Breeze, Fla. U. S. Fish Wildl. Serv. Circ. 335: 18-20.

16. Wolman, A. A. and A. J. Wilson, 1970. Occurrence of Pesticides in Whales. Pestic. Monit. J. 4: 8-10.

17. Zitko, V. and P. Choi, 1971. PCB and Other Industrial Halogenated Hydrocarbons in the Environment. Fish. Res. Board Can. Technical Report No. 272, Biological Station, St. Andrews, N. B.

CONTRIBUTION NO. 207

(16)

Effects of Aroclor® 1254 on Laboratory-Reared Embryos and Fry of *Cyprinodon variegtaus*

STEVEN C. SCHIMMEL, DAVID J. HANSEN
AND JERROLD FORESTER
U.S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory

Eggs of the sheepshead minnow (*Cyprinodon variegatus*) were artificially fertilized (wet method) and maintained at 15° to 30°C and 0 to 35 °/₀₀ to determine efficient culture conditions. Fertilization was not affected within the temperature or salinity ranges tested, but hatching success was greatest (χ^2; $\alpha = 0.01$) within the 24° to 35°C range and 15 °/₀₀ to 30 °/₀₀ range.

Artificially fertilized sheepshead minnow eggs were exposed to logarithmic series of concentrations of the polychlorinated biphenyl (PCB), Aroclor 1254, (0.1 to 10 μg/l) in seawater averaging 30°C and 24 °/₀₀ in a flow-through bioassay. Fertilization was not affected, but significantly fewer embryos developed in the 10 μg/l. Fry were more susceptible to this PCB than were embryos, juveniles or adults. — ® *Registered trademark. Monsanto Co., St. Louis, Mo. Mention of commercial product does not constitute endorsement by the Environmental Protection Agency. — Contribution 207, Gulf Breeze Environmental Research Laboratory.*

Reprinted from *The ASB Bulletin,*
Vol. 21, No. 2, April 1974, p. 81.

CONTRIBUTION NO. 208

EPA-660/3-74-013
September 1974

THEORETICAL MODEL AND SOLUBILITY CHARACTERISTICS OF AROCLOR® 1254 IN WATER:

Problems Associated With Low-Solubility Compounds In Aquatic Toxicity Tests

by

W. Peter Schoor
Gulf Breeze Environmental Research Laobratory
National Environmental Research Center
Gulf Breeze, Florida 32561

Program Element 1EA077
ROAP/Task No. 10AKC/18

National Environmental Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Corvallis, Oregon 97330

ABSTRACT

A theoretical model of the behavior of substances having low water-solubility is presented and discussed with respect to aqueous bioassay. Ultracentrifugal techniques were used in an attempt to study size distributions of Aroclor 1254 aggregates in aqueous emulsions. Results indicate strong adsorption from emulsion by surfaces and a water-solubility at 20°C of less than 0.1µg/ℓ in distilled water and approximately 40% of that value in water containing 30 g/ℓ NaCl. Implications with regard to aqueous bioassay are discussed.

This report was submitted in fulfillment of Program Element 1EA077, ROAP/Task No. 10AKC/18 by the Gulf Breeze Environmental Research Laboratory under the sponsorship of the Environmental Protection Agency. Work was completed as of September, 1974.

CONTENTS

TABLES

ACKNOWLEDGMENTS

The author thanks Messrs. D. Lamb and W. Burgess for assistance with the analytical work and Dr. Ralph Birdwhistell, Dean, School of Chemistry, University of West Florida, for reviewing the manuscript.

Aroclor® 1254 is a registered trademark of the Monsanto Company, St. Louis, Missouri.

Section I

CONCLUSIONS

An extrapolation from the theory presented suggests that the use of "carriers" be continued with caution, because of two independent effects that may be present. One effect can most simply be described as an alteration of the aggregate-solvent interactions by "carriers" forming transition-like links between aggregates and solvent molecules. In such a fashion, solute aggregates are surrounded by "carrier" molecules, thus enhancing the ability of the aggregate to remain in a stable emulsion by permitting greater solute-solvent interaction. This can be illustrated graphically in Fig. 1 by enlarging region "B" over a greater range of aggregate sizes since some aggregates previously belonging to regions "A" and "C" now become more stabilized. It may also be visualized by flattening the two curves in Fig. 2, thereby extending their region of overlap. Thus, when added with a "carrier", more of an insoluble compound may be introduced into a stable water emulsion. The other effect may be due to possible interference with the uptake of a test compound by an organism. Any such uptake must by necessity be preceded by an adsorption to a surface of the organism such as the gills in a fish. If at this time the "carrier" molecules, which are located at the surface of the aggregate, affect the actual process of adsorption in any way, there will be a resultant change in the rate of transfer of the compound into the organism. If the rate of uptake is related to toxicity, there will be a concomitant change in toxicity.

Section II

RECOMMENDATIONS

This study shows, both theoretically and experimentally, that in so far as physical interactions are concerned, emulsions differing in degree of dispersion and stability can be formed, depending on the method of preparation and subsequent treatment. Consequently, the following questions should be answered before conducting bioassays in disperse aqueous systems:

(a) What are the solubility characteristics of the compound under investigation?

(b) To what extent are these characteristics related to field conditions?

(c) How can the solubility characteristics and field conditions be best simulated in the laboratory?

Such information would undoubtably result in more precise data on acute toxicity as well as long-term effects regarding aqueous bioassay of water-insoluble test compounds.

Section III

INTRODUCTION

Laboratory experiments designed to determine the effects of chemicals on aquatic organisms require that the tests be conducted under conditions which reproduce those present in nature as closely as possible. In order to accomplish this in a precise and scientific fashion, the physical state of a compound in an aqueous dispersion must be known. Convenience, time and other factors have in the past often led to the use of techniques in the laboratory which do not take into consideration that the solubility characteristics of a compound may possibly affect the toxicity, necessitating extrapolation from an apparent toxicity established in the laboratory to an expected toxicity under field conditions. In many instances, the practice of using extrapolation in scientific investigations is necessary and has proven to be a valuable tool when certain conditions cannot be met. However, the range through which the extrapolation is carried out must be chosen with great care, because without sufficient experimental and theoretical justification, a resulting extrapolation in this light may well prove to be unrealistic. Since natural water conditions represent a multi-component system, any attempt to quantitatively understand it must be preceded by a study of the system under ideal conditions. While the knowledge thus gained may or may not be of consequence in direct application, it, nevertheless, provides a more precise scientific basis for choosing valid limits for extrapolation.

The physical state of a compound in water is not a simple and straightforward phenomenon, even given the idealized conditions of a

two-component system - a single solute and a single solvent. A definable system should, however, be the starting point of any investigation aimed to scientifically arrive at data which lead to a quantitative understanding of the behavior of a compound in water. With this data a more precise attempt can be made to extrapolate from a system employed in the laboratory to the obviously much more complex system present in natural waters.

The purpose of this work is to provide a working theory on the behavior of substances of low water solubility and to test this theory by investigating the solubility characteristics of Aroclor 1254.

Section IV

THEORY

To explain and predict the characteristics of water-insoluble substances at low concentrations, an attempt is made here to redefine the basic principles underlying a disperse system. No attempts have been made to include in the definition the somewhat obsolete and often vague definitions of emulsions, suspensions, colloids, etc. The characteristics ascribed to each becoming readily apparent as the theoretical treatment of the proposed model continues.

In this paper, an ideal or true solution is defined as a solute dispersed in a solvent so that any single molecule of solute is surrounded by enough solvent molecules to insure that at any instant all solute molecules are distributed statistically equidistant, assuming a dilution at which interactions between solute molecules become negligible.

The ideal solution, under the conditions described, is represented by the presence of single solute molecules. Solute aggregates consisting of two or more molecules may represent a deviation from the ideal solution because, at least theoretically, these aggregates could consist of any number of molecules whose behavior would not necessarily coincide with that of a single molecule. For each solute and a single solvent, there is assumed to exist amongst all aggregates a maximally stable aggregate which, due to its nature, remains statistically equidistant from all other aggregates for at least a certain period of time. The stability of this aggregate depends solely on the molecularly characterized interactions at the solute-solvent interphase and on temperature.

By definition, a single solute molecule in a disperse system possesses a certain sphere of influence, the nature of which governs the fate of the solvent molecules that surround it, which in turn affects the behavior of the solute molecule, and thus determines the characteristics of the solute molecule in the system. While precise information is lacking, it is known, nevertheless, that the range of effect of a solute molecule may extend through several layers of surrounding solvent molecules. This means, of course, an orderly alignment involving either oppositely charged polar regions or non-polar regions on the solute and the solvent molecules. If this interaction between solute and solvent molecules is of significance, the above defined ideal solution can be visualized, provided also that there is no competition among the solvent molecules belonging to respective spheres of influence of two separate solute molecules.

The complexity of the situation is increased in cases where the interactions between solute and solvent molecules (solute-solvent interactions) become less pronounced, and, as a result, the interactions between solute and solute molecules (solute-solute interactions) become more pronounced. This implies that the sphere of influence around the solute molecule is diminished with respect to the solvent molecules which are now no longer attracted to the same degree. As two or more solute molecules start to form aggregates, the factor of size of aggregates versus their stability in a solvent becomes of utmost importance.

A generalized illustration of the size distribution of aggregates that one might expect to find in a suspension is shown in Fig. 1.

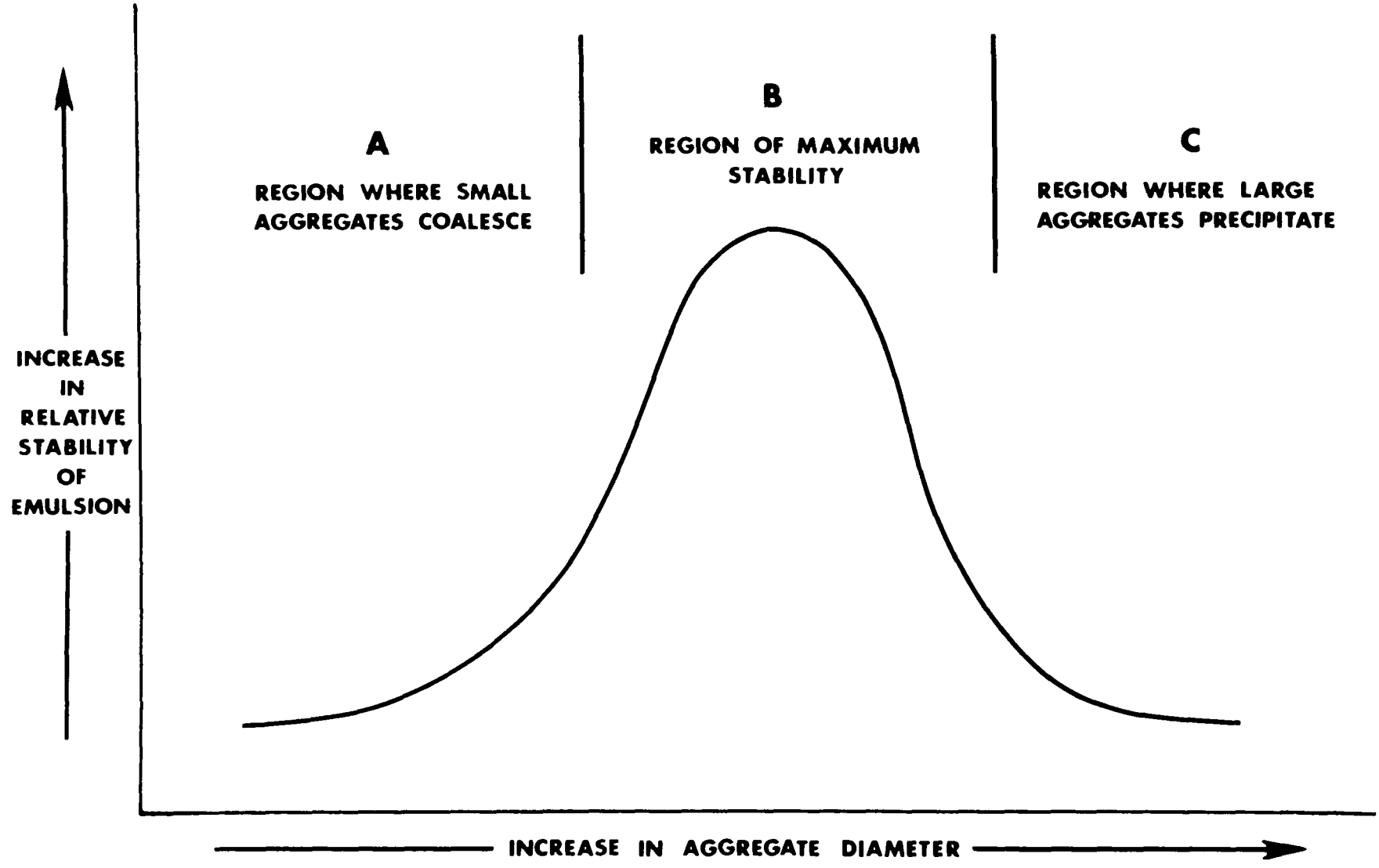


Figure 1. Theoretical relative stability of different sizes of aggregates in an emulsion during a given time interval.

Region "A" describes an area in which the aggregates are too small to exist independently because interactions in the sphere of influence at that point are such that solute-solute interactions, which have now become aggregate-aggregate interactions, are more pronounced than the aggregate-solvent interactions. Therefore, these aggregates are expected to coalesce, moving them into region "B", which describes a range of aggregate sizes of maximum stability. The aggregate-aggregate interactions in this range are weaker than in region "A" for that size of aggregate. Region "C" described aggregates which are too heavy to remain in suspension for a given period of time and will settle out or break into smaller, more stable aggregates. The exact shape of this curve and especially that of region "B", depends on how tightly the solvent is held within the sphere of influence of the solute aggregate, which is a function of the molecular interactions between solute and solvent.

The distribution of different aggregate sizes in terms of molecularly characterized interactions is shown in Fig. 2. The actual equilibrium reaction taking place is described in a simplified manner at the top of the figure. The two curves relate the hypothetical strength of interactions of solute-solvent (aggregate-solvent) type and solute-solute (aggregate-aggregate) type to aggregate size. The region where the curves cross corresponds to a distribution of aggregate sizes of maximum stability.

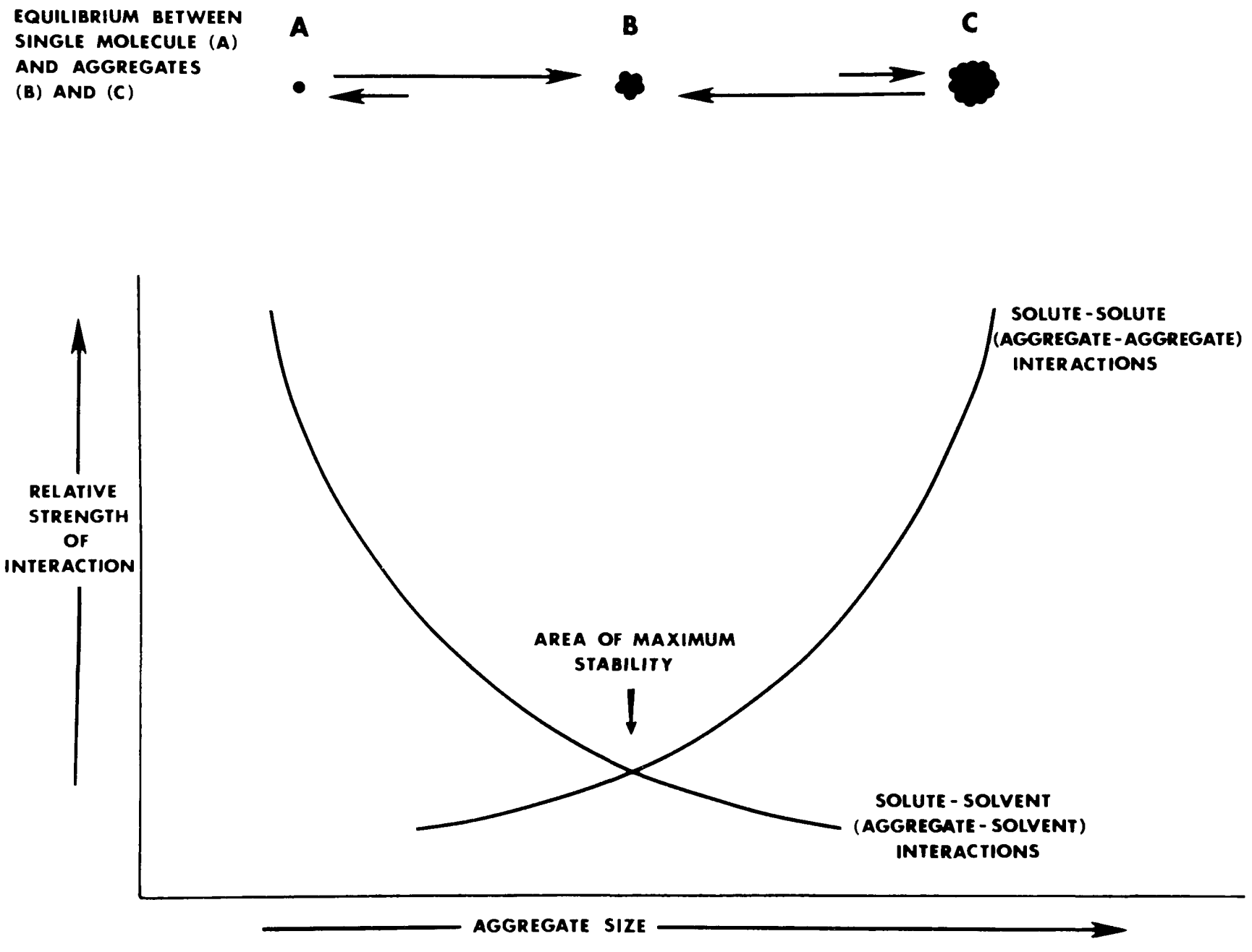


Figure 2. Theoretical strength of interaction between solute and solvent.

Section V

MODEL

Aroclor 1254 was chosen as a model compound because it has been extensively used in bioassay at this laboratory (Duke et al., 1970; Nimmo et al., 1971a; Nimmo et al., 1971b; Hansen et al., 1971; Lowe et al., 1972; Walsh, 1972; Cooley et al., 1972).

One approach to estimate quantitatively the solubility of Aroclor 1254 in water and the behavior of its aggregates is to use ultracentrifugal analysis. This technique permits the selective removal of particles of a certain size. For a spherical particle having a density of (ρ) and a radius of (r) the molecular weight (M.W.) is represented by:

$$M.W. = 4/3\pi r^3 N_o \quad (1)$$

where N_o is Avogadro's Number.[1]

Two opposing forces (f) which determine the fate of a particle in solution:

sedimentation $f = 4/3\pi r^3 (\rho-\rho_o)g$, and (2)

buoyancy $f = 6\pi r\eta$, (3)

where (ρ_o) is the density of the solvent, (g) is gravity, and (η) is the viscosity of the solvent.

To remove a small particle from an emulsion at a reasonable rate, a force larger than gravity must be applied. Using the ultracentrifuge, (g) in equation (2) is replaced with ($\omega^2 x$), the angular velocity of the centrifuge rotor (ω) times the distance of travel (x) of the emulsified particle.

---

1 The equations used are normally found in any textbook on physical chemistry, and their reproduction here is intended merely for the convenience of the reader.

The rate of sedimentation during centrifugation is described by:

$$\frac{dx}{dt} = \frac{2r^2(\rho-\rho_o)\omega^2 x}{9\eta} \tag{4}$$

where (t) is time in seconds to reach equilibrium. Integration yields:

$$\ln x_2 - \ln x_1 = \frac{2r^2(\rho-\rho_o)\omega^2 t}{9\eta} \tag{5}$$

The radius of a spherical particle is then given by:

$$r = \left[\frac{9\eta(\ln x_2 - \ln x_1)}{2(\rho-\rho_o)\omega^2 t}\right]1/2 \tag{6}$$

where

$w = 0.10472\ (\text{rpm})_{\text{rotor}}$

η = g/cm/sec

ρ = g/cm^3

x = cm

t = sec

Knowing the radius of a particle or assuming a radius, the time necessary to remove the particle from an emulsion is given by:

$$t = \frac{9\eta(\ln x_2 - \ln x_1)}{2(\rho-\rho_o)r^2\omega^2} \tag{7}$$

The following are particle size limits calculated using equation (6) for given centrifugation times, with $\eta = 8.94 \times 10^{-3}$ g/sec/cm, $x_1 = 6.7$ cm, $x_2 = 15.3$ cm, $\rho - \rho_o = 0.508$ g/cm^3 at 25,000 rpm.

Time (hrs)	Radius of particle (nm)
1	16.3
2	11.5
3	9.3
4	8.1
6	6.6
8	5.7

The following are particle size limits calculated using equation (6) for given centrifugation times, with $\eta = 8.94 \times 10^{-3}$ g/sec/cm, $x_1 = 6.00$ cm, $x_2 = 10.73$ cm, $\rho - \rho_o = 0.508$ g/cm^3 at 45,000 rpm.

Time (hrs)	Radius of particle (nm)
1	7.6
2	5.4
3	4.4
4	3.8 (208,000 g/mole[1]; 636 molecules)
6	3.1
8	2.7
12	2.2 (40,000 g/mole[1]; 124 molecules)

[1]Average molecular weight Aroclor 1254 = 327 g/mole (Hutzinger et al., (1972).

Section VI

EXPERIMENTS WITH AROCLOR 1254

Wide-mouth jars, 30 cm high and 14 cm wide, were used to produce 3ℓ of Aroclor 1254 emulsion per batch. Mechanical considerations concerning the proper physical agitation of Aroclor 1254 and water made it necessary to use 250 ml of Aroclor 1254 in the jar to submerge the blades of the stirrer. Agitation for 0.5 hr at 60°C and 1,800 rpm produced a cloudy emulsion which was allowed to settle for 48 hrs, when the range of concentration was found to be 1-20 mg/ℓ and the emulsion became almost clear. This emulsion is referred to as type-I. A second homogenization was carried out by transferring to a jar identical to the one used previously volumes of type-I emulsion to produce emulsions of 10-300 µg/ℓ, and stirring 1 hr at 25°C and 1,800 rpm. This emulsion is referred to as type-II. Type-III emulsions were prepared by taking an appropriate volume of type-I emulsion, adding it to a stainless steel blender jar to make a total volume of 500 ml, and homogenizing at high speed for 5 min.

All centrifugations were performed in a Beckman Model L3-50 ultracentrifuge at 20°C using SW 50.1 and SW 25.2 rotors.

The extraction procedure was that of Schoor (1973), with modifications of the ratio of water to hexane. Evaporation was carried out by placing the hexane extracts in a water bath at 35°C and allowing a gentle stream of air to blow across. This method was found superior to distillation in percentage recovery and time involved. When the extract volumes had to be reduced to less than 10 ml, dried, pre-purified nitrogen was used instead of air.

A Hewlett-Packard Model 5700 gas chromatograph with a linear electron-capture detector (^{63}Ni) was used for quantitative determination of the Aroclor 1254. The linearity of this detector eliminated use of different standards at each attenuation or reduction in volume of the sample, either being very time consuming and subject to errors. An OV-101 column (2% OV-101 on Gas Chrom Q, 100-120 mesh) was operated at 195°C with the detector at 300°C and the argon-methane (10:1) carrier gas at a flow rate of 25ml/min. Except where noted, quantitation was performed by comparing total peak heights of sample and standard.

To determine the amount of Aroclor 1254 adsorbed on walls of the 34 ml stainless centrifuge tubes, the water phase was decanted and any adhering droplets removed with a disposable pipet. Since acetone injected with the sample was detrimental to the chromatographic column, a sonic probe and hexane were used for removal of Aroclor 1254 from the walls of the tubes. This was necessary because the thin layer of water remaining on the walls shielded the Aroclor 1254 and prevented it from being desorbed into the hexane phase. Sonification emulsified the water at the boundary layer, thus allowing the hexane to contact the adsorbed Aroclor 1254.

Section VII

RESULTS

A typical chromatogram of an Aroclor 1254 standard in hexane (A) and a hexane extract of a type-II emulsion (B) is shown in Fig. 3. Some of the 11 peaks indicated are multiple peaks. Only peaks 1-7 were used to calculate the "total" peak height on which all quantitations were based. Peaks 8-11 were excluded, because they were often too small to permit accurate calculations.

The effect of storage time on Aroclor 1254 emulsions of type-I and type-II is shown in Table 1. There is a fairly rapid initial decrease in Aroclor 1254 in all cases and it appears that a plateau is reached at around 7 μg/ℓ. This should not be interpreted to mean that solubility is approached at that point, only that perhaps a stable emulsion is reached at that point.

The hexane extract of type-II emulsion (chromatogram B) indicates a relative reduction in peak height for the early eluting peaks. This phenomenon is better described by the results shown in Table 2. For comparison peak 7 was arbitrarily assigned a relative value of 100%. The results indicate that on standing a type-II emulsion shows a reduction of the individual peaks, with the early eluting components, or less chlorinated biphenyls (Zitko, 1970), being reduced much more than the late eluting ones. The degree of reduction depends somewhat on the preparation and initial concentration of individual type-II emulsions (Table 2). Type-III emulsions of comparable "total" concentration show a relative distribution of the isomers identical to that of the standard.

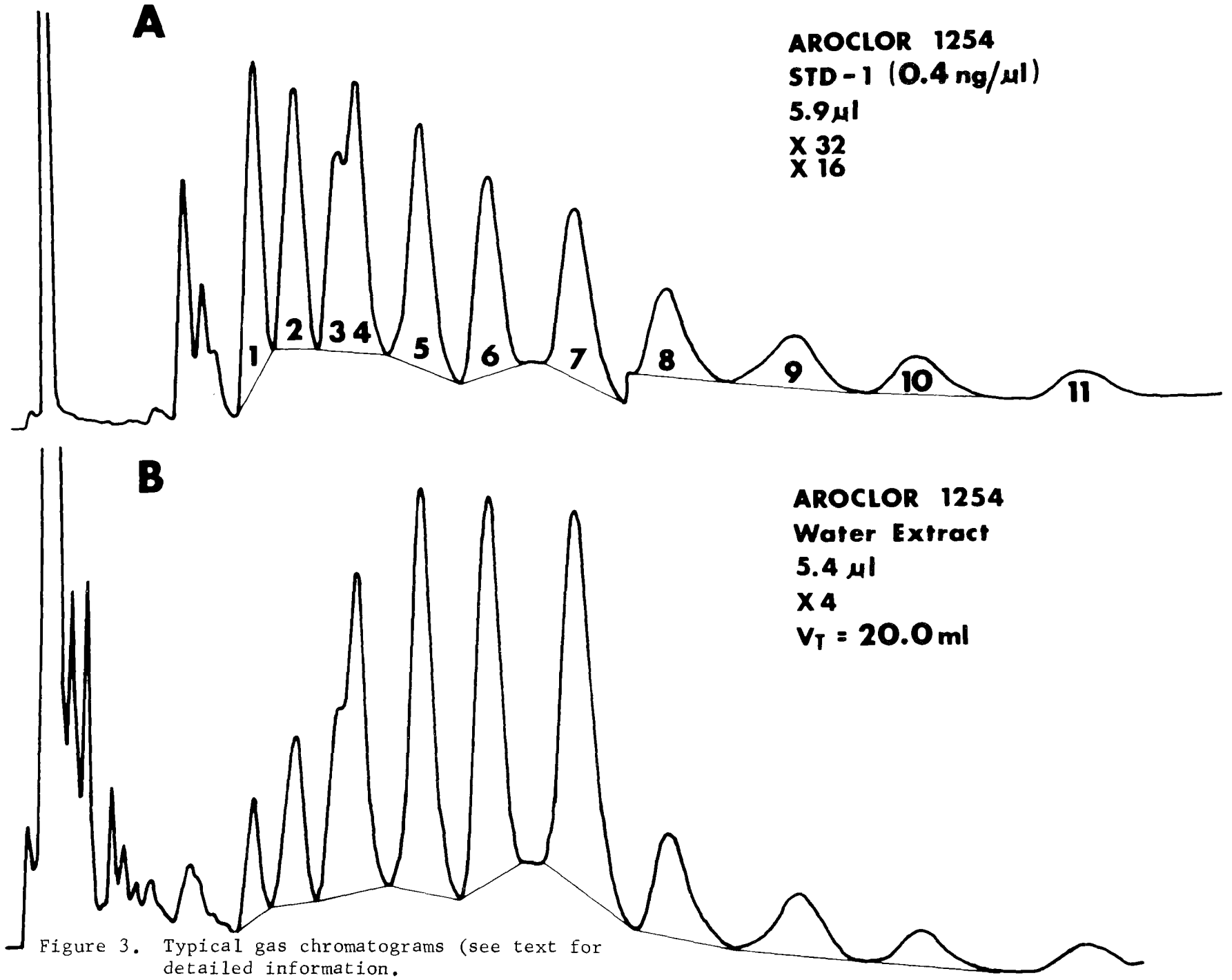


Figure 3. Typical gas chromatograms (see text for detailed information.

Table 1. EFFECT OF STORAGE TIME ON AMOUNT OF AROCLOR 1254 REMAINING IN THE WATER PHASE

Time (days)	µg/ℓ Aroclor 1254			
	Type I		Type II	
0	2300	301		50.2
2			286	
5		115		23.6
6		113		11.3
8		112		
9			123	
13		97	98.5	
15	502			
19	483	87	54.7	6.7
20			48.1	
21			44.5	
23		78		7.1
26				6.5
28	428			7.7
33	355			7.4
34	350			
41			15.5	
43	280			6.8

Table 2. ISOMER DISTRIBUTION OF AROCLOR 1254 TYPE II EMULSION AFTER STANDING FOR VARIOUS PERIODS OF TIME IN 3ℓ GLASS BOTTLE

Time (days)	Total conc. (µg/ℓ)[1]	% Peak Height[1] Peak Numbers[2]						
		1	2	3	4	5	6	7
2	286	76	93	95	95	98	104	100
9	123	79	78	89	94	98	99	100
13	98.5	79	79	93	98	99	96	100
19	54.7	72	75	85	93	91	95	100
20	58.1	64	70	80	89	92	94	100
21	44.5	61	65	82	90	99	93	100
41	15.5	37	41	56	70	77	88	100
21	13.4	16	27	44	55	76	87	100
33	3.6	12	21	39	45	64	82	100
38	1.6	9	10		31	46	63	100
	(3.4 ppm)	(41)		(80)		(87)	(100)	

[1]Calculations are based on the relative height of peak 7 (see below).

[2]Peak numbers are shown on the chromatogram in Fig. 1.

The distribution of isomers in a hexane extract of the gill tissue of a pink shrimp (Penaeus duorarum) exposed to 2.5 µg/ℓ Aroclor 1254 for 20 days is shown in parentheses at the bottom of Table 2. Because peaks 2, 4 and 7 showed obvious contamination, peak 6 was assigned the arbitrary, relative 100% value. The "total" concentration of 3.4 mg/kg was based on the total height of peaks 1, 3, 5 and 6, and on the wet weight of gill tissue (blotted to remove adhering water).

Filtration of type-I emulsion through 450 nm (0.45µ) Millipore^{R} filters revealed obstructed passage of Aroclor 1254 aggregates smaller than 450 nm. Starting with a 1 mg/ℓ emulsion and changing filters after each filtration, less than 0.01 µg/ℓ of the material remained in the water after 15 passages. Since aggregates in the starting emulsion were most likely smaller than 450 nm (calculations using equation 1 lead to roughly 10^{10} times the average molecular weight of Aroclor 1254), the Aroclor 1254 must have been adsorbed on the filter. This was also evidenced by the fact that the filter paper turned slightly transparent after the first passage during which about 95% of the material was removed from the emulsion.

The first centrifugation experiments were carried out by centrifuging 180 ml of 42 µg/ℓ Aroclor 1254 type-II emulsion in 60 ml polyacetate centrifuge tubes for 60 min at 107,000 x g (max.).

At an 85% total recovery the following distribution was found:

Acetone extract of tubes	66%
Hexane rinses of tubes	18%
Top 50 ml water phase	5%
Bottom 10 ml water phase	11%

The low recovery (85%) was probably due to incomplete extraction of the tubes in spite of refluxing with acetone.

Polyallomer^{R} centrifuge tubes were tried next. When 180 ml of 286 µg/ℓ type-II emulsion were centrifuged in 60 ml Polyallomer tubes for 60 min. at 107,000 x g (max.) the following distribution was found:

Acetone extract of tubes	--
Hexane rinses of tubes	22%
Top 25 ml water phase	.5%
Bottom 35 ml water phase	.6%

These percentages were based on the total amount of starting material, i.e., assuming 100% recovery instead of the 85% in the case of the polyacetate tubes. Extraction of the Polyallomer tubes by refluxing with acetone produced too many interfering peaks on the chromatogram, making complete recovery calculations impossible. Direct adsorption on Polyallomer tubes was achieved by permitting type-II emulsions to sit undisturbed in the tubes. Table 3 shows the outcome for two different concentrations.

To permit recovery and study of the material adsorbed on surfaces, 34 ml stainless steel centrifuge tubes were used for static tests,

Table 3. ADSORPTION OF AROCLOR 1254 TYPE II EMULSION ON POLYALLOMER CENTRIFUGE TUBES ON STANDING

Time (hrs)	Aroclor 1254 (µg/ℓ) in water phase
0	125
3	86
72	3.3
0	45
1	35
3	27

Table 4. ADSORPTION OF AROCLOR 1254 ON STAINLESS STEEL CENTRIFUGE TUBES AS A FUNCTION OF TIME AND CONCENTRATION

	Aroclor 1254 type II emulsion					
Time (hrs)	Total (µg)	Total (µg/ℓ)	Water (µg)	Water (µg/ℓ)	S. S. tube (µg)	% adsorbed
0.5	3.83	113	3.63	107	0.18	5
1	3.83	113	3.31	97	0.30	9
2	3.83	113	3.20	94	0.33	13
16	3.83	113	3.14	92	0.51	16
1	0.48	14	0.35	10	0.08	23
2	0.06	2	0.03	1	0.02	67

[1]Stainless steel centrifuge tubes.

as well as for ultracentrifugal analysis. Table 4 shows the amounts of Aroclor 1254 adsorbed on the wall of a stainless steel centrifuge tube in relation to starting concentration and time. The amounts adsorbed from the 14 μg/ℓ and 2 μg/ℓ emulsions were greater than that adsorbed from the 113 μg/ℓ emulsion during the same time period. It should be pointed out that 0.100 μg of Aroclor 1254 adsorbed as a monomolecular layer per tube represents about 2% of the minimum area available. The calculated inside area of a stainless steel centrifuge tube was 60.8 cm^2. This area must be considered minimum because the surface was assumed to be ideally smooth, which certainly is not the case. However, for the approximations involved, this figure was used.

A simple calculation using equation (1) yields 0.613 nm^2 for the cross-sectional surface area of an average Aroclor 1254 molecule using the average molecular weight of 327 (Hutzinger et al., 1972), and $\rho = 1.505$ g/cm^3 (W. B. Papageorge, Monsanto Company, St. Louis, Missouri, personal communication). Utilizing a molecular model with the phenyl groups at right angles to each other and bond length (Pauling, 1940) as the basis for calculations, a cross-sectional area of 0.643 nm^2 for the fully chlorinated and 0.356 nm^2 for the unchlorinated or biphenyl molecule was obtained. Values falling between are not linearly related to amount of chlorination. Using 0.613 nm^2 as an approximate, average cross-sectional area, 0.100 μg of Aroclor 1254 occupies 1.13 cm^2 in the form of a monomolecular layer. This corresponds to approximately 3 μg/ℓ in a 34 ml stainless steel centrifuge tube.

It can be seen that even at 50% adsorption from a 3 μg/ℓ emulsion only about 1% (maximum) of the available surface area is occupied, and surface saturation was not a factor.

The amounts of Aroclor 1254 in the form of emulsions of type-II and type-III adsorbed on the walls of the stainless steel centrifuge tubes are shown in Table 5. There is a difference in adsorption of the two different types of emulsion in the absence of NaCl. At least for type-III emulsions, the introduction of 30 g/ℓ NaCl appears to have no effect on the amount of Aroclor 1254 adsorbed. However, centrifugation reveals a difference in the size of the aggregates formed in the presence of NaCl, as shown in Table 6.

In comparison with an Aroclor 1254 standard, the relative distribution of the isomers in emulsions of type-II and III is quite different, as shown in Tables 7 and 8. However, in all cases the adsorbed Aroclor 1254 had a higher percentage of early eluting (gas chromatography) isomers than did that which remained in solution.

Table 5. ADSORPTION OF AROCLOR 1254 ON STAINLESS STEEL CENTRIFUGE TUBES

Time ((hrs)	µg Aroclor 1254[1] adsorbed		
	Type II Emulsion	Type III Emulsion	
	0 g/ℓ NaCL	30 g/ℓ NaCl	0 g/ℓ NaCl
0.5	0.19	0.09	
1.0	0.30	0.10	0.10
2.0	0.33	0.14	0.14
4.0	0.42	0.19	
19		0.39	
22			0.45

[1]Data adjusted to 4.00 µg total starting amount.

Table 6. CENTRIFUGATION OF AROCLOR 1254 IN WATER OF VARYING SALINITIES AT 69,000 x g (MAX.).

Time (hrs)	µg/ℓ Aroclor 1254 remaining in water phase		
	g/ℓ NaCl		
	0	15	30
0.5	13.9	7.1	6.0
1.0	12.5	6.6	4.9
2.0	7.2	4.6	2.9

[1]Started with 50 µg/ℓ Type III emulsion.

Table 7. DISTRIBUTION OF ISOMERS OF AROCLOR 1254 TYPE II EMULSION ON STANDING IN STAINLESS STEEL CENTRIFUGE TUBES

Storage (days)	Hrs in tube	µg/ℓ	% Peak heights[1] Peak number[2] 1	2	3	4	5	6	7
1	0	310 water	93	90	98	99	98	100	100
5	0	115 water	53	71	73	91	98	98	100
	2	97 water phase	49	67	69	83	100	100	100
	2	12 adsorbed	96	106	103	127	119	100	100
8	0	112 water	51	67	71	82	96	97	100
	2	102 water phase	48	66	68	79	98	98	100
	2	8.0 adsorbed	69	82	85	104	107	100	100
13	0	97 water	47	64	68	81	97	98	100
	2	86 water phase	43	59	66	78	92	96	100
	2	6.1 adsorbed	47	68	77	94	101	98	100

[1]Compared to standard Aroclor 1254 (Fig. 1). Calculations are based on the relative heights of peak 7.

[2]Peak numbers are shown on the chromatogram in Fig. 1.

Table 8. DISTRIBUTION OF ISOMERS IN THE ABSORBED FRACTION OF AROCLOR 1254 TYPE III EMULSION ON STANDING IN STAINLESS STEEL CENTRIFUGE TUBES

NaCl (g/ℓ)	hrs in tube	water phase (µg/ℓ)	adsorbed (µg)	% Peak heights[1] Peak number[2] 1	2	3	4	5	6
0	2	47.4	0.122	149	127	135	130	98	100
30	1	46.9	0.075	144	121	129	129	105	100
0	22	39.7	0.190	139	118	113	122	127	100

[1]Compared to standard Aroclor 1254 (Fig. 1). Calculations are based on the relative heights of peak 6.

[2]Peak numbers are shown on the chromatogram in Fig. 1.

Section IX

DISCUSSION

The original intent for conducting the work described was to find the absolute solubility of Aroclor 1254 in fresh and salt water. This, unfortunately, was not completely accomplished to any accurate degree, because a series of significant problems occurred at the beginning of the centrifugation experiments. Recovery of Aroclor 1254 after centrifugation was low and, hence, led to the discovery that adsorption occurred on the walls of the polyacetate centrifuge tubes as well as on Polyallomer and stainless steel centrifuge tubes. Ultimately, only the stainless steel centrifuge tubes were used in the adsorption and ultracentrifugal studies.

The apparent disappearance of early eluting isomers, such as shown in Table 2, has been observed by others. It was found to occur in the eggs of the double-crested cormorant and regarded as possibly due to metabolic breakdown (Hutzinger et al., 1972). Similar behavior in the carcasses of bobwhite quail after exposure to Aroclor 1254 was observed and believed to be because of isomeric transformations (Bagley and Cromartie, 1973). Application of Aroclor 1254 to different types of soil showed a reduced recovery of the early eluting, lower chlorinated biphenyls (Iwata et al., 1973), and it was postulated that this may have been due to evaporation from the soil. My studies did not substantiate the observations by Zitko (1970) that when Aroclor 1254 emulsions are centrifuged the dissolved fraction is richer in the lower chlorinated biphenyls than is the original preparation. However, the difference could be due to the method of the preparation of his emulsion, which was similar to my type-III emulsion. In both type-II and type-III emulsions the distribution

of isomers in the water phase shows a loss of the lower chlorinated biphenyls on standing (Tables 7 and 8). This loss was accounted for in all cases by adsorption on the stainless steel centrifuge tubes, the "lost" lower chlorinated biphenyls always being found in the adsorbed fraction. Thus, at least from water emulsions of Aroclor 1254, loss of the lower chlorinated biphenyls is due to their relatively greater affinity for surfaces.

The published values for solubility of Aroclor 1254 in fresh and salt water of 2-3 mg/ℓ and 1-1.5 mg/ℓ, respectively (Zitko, 1970), appear much too high. A conservatively high estimate based on my ultracentrifugal experiments indicates the average solubility of the isomers to be less than 0.1 μg/ℓ for fresh water and approximately 0.04 μg/ℓ (calculated from Table 6) in water containing 30 g/ℓ NaCl. It is extremely difficult, in my opinion, to obtain an absolute value for the true solubility of the average molecular weight isomer of Aroclor 1254. The problem lies in the fact that at low concentrations, long centrifugation times (in excess of 12 hrs at 243,000 x g (max.) theoretically are necessary to eliminate aggregates from the emulsion. At the low concentrations necessary to eliminate undesirable stirring back after completion of the centrifugation (Bowman et al., 1960), adsorption on the walls of the stainless steel centrifuge tubes (67% at 2 μg/ℓ for 2 hrs, Table 4) makes it all but impossible to employ ultracentrifugation for extended periods of time.

It appears that at least in the case of type-III emulsions the adsorption from water emulsions containing 0 and 30 g/ℓ NaCl was the same (Table 5), although the rate of sedimentation was quite different. The

explanation for this lies in the fact that the size of the Aroclor 1254 aggregate is much larger in the presence of salt and, while this is not apparent at 1 x g, the larger aggregates are removed more quickly from the salt-containing emulsion during ultracentrifugation. This agrees very well with my hypothesis that a larger aggregate is more stable under the given conditions and in the presence of salt, which is conducive to greater solute-solute (aggregate-aggregate) interaction.

REFERENCES

Bagley, G. E., and E. Cromartie. Elimination Pattern of Aroclor 1254® Components in the Bobwhite. J. Chromatogr. Sci. 75:219-226, 1973.

Bowman, M. C., F. Acree, Jr., and M. K. Corbett. Solubility of Carbon-14 DDT in Water. J. Agric. Food Chem. 8(5):406-408, Sept. 1960.

Colley, N. R., J. M. Keltner, Jr., and J. Forester. Mirex and Aroclor 1254 : Effect On and Accumulation by Tetrahymena pyriformis Strain W. J. Protozool. 19(4):636-638, 1972.

Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. A Polychlorinated Biphenyl (Aroclor® 1254) in the Water, Sediment, and Biota of Escambia Bay, Florida. Bull. Environ. Contam. Toxicol. 5(2):171-180, 1970.

Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr., and P. D. Wilson. Chronic Toxicity, Uptake and Retention of Aroclor 1254® in Two Estuarine Fishes. Bull. Environ. Contam. Toxicol. 6(2): 113-119, 1971.

Hutzinger, O., S. Safe, and V. Zitko. Polychlorinated Biphenyls. Analabs Res. Notes. 12(2):1-11, July 1972.

Iwata, Y., W. E. Westlake, and F. A Gunther. Varying Persistence of Polychlorinated Biphenyls in Six California Soils Under Laboratory Conditions. Bull. Environ. Contam. Toxicol. 9(4):204-211, 1973.

Lowe, J. I., P. R. Parrish, J. M. Patrick, Jr., and J. Forester. Effects of the Polychlorinated Biphenyl Aroclor® 1254 on the American Oyster (Crassostrea virginica). Mar. Biol. 17(3):209-214, Dec. 1972.

Nimmo, D. R., R. R. Blackman, A. J. Wilson, Jr., and J. Forester. Toxicity and Distribution of Aroclor® 1254 in the Pink Shrimp (Penaeus duorarum). Mar. Biol. 11(3):191-197, Nov. 1971(a).

Nimmo, D. R., P. D. Wilson, R. R. Blackman, and A. J. Wilson, Jr. Polychlorinated Biphenyl Absorbed from Sediments by Fiddler Crabs and Pink Shrimp. Nature 231:50-52, May 1971(b).

Pauling, L. Nature of the Chemical Bond. Ithaca, Cornell University Press, 1940. 164 p.

Schoor, W. P. In Vivo Binding of p,p'-DDE to Human Serum Proteins. Bull. Environ. Contam. Toxicol. 9(2):70-74, 1973.

Walsh, G. E. Insecticides, Herbicides and Polychlorinated Biphenyls in Estuaries. J. Wash. Acad. Sci. 62(2):122-139, 1972.

Zitko, V. Polychlorinated Biphenyls Solubilized in Water by Nonionic Surfactants for Studies of Toxicity to Aquatic Animals. Bull. Environ. Contam. Toxicol. 5(3):219-226, 1970.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA 660/3-74-013		

4. TITLE AND SUBTITLE	5. REPORT DATE
Theoretical model and solubility characteristics of Aroclor® 1254 in water: Problems associated with low-solubility compounds in aquatic toxicity tests.	September 1974
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
W. Peter Schoor, Ph.D.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
U. S. Environmental Protection Agency Gulf Breeze Environmental Research Laboratory Sabine Island Gulf Breeze, Florida 32561	1 EA077 / 10AKC / 018
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED Final
	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

A theoretical model of the behavior of substances having low water-solubility is presented and discussed with respect to aqueous bioassay. Ultracentrifugal techniques were used in an attempt to study size distributions of Aroclor® 1254 aggregates in aqueous emulsions. Results indicate strong adsorption from emulsion by surfaces and a water-solubility at 20°C of less than 0.1 µg/ℓ in distilled water and approximately 40% of that value in water containing 30 g/ℓ NaCl. Implications with regard to aqueous bioassay are discussed.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Solubility Adsorption Aroclor® 1254 Theoretical Model Water Aquatic Toxicity Tests Low-Solubility Compounds Emulsion Aqueous Dispersion		

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	Unclassified	
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA

U. S. GOVERNMENT PRINTING OFFICE: 1975—698-089/103 REGION 10

G 207 Observations of Luminescent Bacteria in Continuous Culture. L. KIEFER*, H. JANNASCH, K. NEALSON, and A. BOURQUIN, Univ. W. Fla., Pensacola, FL; Marine Biol. Lab., Woods Hole, MA; Environ. Prot. Agy., Gulf Breeze, FL.

Free-living marine luminous bacteria have never been observed to luminesce in the open ocean. The absence of this activity is postulated to be a result of an insufficent concentration of extracellular inducer substance (responsible for auto-induction in batch culture) in the environment.

To investigate this model, Photobacterium fisheri, str. 121, was cultured in a glycerol-limited chemostat apparatus. Light production was shown to be sustainable for several days when a cell density greater than the induction density was maintained. Thus, a potential for continuous light emission was demonstrated.

After steady-state conditions were achieved at high cell density, dilutions of the limiting substrate resulted in proportional and predictable decreases in cell density. Light emission, on the other hand, was proportional to dilution only at or above the cell density of induction. Thereafter, light emission was rapidly extinguished while cell density remained at the predicted value, thus supporting the critical concentration model for inducer activity.

CONTRIBUTION NO. 210

G 264 Microbial Response to Malathion Treatments in Salt Marsh Microcosms. AL BOURQUIN*, L. KIEFER, and S. CASSIDY. U.S. Environmental Protection Agency, GBERL, Gulf Breeze, FL.

Battery jars (6.5ℓ) were filled with salt marsh mud and water and placed at a constant temperature (28C) and a 12 h diurnal light cycle. The slowly aerated microcosms were stabilized for 1 week before treating with malathion at 1X and 10X field application rate. Application of the toxicant was repeated every 10 days for 30 days. Sediment and water samples were analysed at appropriate intervals for total aerobic heterotrophs and malathion degrading organisms (sole carbon source, SCS, and added growth substrate, MN). Varience analysis of the MN data showed significant differences between control and treatment levels for both sediment and water samples. No significant differences were noted between treatment periods. Numbers of MN organisms increased rapidly (7 days) after the first treatment, remaining at or over 70% (sediment) or 80% (water) of the total heterotrophic community. Although numbers of SCS degraders appeared to increase with malathion treatments and increase over the control in both sediment and water, no statistically significant differences were noted, due to fluctuations after treatments. No changes in total numbers of heterotrophs over the controls were noted.

No differences in populations of amylase, chitinase, lipase and casease producers were observed between controls and treated microcosms.

JOURNAL OF INVERTEBRATE PATHOLOGY 23, 389–396 (1974)

Pathological Effects of *Urosporidium* (Haplosporida) Infection in Microphallid Metacercariae[1]

JOHN A. COUCH

U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory,[2] Sabine Island, Gulf Breeze, Florida 32561 (Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon)

Received November 30, 1973

Extensive pathological changes occur in *Megalophallus* metacercariae as a result of natural infections by the haplosporidan hyperparasite *Urosporidium crescens*. Infected and uninfected metaceriae, recovered from blue crabs from Chincoteague Bay, Maryland, were examined and compared histologically in regard to condition of metacercarial cyst wall, tegument, and specialized parenchymal cells. Changes from normal found in heavily infected metacercariae were (1) suppression and replacement of possible secretory and parenchymal cells by the hyperparasite, (2) lack of reticulin stromata, polysaccharides, and acid mucopolysaccharides, (3) reduction in thickness of cyst wall, tegumental, and connective tissue structures, and (4) loss of mobility and resistance to mechanical pressures. Though severe pathological changes occur in heavily infected metacercariae, most infected metacercariae remain viable within the blue crab and thereby serve as a vector for *Urosporidium* until the death of the blue crab. At the time of the crab's death and disorganization, infected metacercariae rupture and release spores of the hyperparasite.

INTRODUCTION

DeTurk (1940) described a hyperparasite, *Urosporidium crescens* (Protozoa: Haplosporida), in the metacercariae of a species of microphallid trematode that parasitized the blue crab, *Callinectes sapidus*, in the Beaufort, North Carolina, area. He identified the trematode as the metacercaria of *Spelotrema nicolli*, which has since been transferred to the genus *Microphallus* by Baer (1943). During my studies of a species of microphallid trematode from blue crabs in Chincoteague Bay, Maryland, I found metacercariae of the genus *Megalophallus*[3] parasitized by a species of *Urosporidium* (Fig. 1) which appears identical to *U. crescens*. Sprague (1966), in a detailed review of haplosporidan parasites of trematodes, and Sprague and Couch (1971) reported finding similar infected metacercariae of a microphallid trematode in blue crabs from Chincoteague Bay, Maryland.

The infected *Megalophallus* metacercariae are readily recognized because they become enlarged, darkly pigmented, and rupture upon application of slight mechanical pressure (coverslip pressure). The uninfected and lightly infected metacercariae are smaller, white to cream color, and withstand relatively intense mechanical pressure.

A histological study was undertaken to examine the *Megalophallus–Urosporidium* relationship.

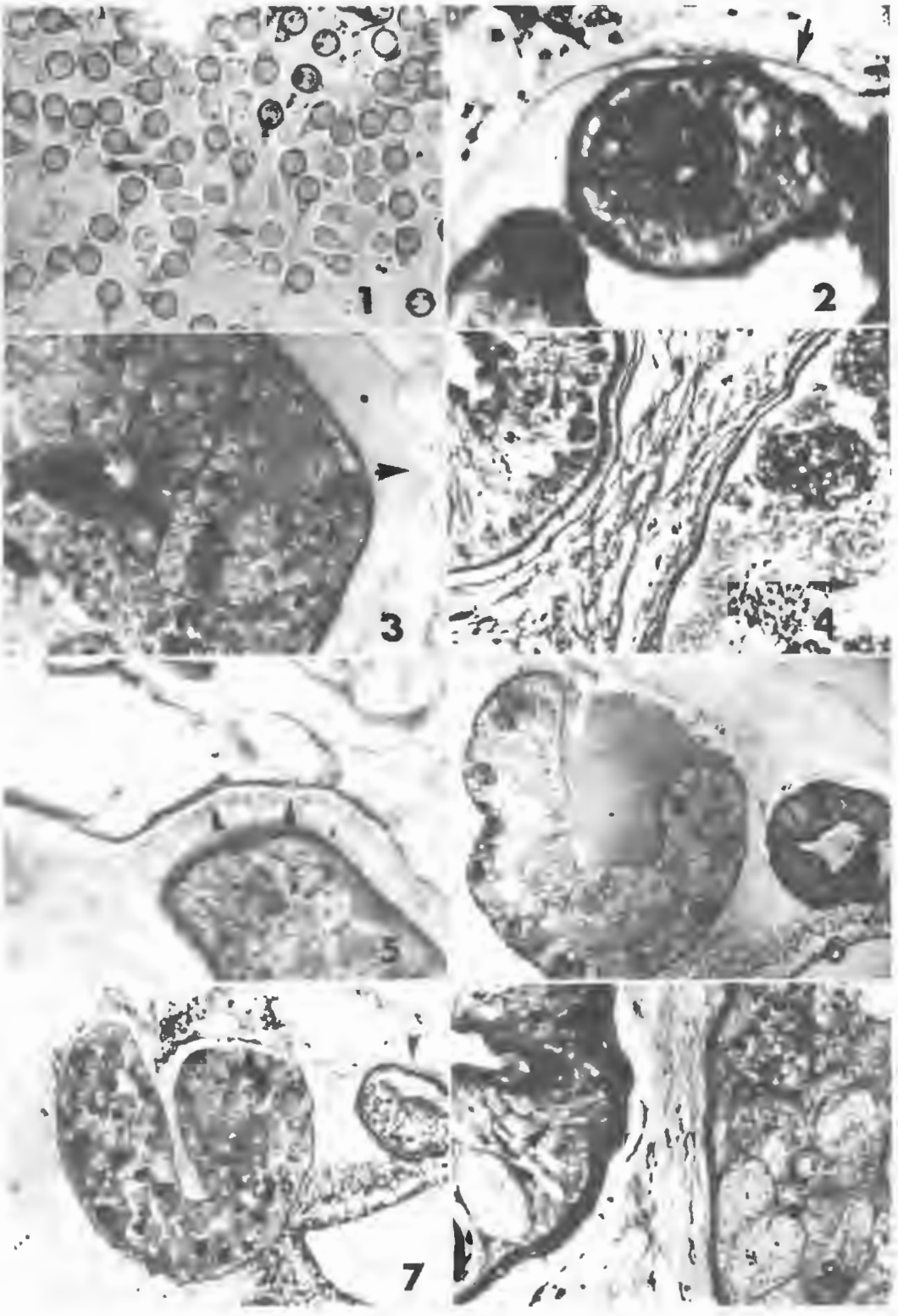
MATERIALS AND METHODS

Blue crabs harboring uninfected and infected metacercariae were collected monthly

[1] Most of the work reported here was done at the National Marine Fisheries Service Laboratory, Oxford, Maryland.

[2] Contribution No. 211, Gulf Breeze Environmental Research Laboratory.

[3] I wish to thank Dr. R. M. Cable for valuable aid in identifying the trematode genus.



FIGS. 1–8. *Urosporidium crescens* spores and sections of metacercariae.

from Chincoteague Bay in Maryland and Virginia during 1968 and 1969. Most of these crabs were caught in commercial crab traps during a crab survey by the U.S. National Marine Fisheries Service. Crabs were prepared by removing the dorsal carapace and inspecting all internal tissues and organ surfaces for metacercariae. Muscle and hepatopancreatic tissues were teased apart under a dissecting microscope; uninfected and infected metacercariae were fixed in Davidson's (Shaw and Battle, 1957), Bouin's (aqueous), or AFA fixatives.

Uninfected metacercariae were allowed to excyst in petri plates, filled with filtered seawater, over periods of 24–36 hr at room temperature. The complex male reproductive system was studied in living and stained whole mounts (Semichon's acetocarmine) in order to make generic identification.

Fixed, uninfected and infected metacercariae were carefully embedded together in paraffin blocks, sectioned at 3μm, and the sections stained by one of the following methods. Total protein was studied with the mercury bromophenol blue method of Mazia et al. (1953), with the exception that, instead of dehydrating with an aqueous ethanol series, a 1-butanol (2 changes) dehydration was used[1] to prevent excessive loss of stain. Scleroproteins, i.e., reticulin and collagenous materials, were studied by Lillie's (1965) silver oxide reticulin technique. Acid mucopolysaccharides were stained by a modified alcain blue method of Lison (1954) (*p*H 2.5; no counterstain) using 1-butanol for dehydration. Periodic acid-Schiff (PAS) methods with or without 0.5% malt diastase digestion were used to compare total polysaccharide patterns and to determine location and condition of certain cell types in uninfected and infected metacercariae. Mallory's triple stain and standard iron hematoxylin-eosin were used for general histological study.

Measurements were made of 20 normal and 20 infected metacercariae in sections, and averages and ranges given are based on these measurements.

Results

Cysts of uninfected metacercariae (in section) range from 189 to 266 μm in diam-

[1] Pearse (1968; p. 607) recommends tertiary butyl alcohol following staining by this method.

Fig. 1. Arrows point to immature and fully developed, tailed spores of hyperparasite; round sporoplasms with nuclei visible in most spores. ×970.

Fig. 2. Oral sucker region of uninfected metacercaria stained with Hg-BPB; arrow points to normal cyst wall with moderate staining. ×430.

Fig. 3. Oral sucker region of heavily infected metacercaria; arrows point to thin cyst wall and Hg-BPB-positive pharynx; note complete replacement of oral sucker cells by sporocysts of *Uro-Sporidium*, but lack of infection of pharynx. ×430.

Fig. 4. Dense fibers of silver-positive stroma in uninfected worm (upper arrow); lack of silver-positive stroma in infected worm (lower arrow); note the silver-positive walls of *Urosporidium* spores as well as dense meshwork of connective tissue stroma of crab host between metacercarial cysts (Lillie's silver oxide stain). ×430.

Fig. 5. Lack of reticulin-collagen-positive material in clear, single-layered cyst wall of uninfected worm (arrowheads); note dense layer of reticulin-positive host material closely abutting negative cyst wall (Lillie's silver oxide stain). ×430.

Fig. 6. PAS-treated infected metacercariae (large specimen); note the more intense staining of uninfected metacercaria (smaller specimen), and the difference in sizes of uninfected and infected metacercariae. ×100.

Fig. 7. PAS (treated with 0.5% diastase) section of infected and uninfected metacercariae (compare with Fig. 6); note PAS-positive diastase-resistant cyst wall (arrowhead) and tegument. ×100.

Fig. 8. PAS-treated uninfected (arrow) and infected metacercariae; compare differences in thickness and intensity of staining in tegument, and parenchyma; particularly note the reticulated PAS pattern (arrow) in uninfected metacercaria. ×430.

TABLE 1
RESULTS OF SELECTED STAINING METHODS ON UNINFECTED AND INFECTED METACERCARIAE[a]

Staining method	Cyst wall		Tegument		Parenchyma		Type I cells		Type II cells	
	U[b]	I[b]	U	I	U	I	U	I	U	I
Hg-BPB	2	2	3	3	3	0,1	0	0	2	0,1
Lillie's silver oxide	0	0	3	1	3	1	2	0	0	0
PAS	2	2	3	1	3	0,1	2	0	3	0
PAS (0.5% diastase)	2	2	3	1	0	0	2	0	3	0
Alcian blue (pH 2.5)	0	0	3	0	0	0	3	0	0	0

[a] Staining reaction scale: 0 = negative staining or absence of structure; 1 = light staining; 2 = moderate staining; 3 = strong staining.
[b] U = uninfected metacercariae; I = infected metacercariae.

eter, whereas those of infected metacercariae are usually 410 to 654 μm in diameter. The difference in size is a result of enlargement of infected metacercariae when they become filled with sporocysts of *Urosporidium* (Figs. 6, 7, 10).

Encysted metacercariae, both uninfected and infected, are surrounded by a single-layered cyst wall of apparent parasite origin (Figs. 2, 5, 7). Often a layer of host tissue adheres to cysts removed from the host. Usually, the major difference between cyst walls of normal and infected *Megalophallus* metacercariae is their relative thicknesses, the latter often having a much thinner wall (average thickness of normal, 2.3 μm; infected, 0.8 μm).

The tegument of normal metacercariae is spinous from the anterior end of the worm to a point slightly posttesticular. It is 1.7–4.5 μm thick, with an obvious underlying basement membrane. In sections of heavily infected metacercaria, the tegument is thinner ($<$0.5 to rarely 2.0 μm), spines are less obvious, and the basement membrane appears thinner than normal.

The parenchyma (cells and spaces between tegumental basement membrane and internal organs) of normal worms is compact in sections stained with iron hematoxylin and eosin, whereas the normal relationships of specific parenchymal cells, connective tissue elements, and organs are altered by hyperparasites in heavily infected metacercariae.

Table 1 gives the results of selected staining methods on specific tissues of uninfected and infected metacercariae. The tissues studied were the cyst wall, tegument, parenchyma, and certain specialized cell types in the peripheral and central parenchymal areas.

The cyst wall surrounding the uninfected metacercaria stains blue with mercury bromophenol blue (abbreviated Hg-BPB hereafter) (Fig. 2). In cysts of the infected worm, the wall is reduced in thickness and is often broken, (Fig. 3). However, the cyst wall of heavily infected metacercariae, though thinner than normal, stains with Hg-BPB.

As noted earlier, the single cyst wall of *Megalophallus* is intimately surrounded by host tissue. The nature of the fine layer of host material adjacent to the cyst wall becomes clear when one studies sections stained with Lillie's silver oxide reticulin stain. The fibrous material deposited against the cyst wall stains black (Fig. 5), and corresponds in stain affinity to reticulin fibers in the vertebrates. The presence of this fine reticulin layer, which contains some fibroblastlike cells, is the only histological evidence of a host response to the presence of metacercariae, and may be considered a very slight encapsulation reaction. There appears to be no difference in the amount of reticulin-positive material surrounding uninfected and infected cysts (Fig. 4).

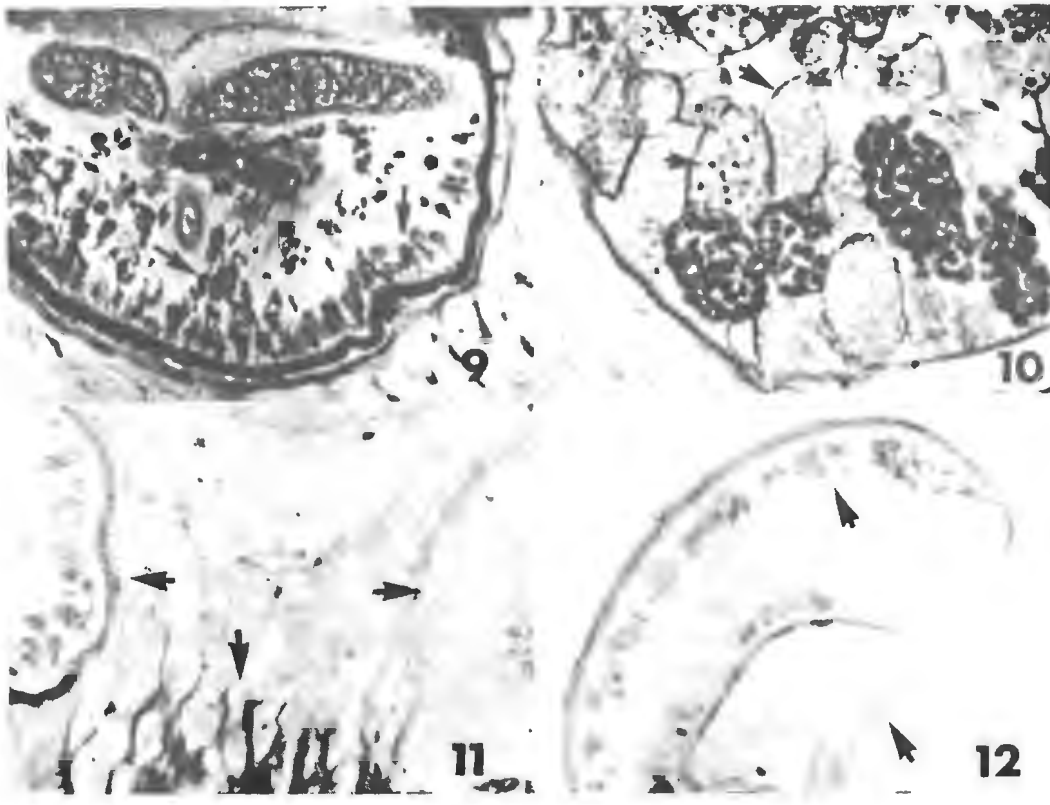


FIG. 9. Distribution of cells of type I (right arrow) and type II (left arrow) in uninfected metacercaria; note difference in stain (PAS-diastase treated) intensity in two cell types. ×430.

FIG. 10. Infected metacercaria (PAS-diastase treated) to be compared with Fig 9· parenchyma completely filled with sporocysts. ×430.

FIG. 11. Alcian blue-treated section; middle arrow points to alcian blue-staining material in *crab host tissue (ground s*[illegible]*)*, *arrow on reader's left points to acid mucopolysaccharide-positive* tegument and type I cells of uninfected metacercaria; arrow at right points to comparable section of infected worm which lacks alcian blue-positive material and type I cells. ×430.

FIG. 12. Longitudinal section of uninfected metacercaria stained with alcian blue only; note concomitant distribution of acid mucopolysaccharide material in tegument and type I cells beneath tegument; heavier alcianophilia in anterior tegument (upper arrow) and lesser alcianophilia in posterior regions of metacercaria (lower arrow) 430

The cyst wall proper is completely negative for reticulin and collagen. It appears as a translucent narrow band within the fine framework of host reticulin (Fig. 5).

The cyst walls of uninfected and infected metacercariae are PAS-positive after diastase digestion (Figs. 7, 9). Because of its Hg-BPB staining and PAS-positiveness, diastase-resistance, the single-layered wall probably consists of a carbohydrate pro ein complex.

The ground substance of the crab host shows considerable alcian blue-positive material (Fig. 11), with substantial amounts often closely abutting the outer surface of the cyst wall. The cyst walls proper of uninfected and infected metacercariae are alcian blue-negative. Often, small amorphous amounts of alcian blue-positive material are found against the inner surface of the cyst wall, but this material does not form a distinct inner layer.

In both uninfected and infected worms, the tegument stains positively for protein (Figs. 2 and 3), but the reduced thickness of the tegument of infected worms indicates less total protein (Fig. 3).

The basement membrane of the tegument of uninfected worms apparently reduced more silver (per unit length) than that of the infected worms, thus showing a greater concentration of basement membrane constituents per unit length of membrane in section. When treated with Lillie's silver oxide method and other connective tissue stains, i.e., Mallory's triple stain and Hg-BPB, the tegumental basement membranes of the infected worm appear to have undergone a thinning or "stretching," as had the tegument and cyst wall. This is probably a result of the remarkably enlarged condition of heavily infected metacercariae.

The tegument of uninfected and infected worms is PAS-positive and diastase-resistant. The tegumental layer, including the basement membrane, of infected worms shows less staining for PAS-positive, diastase-resistant material than does that of the uninfected worms (Figs. 9, 10).

The outer tegument of uninfected metacercariae stains intensely with alcain blue (Figs. 11, 12), indicating that a heavy concentration of acid mucopolysaccharides exists in this layer. The basement membrane is negative for aid mucopolysaccharides. Heavily infected worms do not stain with alcian blue (Fig. 11).

Hg-BPB-positive fibrous tissue between parenchymal cells gives an overall blue cast to sections of uninfected metarcercariae (Fig. 2). Comparison of the same general regions in infected worms suggests a loss of protein. Figure 3 shows the almost complete replacement of host tissue by plasmodia and spores of the hyperparasite. Note particularly the oral sucker of the uninfected (Fig. 2) and infected (Fig. 3) metacercariae. In the heavily infected worm, the only tissue that shows a significantly normal protein concentration is the pharynx (arrow), the only organ not invaded by *Urosporidium*.

In their parenchyma, uninfected worms have fibers of acellular, reticulin-positive material. This material (Fig. 4) may compose part of an interstitial tissue similar to that described by Threadgold and Gallagher (1966) as ramifying among and between parenchymal cells of *Fasciola*. These fibers appear to be identical with, and occupy the same space as, the Hg-BPB-positive fibrous tissue in the parenchyma. In infected worms, this Hg-BPB-positive and reticulin-positive tissue is less obvious (Fig. 4) because masses of sporocysts and plasmodia replace or occupy large spaces in the parenchyma. In infected metacercariae, there is no evidence of increase in reticulin or other connective tissue acellular elements concomitant with enlargement of the worm.

The parenchymata of uninfected worms show much heavier positive PAS reactions than do those of infected worms (Figs. 6, 8). The PAS-positive substances in uninfected worms seem to be distributed along the paths of the supportive stromata, rather than in the general interstromal spaces occupied by parenchymal cells. This may give the PAS-positive material a reticulated appearance (Fig. 8).

Certain cells found in the peripheral and central parenchymata of metacercariae are designated here as types I and II. These two cell types are characterized by the staining reactions of their cytoplasms (Table 1). These cell types are used mainly as "markers" of normal conditions in uninfected metacercariae and to demonstrate pathological changes that take place in infected metacercariae. In this paper, these cells are not compared with specific cell types described for other larval and adult Digenea, e.g., Dixon (1966), Thakur and Cheng (1968), because of the lack of background cytological studies of microphallid metacercariae needed as a basis for comparison.

TABLE 2
THREE PATHOLOGICAL CHANGES AND POSSIBLE RELATED EFFECTS IN METACERCARIAE INFECTED BY *Urosporidium crescens*

	I	II	III
Pathological change	Reduction in thickness of cyst wall and in thickness of tegument	Enlargement of metacercariae without concomitant increase in supportive connective tissue stromata (total protein; reticulin)	Replacement and suppression of activity of cell types I and II
Final effect	Rupture of cyst wall upon application of pressure	Collapse of parenchyma and release of hyperparasite; loss of mobility of preencysting metacercaria	Loss of possible protective layer of acid mucopolysaccharides and mucoproteins

Cell type I is found immediately beneath the basement membrane, mostly in the anterior dorsal and ventral regions of the parenchyma of uninfected worms (Figs. 9, 12). The absence of Hg-BPB staining and the occurrence of a distinct PAS reaction (diastase-resistant) and alcianophilia in the cytoplasm of cells of this type indicates that they possess neutral, nonglycogen polysaccharides and acid polysaccharides. Infected worms (Figs. 7, 8, 10) show a loss of these cells or replacement of them by the hyperparasite.

The degree of alcianophilia in a given section of the tegument appears to be proportional to the concentration of neutral and acid mucopolysaccharide-positive cells (type I) underlying that section (Fig. 12). The tegument in the anterior half of uninfected metacercariae gives the strongest reaction with alcian blue, and the largest number of type I cells is found there. These observations suggest a possible relationship between the distribution of acid mucopolysaccharides in the outer tegumental layer and a concomitant distribution of type I cells in the anterior peripheral parenchyma (see particularly Figs. 11 and 12). The identity of staining reactions of these cells with staining reactions (PAS+, alcian blue+) of materials in the tegument of the worm suggests that the type I cells may secrete the tegumental mucopolysaccharides. The concomitant loss of tegumental alcianophilia and absence of cell type I in parenchymata of heavily infected metacercariae further strengthens this concept. The presence of large amounts of acid mucopolysaccharides in the tegument (particularly around the oral sucker) could protect the worm from the definitive host's digestive enzymens, a function suggested for tegumental acid mucopolysaccharides in helminths by Monné (1959) and Lee (1966).

Cell type II, found in the anterior central and marginal parenchyma (Figs. 7, 9), is positive for protein and is intensely PAS-positive following diastase treatment (Table 1). These staining reactions suggest the presence of mucoproteins. In infected metacercariae, these cells are replaced or destroyed by the hyperparasites (Figs. 7, 10).

Effects of *Urosporidium crescens* infection on metacercariae of *Megalophallus* sp. are summarized in Table 2.

DISCUSSION

DeTurk (1940) was the first to note that *Urosporidium* occurs in such large numbers that the tissues of the trematode host are often replaced. I have observed that even though the trematode becomes intensely invaded and most of its parenchyma is replaced by the hyperparasite, the enlarged

and heavily infected metacercariae retain the ability to move slowly, thereby demonstrating their viability. It is possible that a small percentage of infected metacercariae are destroyed in the crab. There is only a slight host response to living uninfected and infected metacercariae in blue crabs.

Death of infected metacercariae in the tissues of the crab would result in a premature release of *Urosporidium crescens* into a hostile environment, exposing the hyperparasite to possible destruction by crab hemocytes or other defense mechanisms. Therefore, I suggest that, usually, fragile infected metacercarie while in the living crab host serve as a vehicle for the hyperparasite until the crab dies or is killed and disorganized, at which time the spores of *Urosporidium* are freed by the rupture of the metacercaria.

The replacement, failure, or reduction of supportive tissue complexes, e.g. reticulin, and the specific loss of possibly protective acid mucopolysaccharide layers could greatly reduce the probability of any given heavily infected metacercaria establishing itself in a definitive host, even if it were released intact from the crab. The suppression or elimination of possible secretory cells in heavily infected metacercariae suggests that the specific roles of these cells could be studied further by using uninfected metacercariae and infected metacercariae as experimental systems.

References

Baer, J. G. 1943. Les trématodes parasites de la musaraigne d'eau *Neomys fodiens* (Schreb.). *Bull. Soc. Neuchatel Sci. Natur.*, **68**, 34–84.

DeTurk, W. E. 1940. The occurrence and development of a hyperparasite *Urosporidium crescens* sp. nov. (Sporozoa, Haplosporidia) which infests the metacercariae of *Spelotrema nicolli* parasite in *Callinectes sapidus*. *J. Elisha Mitchell Sci. Soc.*, **56**, 231–232.

Dixon, K. E. 1966. A morphological and histochemical study of the cystogenic cells of the cercaria of *Fasciola hepatica* L. *Parasitology*, **56**, 287–297.

Lee, D. L. 1966. The structure and composition of the helminth cuticle. *Advan. Parasitol.*, **4**, 187–254.

Lillie, R. D. 1965. "Histopathologic Technic and Practical Histochemistry," 3rd ed. McGraw-Hill, New York.

Lison, L. 1954. Alcian blue 8G with chlorantine fast red 5B. A technic for selective staining of mucopolysaccharides. *Stain Technol.*, **29**, 131–138.

Mazia, D., Brewer, P. A., and Alfert, M. 1953. The cytochemical staining and measurement of protein with mercuric bromophenol blue. *Biol. Bull.*, **104**, 57–67.

Monne, L. 1959. On the external cuticles of various helminths and their role in the host-parasite relationship. A histochemical study. *Ark. Zool.*, **12**, 343–385.

Pearse, A. G. E. 1968. "Histochemistry, Theoretical and Applied," Vol. 1, 3rd ed., Little, Brown, Boston, Massachusetts.

Shaw, B. L., and Battle, H. I. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). *Can. J. Zool.*, **35**, 325–347.

Sprague, V. 1966. Haplosporidan parasites of trematodes. Proc. Symp. Anim. Parasites Invertebr., Washington, D.C., sponsored by Am. Soc. Zool. Ref. No. 66–100, Natur. Resour. Inst., Univ. Maryland, Solomons, Maryland (Mimeo.).

Sprague, V. and Couch, J. A. 1971. An annotated list of protozoan parasites, hyperparasites, and commensals of decapod crustacea. *J. Protozool.*, **18**, 526–537.

Thakur, A. S., and Cheng, T. C. 1968. The formation, structure, and histochemistry of the metacercarial cyst of *Philophthalmus gralli* Mathis & Leger. *Parasitology*, **58**, 605–618.

Threadgold, L. T., and Gallagher, S. E. 1966. Electron microscope studies of *Fasciola hepatica*. I. The ultrastructure and interrelationship of the parenchymal cells. *Parasitology*, **56**, 299–304.

(Reprinted from Nature, Vol. 247, No. 5438, pp. 229 231, January 25, 1974)

Free and Occluded Virus, similar to *Baculovirus*, in Hepatopancreas of Pink Shrimp

VIRUSES or virus-like particles have been reported infrequently from cells of estuarine and marine organisms[1-4]. Herpes-type viruses were described from an estuarine fungus[3] and oysters[4]. Helical or rod-shaped viruses have not been reported from most aquatic invertebrates, although rod-shaped virus-like particles were reported from a microannelid[5] and aquatic beetle[6].

Recently, I observed rod-shaped virus particles and related inclusion bodies in cells of pink shrimp (*Penaeus duorarum*) that were experimentally exposed to the polychlorinated biphenyl (PCB), Aroclor 1254. This report describes these particles and demonstrates their similarity to certain non-inclusion viruses and to nuclear polyhedrosis viruses of the *Baculovirus* group previously described only from insects and mites[6-10].

Adult pink shrimp, from the Gulf of Mexico near Cedar Key, Florida, were kept in flowing seawater and exposed to 3–5 $\mu g\ l^{-1}$ PCB for 30–52 d as described elsewhere[11]. Control shrimp from the same stock were maintained in PCB-free flowing seawater. Non-experimental pink shrimp which were collected periodically from near Cedar Key and Pensacola, Florida, and prepared immediately for cytology are considered here as feral or wild shrimp. Hepatopancreatic tissues from forty exposed shrimp, forty control shrimp and fifty feral shrimp were fixed for histology in Davidson's fixative[12], sectioned and stained with Harris haematoxylin and eosin, mercury bromophenol blue or the PAS method.

Hepatopancreatic tissues from five exposed, five control and ten feral shrimp were fixed for electron microscopy (2.5% glutaraldehyde and 1% osmium) and embedded in Epon 812. Sections 50–100 nm thick were cut, collected on unsupported 300 mesh copper grids and stained with uranyl acetate and Reynold's lead citrate according to the short method of Hayat[13]. Approximately 3,000 hepatopancreatic cell profiles from feral and control shrimp and 1,500 hepatopancreatic cell profiles from exposed shrimp were examined with the electron microscope.

Fig. 1 Light micrograph of cross section of hepatopancreatic tubule from pink shrimp exposed to PCB; note normal nucleus of epithelial cell (one arrow), and crystalloid, tetrahedral inclusion bodies in hypertrophied nucleus of affected cells (two small arrows).

Light microscopy of fresh squashes and fixed, stained hepatopancreatic tissues of control shrimp revealed normal histological and cytological structure. Thirty per cent of shrimp exposed to PCB, and approximately 20% of feral shrimp from near Cedar Key, however, had crystalloid inclusion bodies in distorted, hypertrophied nuclei of hepatopancreatic epithelial cells (Fig. 1). These nuclear inclusion bodies were usually tetrahedral (pyramidal) in form in fresh squashes and triangular in sections. They ranged in size from 0.5 μm to 12 μm from base to point, and stained light blue with mercury bromophenol blue, and were PAS-negative.

Electron microscopy demonstrated normal ultrastructure of hepatopancreatic tubule cells and nuclei in feral and control shrimp. However, approximately 8% of the nuclear profiles of hepatopancreatic tubule cells from three PCB-exposed shrimp were hypertrophied, and contained rod-shaped, free (non-occluded) and occluded virions (Figs 2–4). Light microscopy showed that these exposed shrimp had numerous tetrahedral, crystalloid inclusion bodies in nuclei of hepatopancreatic cells.

A typical free virion within a cell nucleus consisted of a rod-shaped, electron-dense nucleocapsid surrounded by an electron-lucid area enclosed by one or two envelopes (Figs 2*a*, inset, 2*b* and 5). Nucleocapsids varied in length

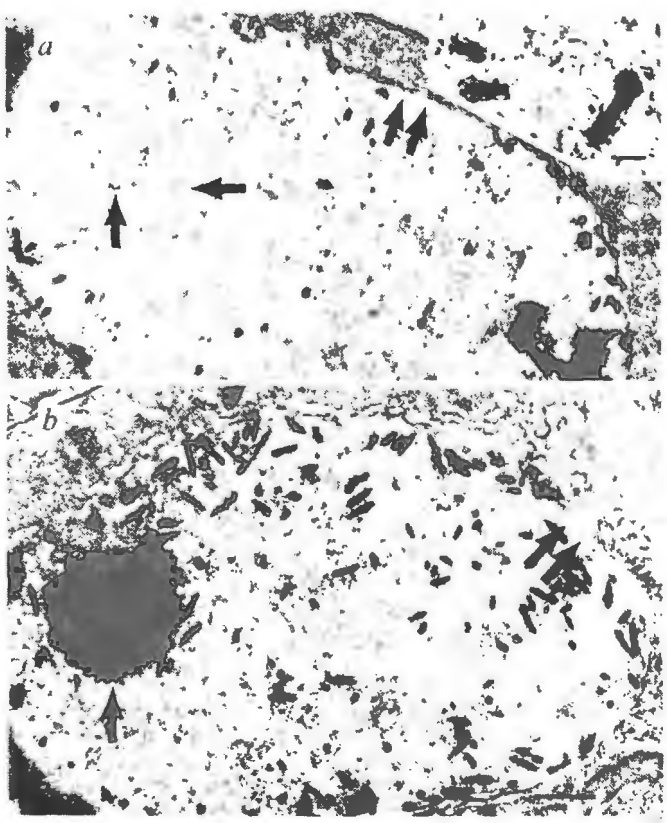


Fig. 2 *a*, Hypertrophied nucleus in epithelial, absorptive cell from hepatopancreas of shrimp exposed to PCB; non-occluded rod-shaped virions in longitudinal and cross section; single arrows point to intranuclear membranes associated with early virion formation, double arrows indicate abnormal multilaminate nuclear envelope. Inset: high magnification of rod-shaped virion in nucleus (bar = 0.1 μm); note single envelope surrounding nucleocapsid. *b*, Moderately heavily affected nucleus; virions distributed mostly around inner periphery of nucleus; arrow points to degenerate nucleolus; double arrows point to envelope membranes entering the cytoplasm. Note many free ribosomes in cytoplasm (bar = 1 μm).

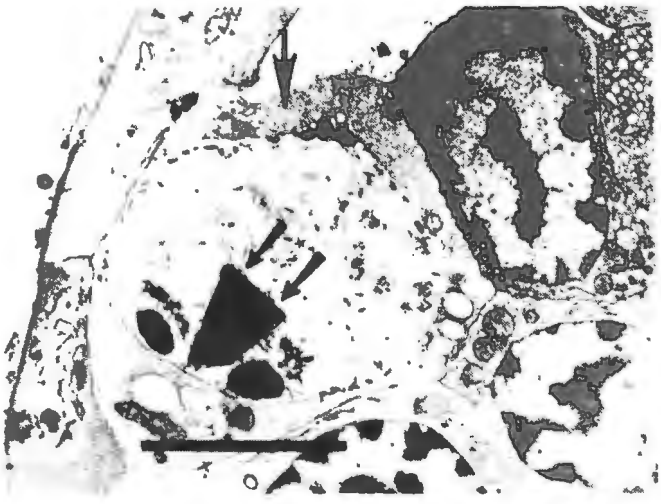


Fig. 3 Low magnification electron micrograph of section through hepatopancreatic tubule from exposed shrimp Note profile of epithelial cell and nucleus (one arrow) containing free virions and intranuclear, crystalline occlusion body (two arrows); note hypertrophy of affected nucleus (bar = 5 μm).

(from 210 to 316 nm; average 288 ± 31 nm, n=30) but had a relatively narrow range of diameters (from 44 to 64 nm; average, 59 nm + 6 nm, n=30). In cross section, the average diameter of nucleocapsids plus their containing envelopes was 75 nm + 7.5 nm (n=30). The envelopes surrounding the nucleocapsids (Fig. 5) seemed to be membranous with spatial and structural relationship to the nucleocapsid similar to that of envelopes in nuclear polyhedrosis virus (a *Baculovirus*) from *Bombyx mori*[9].

Virions were distributed in most nuclei in greater numbers near the inner nuclear membrane (Fig. 2*a* and *b*); however, they were associated with or occluded in crystalline bodies at the ultrastructural level in some nuclei (Figs 3 and 4). These crystalline, occlusion bodies were always triangular in section, paracrystalline in structure, and were the only structures observed with the electron microscope that could be related to the intranuclear, tetrahedral, crystalloid bodies observed by light microscopy (Fig. 1). The occluded virions were similar in dimensions and structure to the non-occluded virions.

Evidence suggesting early stages of virion formation was found in altered nuclei (20% of cell profiles from exposed shrimp) that contained few or no completely formed virions. U-shaped membranous arrays occurred in these hypertrophied nuclei (Fig. 2*a*). These arrays and associated immature virions were similar to certain stages in early virogenesis of *Rhabdionvirus oryctes* in the rhinoceros beetle[7], and of the virus reported from the whirligig beetle[6].

Ultrastructural cytopathic alterations were associated with the presence of virions. Nuclei that contained virions in large numbers were often 1.5–2 times greater (hypertrophied) in profile area than normal nuclei (Fig. 3). Their cytoplasms, however, usually exhibited no parallel difference in profile area. Heavily affected nuclei had a distinct loss of heterochromatin (Fig. 2*a* and *b*), and some affected nuclei had no heterochromatin. Nucleolar degeneration was evident in nuclei that contained many virions (Fig. 2*b*). The most remarkable change associated with the presence of large numbers of virions was a proliferation of nuclear membranes (Fig. 2*a* and *b*), and their eruption into the cytoplasm to form a multimembranous labyrinth (Fig. 2*b*). Cells whose nuclei contained many virions possessed cytoplasms filled with ribosomes, but without normal numbers of mitochondria or endoplasmic reticulum vesicles (Fig. 2*a* and *b*). Most of these changes have been observed in cells of insects that were infected by *Baculovirus*. At present it is not known if exposure of infected shrimp to PCB enhances the cytopathic effects of the virus.

The following findings suggest a close relationship of the shrimp virus to the *Baculovirus* group[10] of invertebrate viruses. The virions were found in nuclei of hepatopancreatic cells of an arthropod (these cells are analogous in many aspects to fat body and midgut cells of insects). They are rod shaped (bacilliform), enclosed by one to two envelopes or capsids, found both free in nuclei and occluded in crystalline bodies within nuclei[10], and were 288 nm long by 75 nm in diameter, thus falling within the size range of *Baculovirus*[9,10]. Stromata which seemed to be virogenic were present in affected nuclei, and cytopathic alterations of affected cells and nuclei were similar to those reported for *Baculovirus* infections in insects.

In summary, a rod-shaped, free and occluded virus exists in a marine shrimp, indicating that marine crustacea are potential hosts for viruses similar to certain viruses infecting insects and mites. So far, the virus has been found only in shrimp taken from near Cedar Key and experimentally

Fig. 4 Higher magnification (EM) of occlusion body from Fig. 3. Note virions occluded randomly within body. This body has a two-dimensional triangular form of crystalloid inclusion body observed with light microscopy in nuclei of hepatopancreatic cells; compare with Fig. 1 (bar = 1 μm).

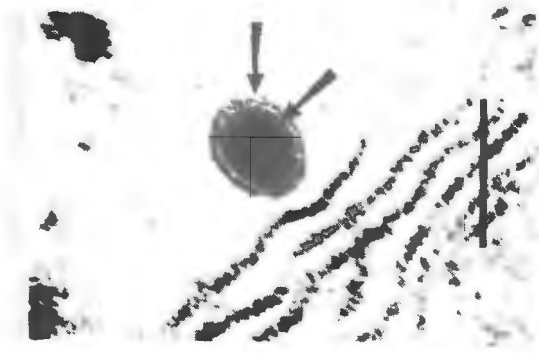


Fig. 5 Cross section of free virion in nucleus near abnormal, multimembranous (six membranes) nuclear envelope; note outer envelope and capsid of the nucleocapsid of virion (arrows) (bar = 0.1 μm).

exposed to the toxic chemical, Aroclor 1254 (PCB). The virus probably is a natural parasite, however, previously undetected, of estuarine and marine shrimp.

Studies of possible interactions of the PCB and virus in pink shrimp may provide valuable information needed to clarify the relationship between natural infectious diseases and pollutant chemicals in the aquatic environment.

I thank Dr D. R. Nimmo for collecting and exposing the shrimp, Dr Jean Adams for advice, Drs John Briggs, C. Vago and Max Summers for examining electron micrographs and for suggestions. Mrs Leslie Cupp and Mr Steve Foss gave technical assistance.

JOHN A. COUCH

US Environmental Protection Agency,
Gulf Breeze Environmental Research Laboratory,
Sabine Island, Gulf Breeze, Florida 32561

Received September 24; revised November 5, 1973.

[1] Vago, C., *Nature*, **209**, 1290 (1966).
[2] Runnger, D., Rustelli, M., Braendle, E., and Malsberger, R. C., *J. invert. Path.*, **17**, 72 (1971).
[3] Kazama, F. Y., and Schornstein, K. L., *Science, N.Y.*, **177**, 696 (1972).
[4] Farley, C. A., Banfield, W. G., Kasnie, jun., G., and Foster, W. S., *Science, N.Y.*, **178**, 759 (1972).
[5] Dougherty, C., Ferral, J., Brody, B., and Gotthold, M. L., *Nature*, **198**, 973 (1963).
[6] Gouranton, J., *J. ultrastruct. Res.*, **39**, 281 (1972).
[7] Huger, A. M., *J. invert. Path.*, **8**, 38 (1966).
[8] Reed, D. K., and Hall, I. M., *J. invert. Path.*, **20**, 272 (1972).
[9] Berghold, G. H., *Insect Pathology: An Advanced Treatise*, **1**, 413 (Academic Press, New York, 1963).
[10] Wildy, P., *Monog. Virol.*, **5**, 32 (1971).
[11] Nimmo, D. R., Blackman, R. R., Wilson, jun., A. J., and Forester, J., *Mar. Biol.*, **11**, 191 (1971).
[12] Shaw, B. L., and Battle, H. I., *Can. J. Zool.*, **35**, 225 (1957).
[13] Hayat, M. A., and Giaquinta, R., *Tissue and Cell*, **2**, 191 (1970).

CONTRIBUTION NO. 215

Reprinted from JOURNAL OF INVERTEBRATE PATHOLOGY, Vol. 24, No. 3, November 1974

An Enzootic Nuclear Polyhedrosis Virus of Pink Shrimp: Ultrastructure, Prevalence, and Enhancement[1]

JOHN A. COUCH

U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561 (Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon)

Received March 28, 1974

A nuclear polyhedrosis virus exists in pink shrimp, *Penaeus duorarum*, from waters of the northern Gulf of Mexico. This virus is rod-shaped, 269 nm long, and possesses an outer envelope surrounding its nucleocapsid. The nucleocapsid is 50 nm in diameter. The virus occurs in nuclei of host hepatopancreatic and midgut cells, and is both free in the nucleus and occluded within pyramidal-shaped polyhedral inclusion bodies (PIB's). Histochemically and ultrastructurally, the shrimp PIB's appear to be ribonucleoprotein and in fine structure bear close resemblance to polyhedral inclusion bodies of *Baculovirus* species from insects. However, the lattice line-to-line spacing is greater than that usually reported for insect PIB's. Crowding and chemical stress of shrimp in aquaria may enhance and increase the virus infection and prevalence. In limited experiments, shrimp fed heavily infected hepatopancreatic tissues had much higher mortality than controls fed only fish. The virus appears to be enzootic in pink shrimp in nature. Cytopathological changes in infected cells of shrimp appear similar to those in insects infected with certain species of *Baculovirus*. The name *Baculovirus penaei* n.sp. is proposed for the shrimp virus.

INTRODUCTION

In the last decade, several reports of virus diseases, and of viruslike particles in estuarine and marine organisms have been published (Vago, 1966; Runger et al., 1971; Bang, 1971; Bonami and Vago, 1971; Bonami et al., 1971, 1972; Devauchelle and Vago, 1971; Kazama and Schornstein, 1972; Farley et al., 1972; Couch, 1974).

The report by Couch (1974) was concerned with a rod-shaped virus in pink shrimp, *Penaeus duorarum*. This virus is associated with polyhedral (tetrahedral) inclusion bodies visible with the light microscope in nuclei of host hepatopancreatic cells. The virions are free and occluded within the polyhedral bodies which possess a crystalline lattice fine structure similar to that of nuclear polyhedral inclusion bodies associated with *Baculovirus* (subgroup A) in insects. Therefore, the designation "nuclear polyhedrosis virus of pink shrimp" appears appropriate. This was the first virus recognized in a host of the crustacean suborder Natantia, and the first nuclear polyhedrosis virus reported in animals other than insects or mites (Wildy, 1971; Couch, 1974). Other viruses reported to date from crustacea have been icosahedral viruses, all from portunid crabs as described by Vago (1966), Bonami et al. (1971), and Bang (1971).

The purpose of the present paper is to confirm and enlarge upon original observations on the nuclear polyhedrosis virus in pink shrimp and to present new evidence for the enzootic nature, laboratory transmission, and enhancement of the virus.

MATERIALS AND METHOD

Source of virus. Polyhedral inclusion bodies were first observed in hepato-

[1] Contribution No. 215, Gulf Breeze Environmental Research Laboratory.

pancreatic epithelial cell nuclei of shrimp that had been taken originally from Apalachee Bay near Cedar Key, Florida (actually landed at Keaton Beach, Florida). Prior to examination, the shrimp had been exposed experimentally to 3–5 μg/liter of the polychlorinated biphenyl (PCB), Aroclor 1254, by Nimmo (personal communication). These shrimp, examined by Couch (1974) and Couch and Nimmo (1973), possessed pyramidal or tetrahedral (fresh squash), and triangular (sections) inclusion bodies in hypertrophied nuclei. Later, electron microscopy (Couch, 1974) revealed the presence of rod-shaped virions, both free and occluded in and associated with the inclusion bodies.[2] These inclusion bodies will be called polyhedral inclusion bodies (PIB's) in comparison with, and in the sense of similar, well-studied inclusion bodies associated with *Baculovirus* (subgroup A) in insects (Wildy, 1971).

Subsequent to the initial findings, over 400 pink shrimp have been examined from the Gulf of Mexico, taken from near the following localities: Keaton Beach, Apalachee Bay, Port St. Joe, and Pensacola, all in Florida.

Histological Methods

Hepatopancreatic tissues were removed from shrimp, and fresh squashes were prepared immediately for microscopical study. Hepatopancreas containing PIB's was fixed in Davidson's fixative or in neutral, buffered 10% formalin, processed, and embedded in paraffin. Sections (5–7 μm) were stained with Harris hematoxylin and eosin, mercury bromophenol blue (Mazia and Brewer, 1953), periodic acid-Schiff (PAS) (with and without diastase digestion), methyl green-pyonin (Luna, 1968), and the Feulgen method. Normal hepatopancreas, i.e., without PIB's, was processed as above for comparison with infected tissue.

For electron microscopy (EM), hepatopancreas containining heavy concentrations of PIB's was diced in 2.5% glutaraldehyde in plastic Petri plates. Diced tissue was fixed for 30 min in fresh 2.5% glutaraldehyde and postfixed in 1% OsO_4 for 30 min at 0–4°C. The fixed tissues were then processed and finally embedded in Epon 812 according to the method of Hayat (1970). Sections 50–100 nm thick were collected on 300 mesh, unsupported copper grids, and stained with uranyl acetate and lead citrate. Normal, uninfected hepatopancreatic tissue was prepared similarly for electron microscopy. Several thousand hepatopancreatic cell profiles from 30 pink shrimp were examined during this study with a Zeiss EM 9S2 electron microscope.

Prevalence and Relative Concentrations of PIB's

Prevalence of polyhedral inclusion bodies, hence prevalence of patent virus infections,[3] is herein expressed as the proportion of any given sample of pink shrimp that possesses PIB's demonstrable in fresh hepatopancreatic squash preparations or in stained sections of hepatopancreas. To find a single PIB in a squash preparation with light microscopy is to diagnose the presence of the virus in a shrimp. The certainty of this conclusion is based on a 100% association of virions with the PIB's in over 1000 PIB-containing cell profiles examined by EM.

Known volumes of hepatopancreatic tissues containing PIB's were diluted with exact volumes of distilled water (usually 0.25 ml hepatopancreas in 5 ml of distilled

[2] Dr. Max Summers has subsequently confirmed the viral structures in infected shrimp cells through his own EM studies of shrimp tissues supplied by our laboratory. Certain electron micrographs in this paper were prepared by Dr. Summers; he is gratefully acknowledged for this and his generous and helpful comments on fine structure of the virus.

[3] Patent virus infection herein refers to the situation in which virus development has proceeded to the point of polyhedral inclusion body production, thus making infections detectable with the light microscope.

water). This mixture was homogenized with a sonic probe and relative concentrations of PIB's in individual shrimp were determined by hemocytometer counts. These counts provided an index of relative intensities of patent infections in shrimp because the number of PIB's/mm³ of hepatopancreatic tissue is proportionate to the number of host cells/mm³ that have patent virus infections (a single PIB per patently infected nucleus is the dominant histological and EM finding; see Figs. 4, 8, and 11). PIB's can be harvested by homogenizing hepatopancreas and centrifuging the homogenate at 2000–3000*g*. The pellet thus obtained can be resuspended and cleaner pellets may be obtained with further centrifugation.

Enhancement experiments

Pink shrimp samples that were collected periodically from near Keaton Beach, Florida, in the Gulf of Mexico, and from Santa Rosa Sound, near Pensacola, Florida, were examined by the fresh squash method to determine base prevalence[4] of PIB-containing shrimp. These samples usually consisted of 100 shrimp of which 40 were directly examined to determine base prevalence. Fifty to sixty of the surviving shrimp from these samples were placed in 100-gal aquaria in premixed artificial seawater of controlled salinity and temperature. These shrimp were held under crowded conditions for 30–40 days and fed only frozen fish purchased from a local seafood market. To detect any increase over the initial base prevalence found in the original stock of shrimp, samples of shrimp were removed at periodic intervals after 30 days holding, and PIB prevalence in each sample was determined. Daily attention was given to mortality in the shrimp held for 30–40 days. Dead or moribund shrimp, upon detection, were examined for presence and relative intensity of PIB's in hepatopancreas.

Juvenile pink shrimp from a stock that showed no patent infections were kept individually in 1000 ml beakers for 20–40 days and fed only hepatopancreatic tissues that contained large numbers of PIB's (>1000 PIB's/mm³ tissue). Other shrimp from this stock, kept as controls in like manner, were fed only frozen fish. Shrimp that died were immediately examined for the presence of PIB's or hypertrophied nuclei in hepatopancreatic cells.

Chemical Exposure of Shrimp

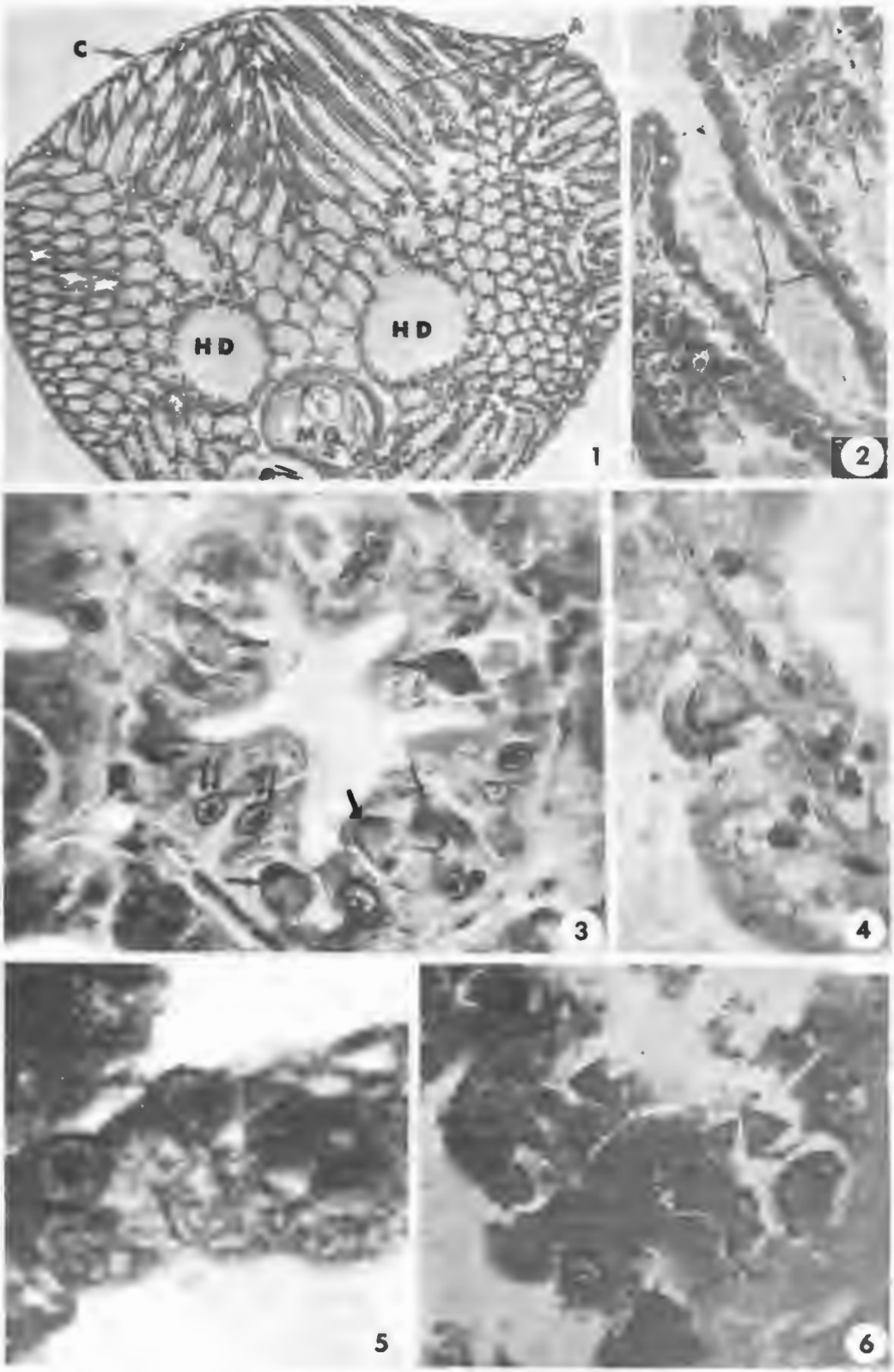
Samples of pink shrimp from populations in Apalachee Bay were exposed to the polychlorinated biphenyl (PCB), Aroclor 1254, in flowing seawater by Nimmo et al. (1971). Samples of pink shrimp from near Pensacola were exposed to the chlorinated hydrocarbon insecticide, mirex, in flowing seawater, laboratory experiments by Tagatz (personal communication). Control shrimp in both of the preceding experiments were kept in toxicant-free flowing seawater. After approximately 30 days, exposed and control shrimp samples were examined histologically for prevalence of PIB's.

Results and Discussion

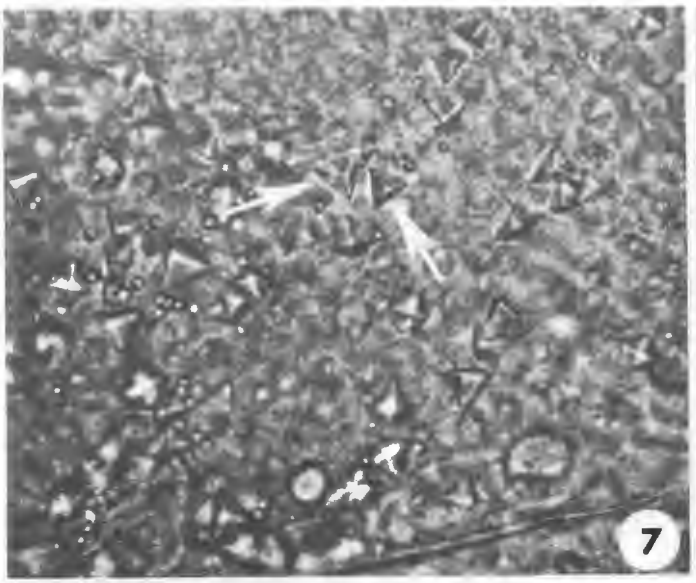
Light Microscopy and Histopathology

The hepatopancreas of pink shrimp (Fig. 1) is histologically similar to the hepatopancreas of other Natantia (Crustacea: Decapoda). Polyhedral inclusion bodies (PIB's) occur in nuclei of epithelial cells of the acini of the hepatopancreas in infected shrimp (Figs. 2, 3). They may be found in cells in proximal, medial, and distal epithelia of acini. Usually, in shrimp with moderate to light patent infections, PIB's occur in foci (Fig. 2). In heavily infected shrimp, homogeneous distribution of PIB's is the rule. PIB's have been found in midgut cell nuclei but not as commonly as in hepatopancreatic cells.

[4] Base prevalence refers to the proportion of shrimp in a sample taken directly from nature and prior to laboratory holding that have patent virus infections.



FIGS. 1–6. Light micrographs of hepatopancreatic tissues, and polyhedral inclusion bodies (PIB's) of virus.



FIGS. 7 and 8. Light micrographs of fresh squash hepatopancreatic preparations showing characteristic pyramidal forms of PIB's.

FIG. 7. Heavy infection showing PIB's; note pyramidal forms (arrows). ×1000.

FIG. 8. Single large PIB in epithelial cell nucleus, *in vivo*. ×3000.

PIB's of the shrimp virus are tetrahedral, or pyramidal in three-dimensional form. They fall into the class of polyhedra that have cubic symmetry. When measured with light microscopy (LM), they range in size from 0.5–20 μm from pyramidal base to peak (Figs. 7, 8), with a modal, vertical length of 8–10 μm.

The polyhedra, when sectioned, always have two-dimensional shapes of triangles (Figs. 3–6). They stain light to dark blue with mercury bromophenol blue, and they stain bright red with methyl green-pyronin, indicating the presence of ribonucleoprotein. They are PAS negative, mostly Feuglen negative, and show variable basophilia when hematoxylin stains are applied, but no acidophilia when eosin is applied.

Neither the virus particles occluded within the PIB's nor the nonoccluded virus particles in hypertrophied nuclei are observable with LM.

The major histopathological effect associated with the PIB's and observable with LM, is the eventual growth of the polyhedral inclusion body to a size that surpasses the infected cell's capacity to retain it. The cell then ruptures, is destroyed, and releases the large PIB into the lumen of the acinus (Figs. 4–6). In histological sections of heavily infected shrimp, it is not unusual to find hundreds of PIB-containing cell

---

FIG. 1. Cross section of hepatopancreas of pink shrimp; A, acinus; C, capsule; HD, hapatopancreatic ducts; MG, midgut. ×40.

FIG. 2. Epithelial layers of acini in hepatopancreas containing PIB's; arrows point to foci of PIB's; E, epithelium ×80.

FIG. 3. Cross section of acinus showing PIB's in nuclei of infected epithelial cells (single arrows); normal nuclei (double arrows). ×800.

FIG. 4. Large, single PIB in nucleus of hepatopancreatic epithelial cell (arrow). ×800.

FIG. 5. Four triangular PIB's in epithelial cell nucleus (arrow); note normal nuclei. ×1600.

FIG. 6. Histopathological effect of PIB's and virus on hepatopancreatic tissues; note rupture and liberation of large PIB's from cells. ×800.

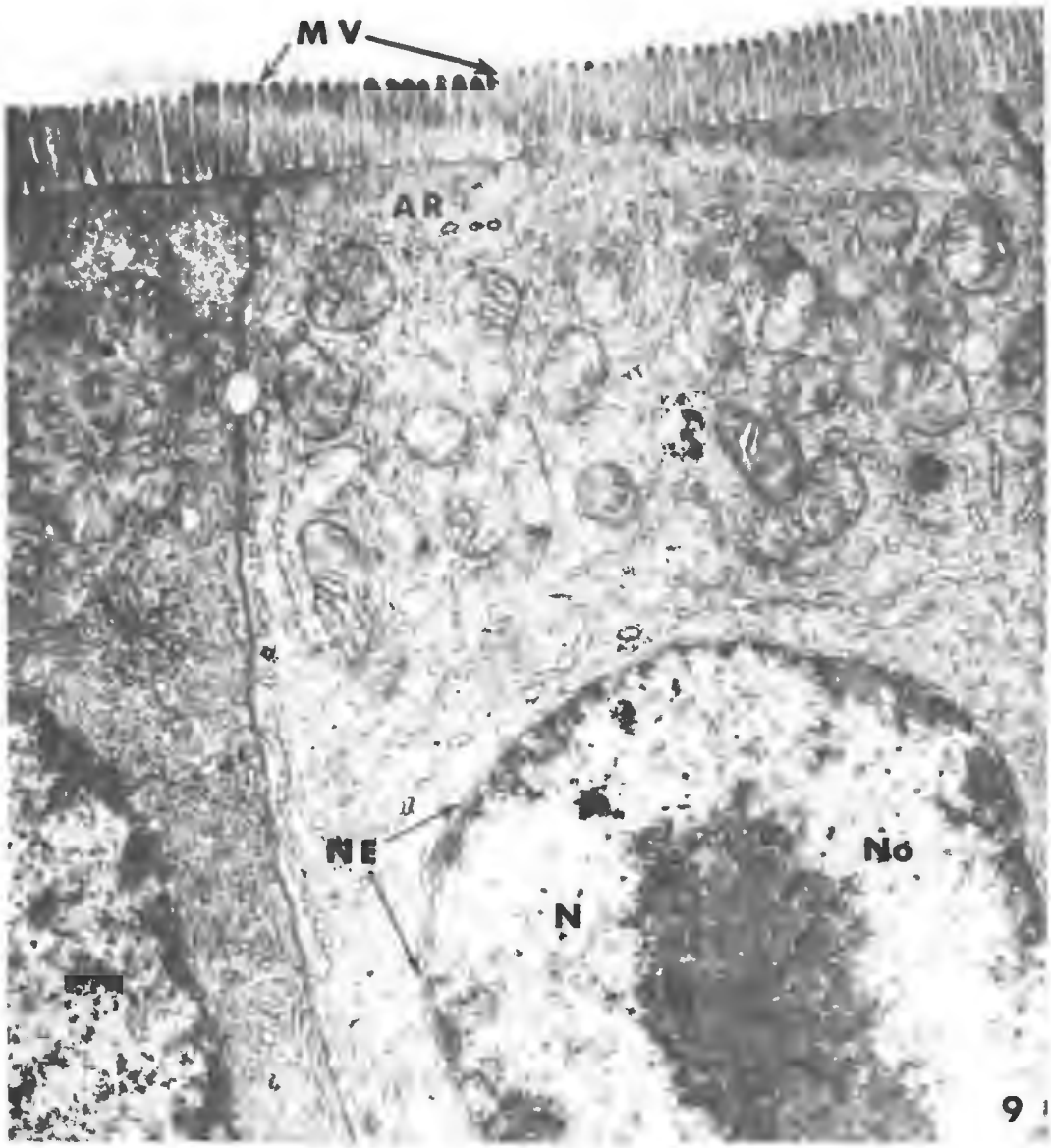


FIG. 9. Portion of normal epithelial cell of pink shrimp; note salient features of microvilli (MV), absorptive region (AR), nucleus (N), Nucleolus (No), and nuclear envelope (NE). ×14,400.

profiles (patent cell infections) ready to rupture, or disintegrating cells with polyhedra being extruded from them (Figs. 6, 7).

Infected shrimp show no gross lesions that would indicate infection. However, lethargic and moribund as well as dead pink shrimp from nature and from laboratory experiments are often infected and contain many PIB's in hepatopancreatic tissues. To date, PIB's have not been found in any tissues other than hepatopancreas. and midgut, although gill, gonad, and muscle have been examined.

Electron Microscopy and Cytopathology

The ultrastructure of virions, PIB's and infected cells was determined following EM

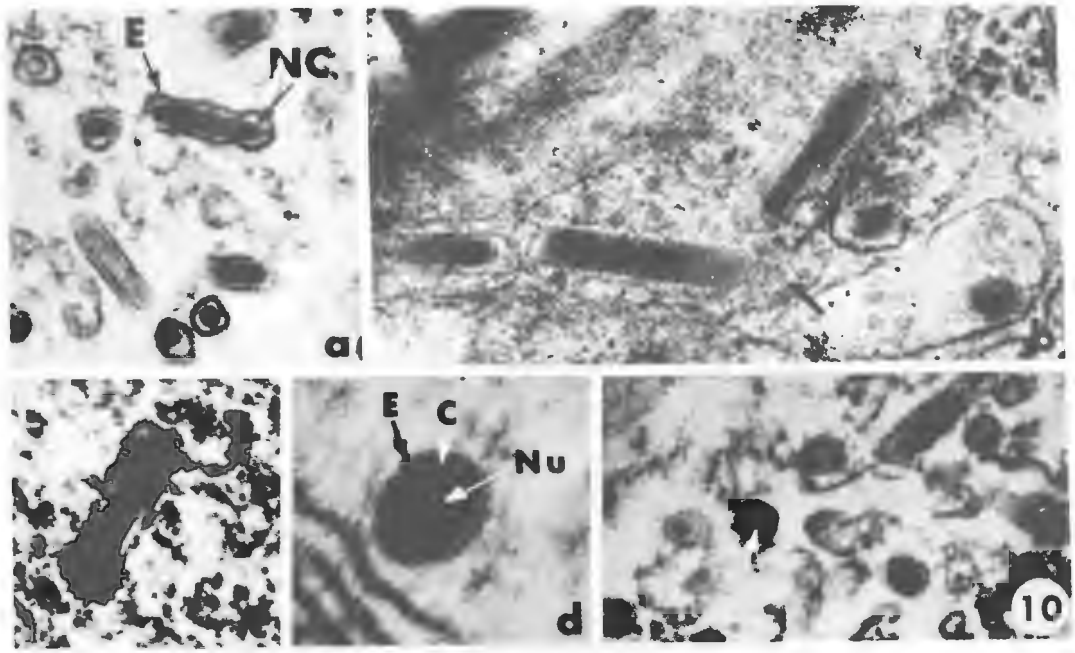


FIG. 10a–e. Pertinent characteristi s of virus particles in shrimp cells. a. Virions in longitudinal and cross sections; E, envelope; NC, nucleocapsid. ×66 500. b. Longitudinal sections of virions; note terminal protrusion of central virion (arrow). ×79,800. c. Single virion in nucleus; note envelope detail. ×79,800. d. Cross section of virus rod; note outer envelope (E), capsid (C), and nucleoid (Nu). ×185,000. e. Cross section of virus rod showing dense central core of nucleocapsid (arrow). ×66,500.

studies of normal uninfected hepatopancreatic cell profiles. The normal hepatopancreatic absorptive cell (Fig. 9), one of several major cell types in hepatopancreatic epithelial tissue, possesses organelles and fine structures remarkably similar to those of absorptive cells of higher animals. Absorptive cells appear to be the cells most commonly infected in pink shrimp, although embryonic and secretory cell profiles frequently have been found to contain virions and PIB's.

The mature shrimp virion consists of a bacilliform, enveloped nucleocapsid (Figs. 10a–e, 14). Often, a dense central core is visible within the nucleocapsid of developing or incomplete virus particles (Figs. 10e, 15, 22). Certain profiles reveal a protruding structure at one extremity of some virions, giving them a "bullet" form (Fig. 10b). The functional significance of the protrusion is not known, but it may only reflect an artifact of the envelope or it may serve as an attachment organelle in mature virions during infectious processes.

The sizes of virions and their components

TABLE 1

AVERAGE SIZE, STANDARD DEVIATIONS, AND SIZE RANGES OF VIRIONS AND VIRION COMPONENTS[a]

Component	Length (nm)	Diameter (nm)	Thickness (nm)
1. Nucleocapsid	269.6 ± 20.7 (228.1–320.2)	50.3 ± 4.8 (40.0–60.7)	—
2. Capsid	—	—	4.0 (3.7–4.2)
3. Nucleocapid and envelope	—	74.5 ± 8.3 (60.3–97.6)	—
4. Envelope	—	—	8.5 (5.3–10.7)

[a] n =50 for 1 and 3; n = 2 for 2; n = 17 for 4.

FIG. 11. Polyhedral inclusion body and virions in advanced infection. Note occluded virions (arrows) and numerous small, round subunits in nucleoplasm (arrows). ×28,500.

are given in Table 1. Nonoccluded and occluded virions have similar dimensions and fine structure.

The fine structure of the shrimp virus, particularly the spatial relationship of the nucleocapsid and outer envelope (Fig. 10d) is similar to that of the nuclear polyhedrosis virus (*Baculovirus*) from *Bombyx mori* described by Berghold (1963). Berghold referred to the envelope of the *B. mori* virus as the "developmental membrane," and to the capsid as the "intimate membrane." In this work, I have selected the presently more acceptable terms "envelope" and "capsid" for these structures, to be used as applied by Summers (1971) to similar structures in granulosis virus.

Light and electron micrographs of thin sections of polyhedral inclusion bodies in tissue and those isolated by centrifugation reveal characteristic triangular or semitriangular forms (Figs. 11, 12, 13). Occasionally, multipointed structures are found (Fig. 12), which appear to be the result of fusion of several triangular PIB's. Virions are fully occluded randomly in the PIB's,

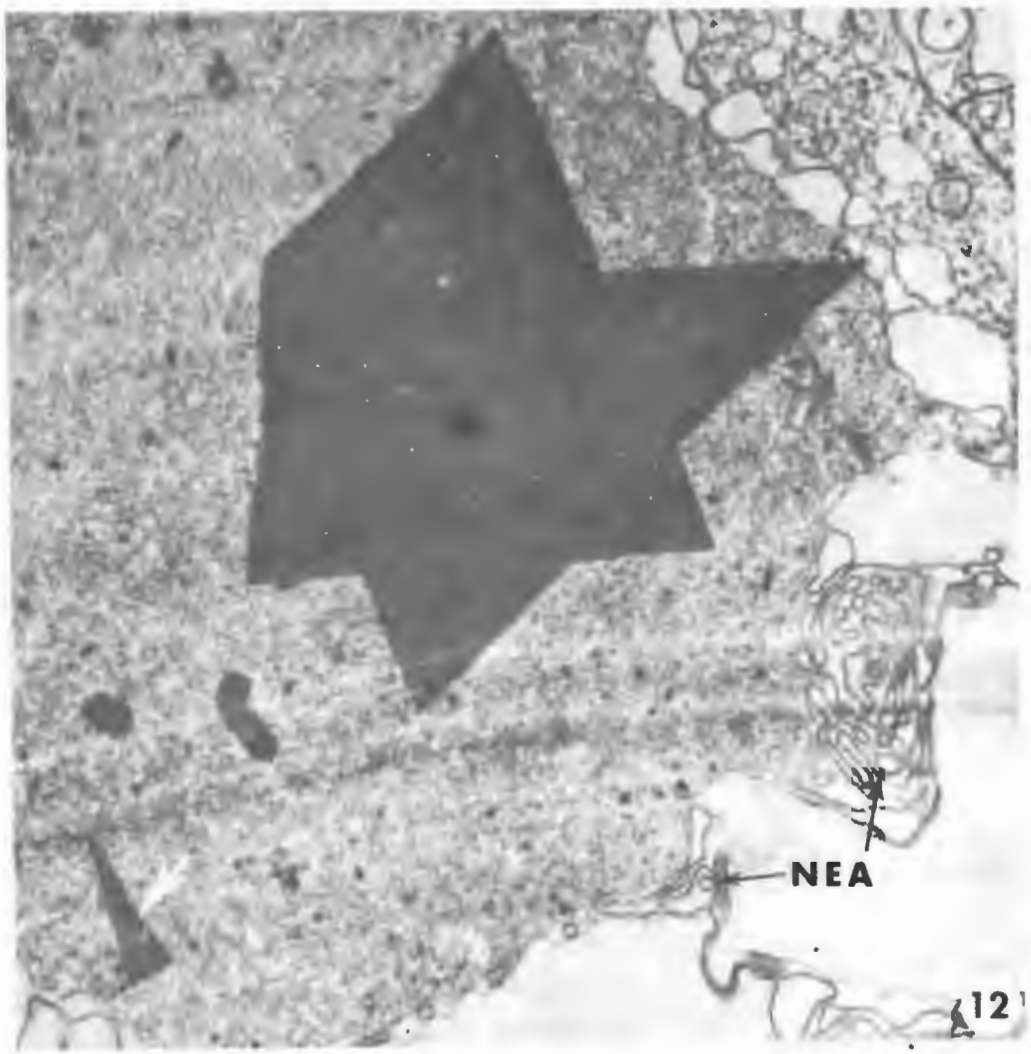


FIG. 12. Unusual coalescence of PIB's in nucleus. Also, note edge of PIB in triangular form caught in thin section (white arrow). Nuclear envelope alterations are obvious (NEA). ×14,400.

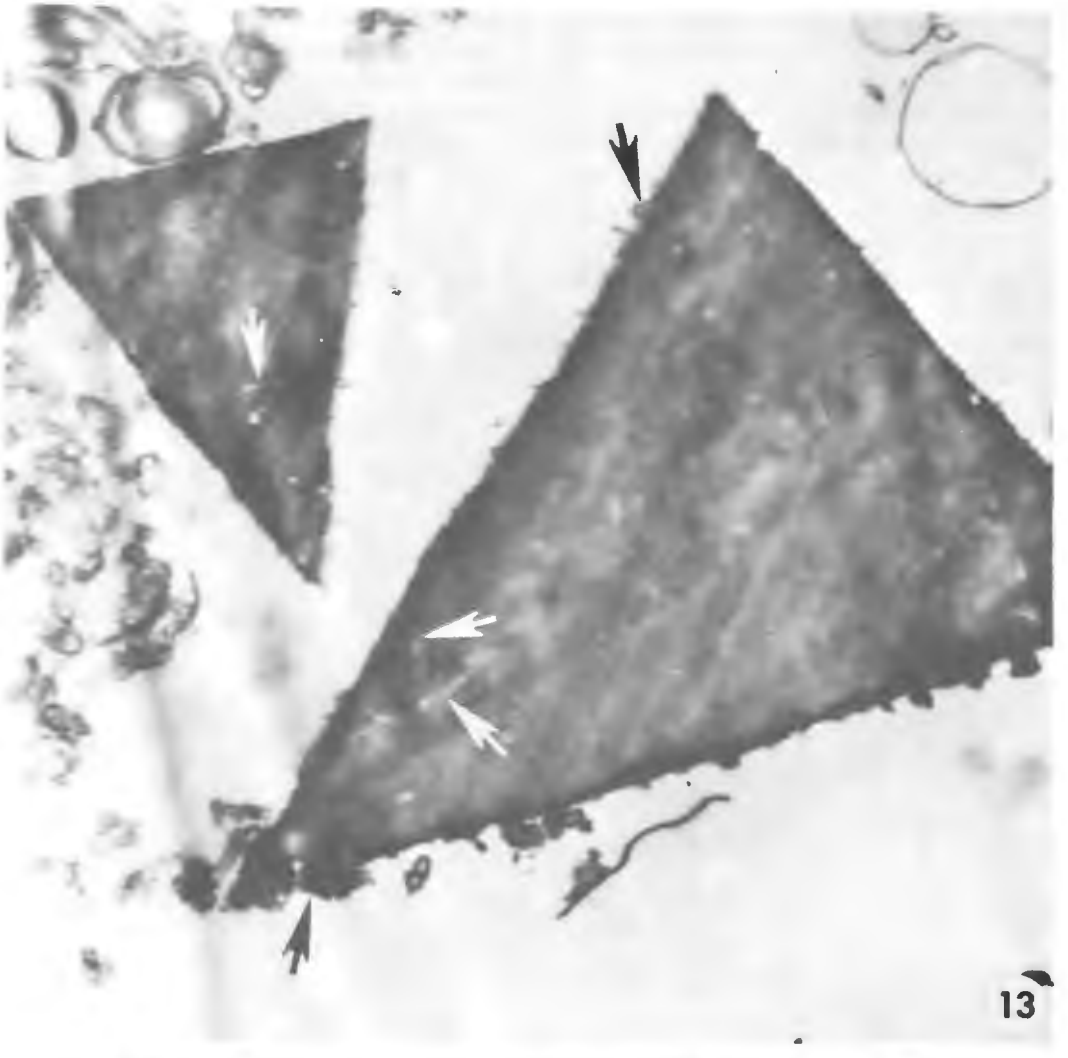


FIG. 13. Isolated PIB's; impure preparation; note occluded virions (arrows). ×28,500.

but partially occluded virions may be observed (Fig. 14).

Rarely, more than one PIB (up to five or six) is found per nucleus (Fig. 5). Often, only small corners are cut from a large PIB, producing, in thin sections, small triangular bodies (Fig. 12).

The fine structure of the PIB, as revealed by EM, has some similarity to that of PIB's from *Baculovirus*-infected insects. However, certain features of the shrimp virus PIB have not been reported for insect virus PIB's.

The crystalline structure of the shrimp virus PIB consists of a linear lattice made up of round subunits, each approximately 11–20 nm in diameter (Fig. 15), spaced in rows approximately 5 nm apart (Fig. 15). Sections at right angles to the linear lattice plane show a cubic lattice substructure (Fig. 16). The subunits, which appear round in Fig. 15, are probably ribonucleoprotein (RNP) bodies that are incorporated into peripheral assembly planes of the PIB's from a large population of similar, but larger subunits (12–21 nm) in the nucleoplasm. Incorporation or assembly of these subunits is strongly suggested in Figs.

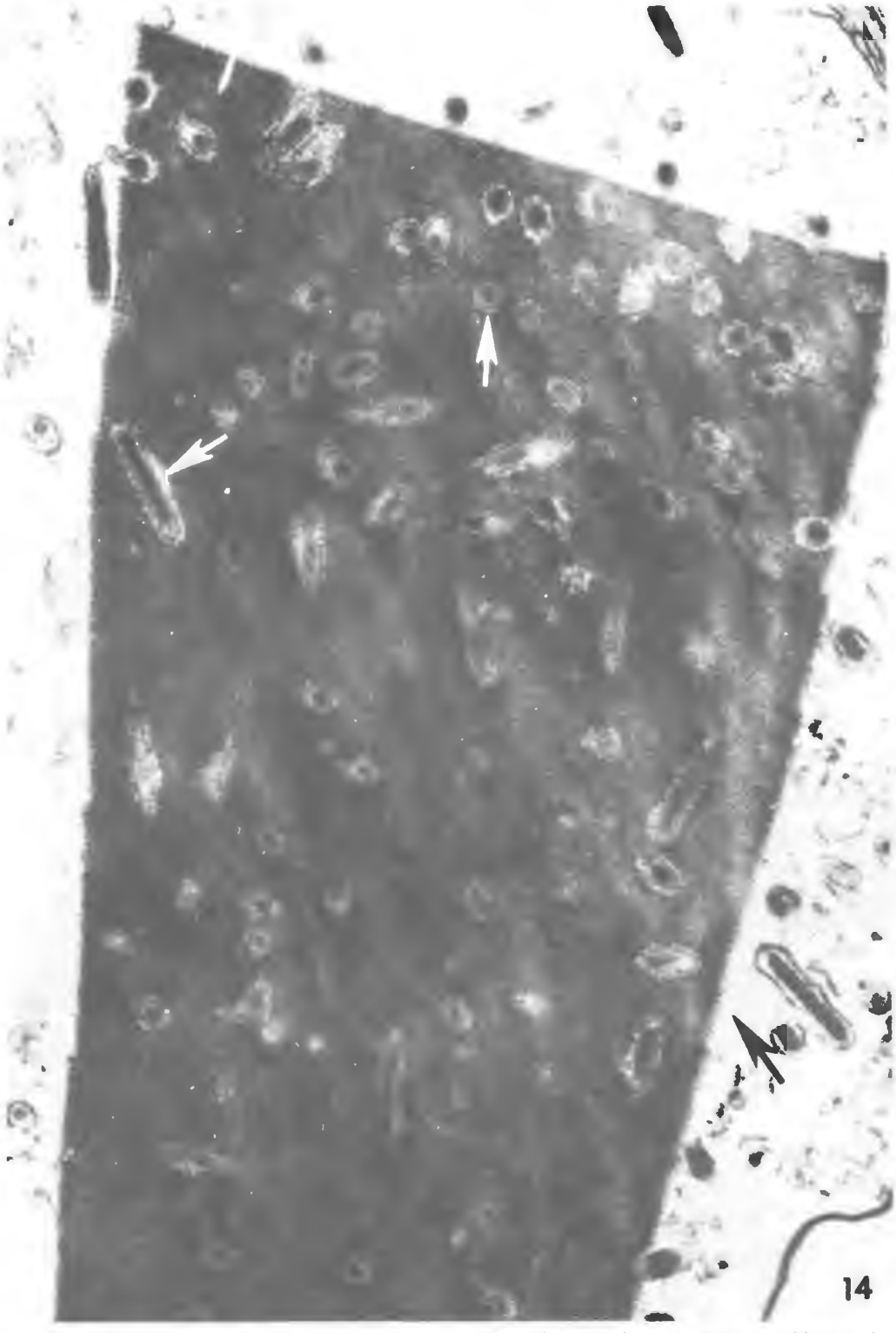


FIG. 14. PIB in section; note numerous occluded virions (white arrows) and evidence of lattice fine structure (black arrow). ×70,000.

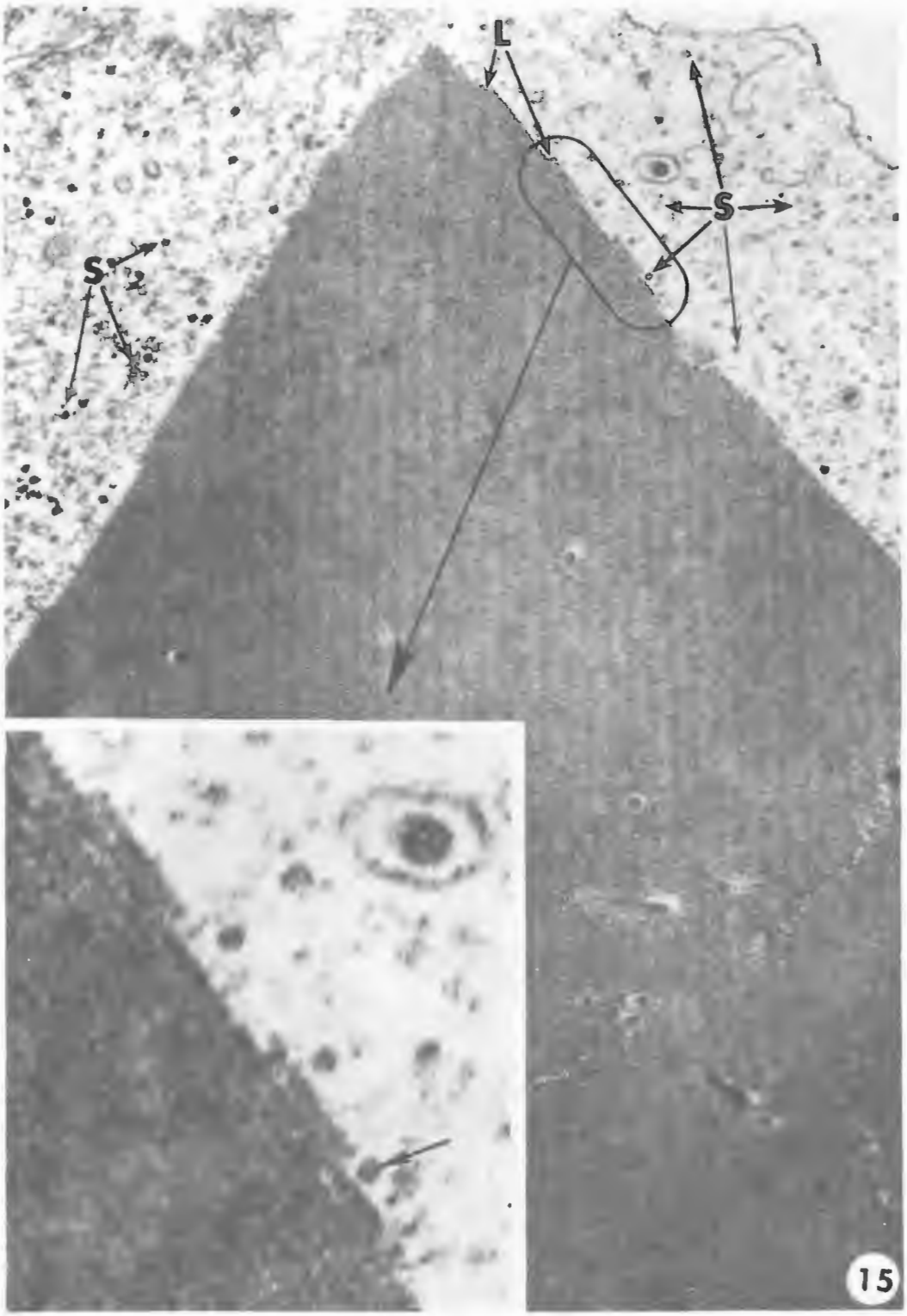
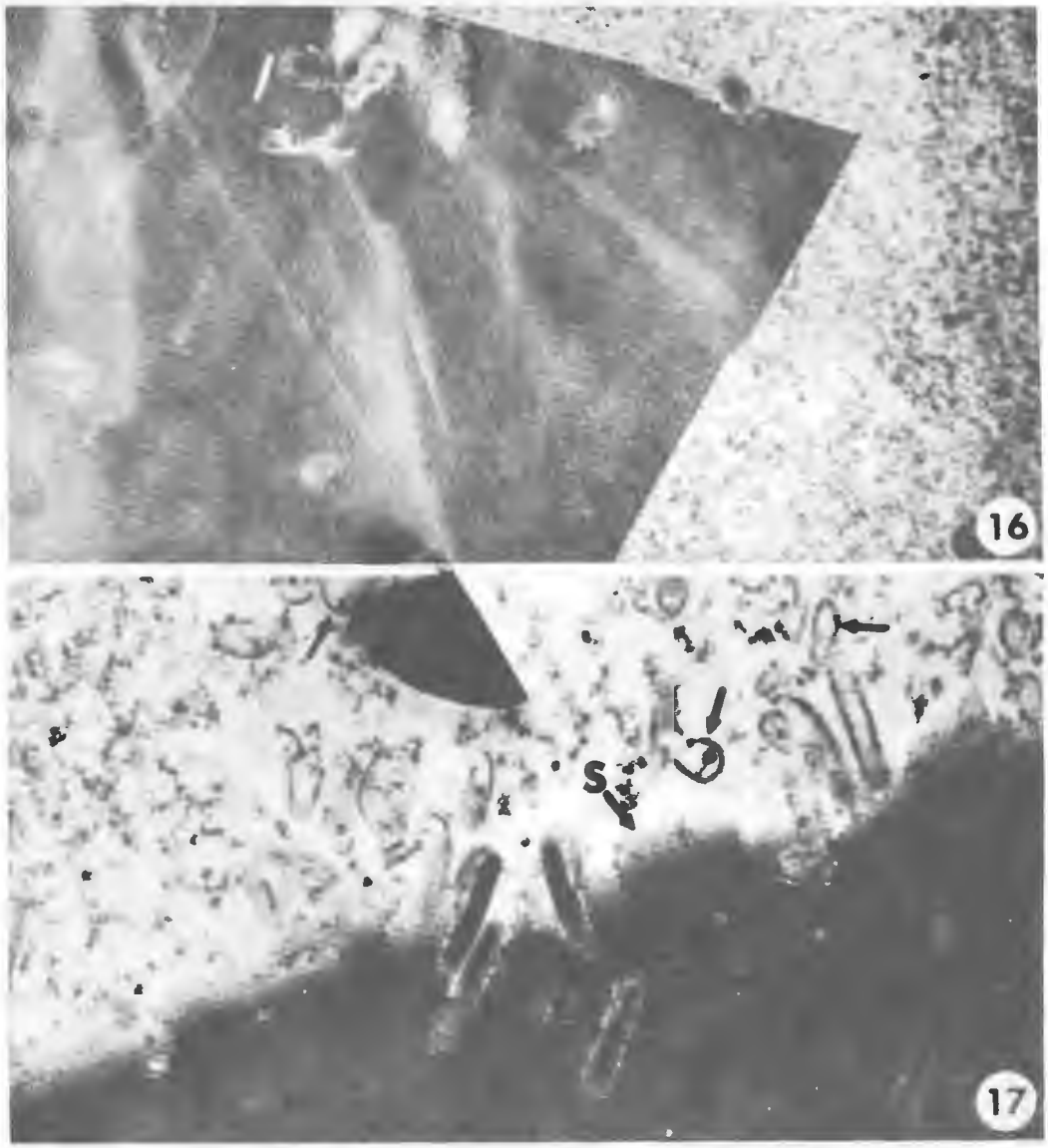


FIG. 15. Section in linear plane of PIB lattice; linear arrangement (L) of round subunits (S) in crystalline lattice cut at right angles to the cubic lattice face (see Fig. 16); note free subunits (S) in nucleoplasm particularly near linear lattice, ×70,000; *Inset:* higher magnification of region near periphery of lattice; note that some subunits may have central core (arrow). ×210,000.

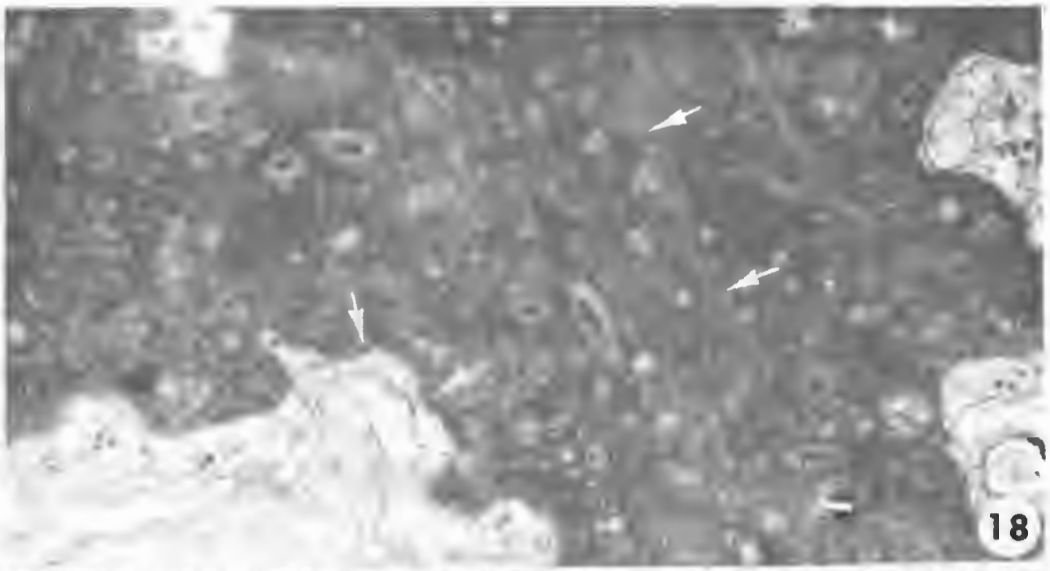


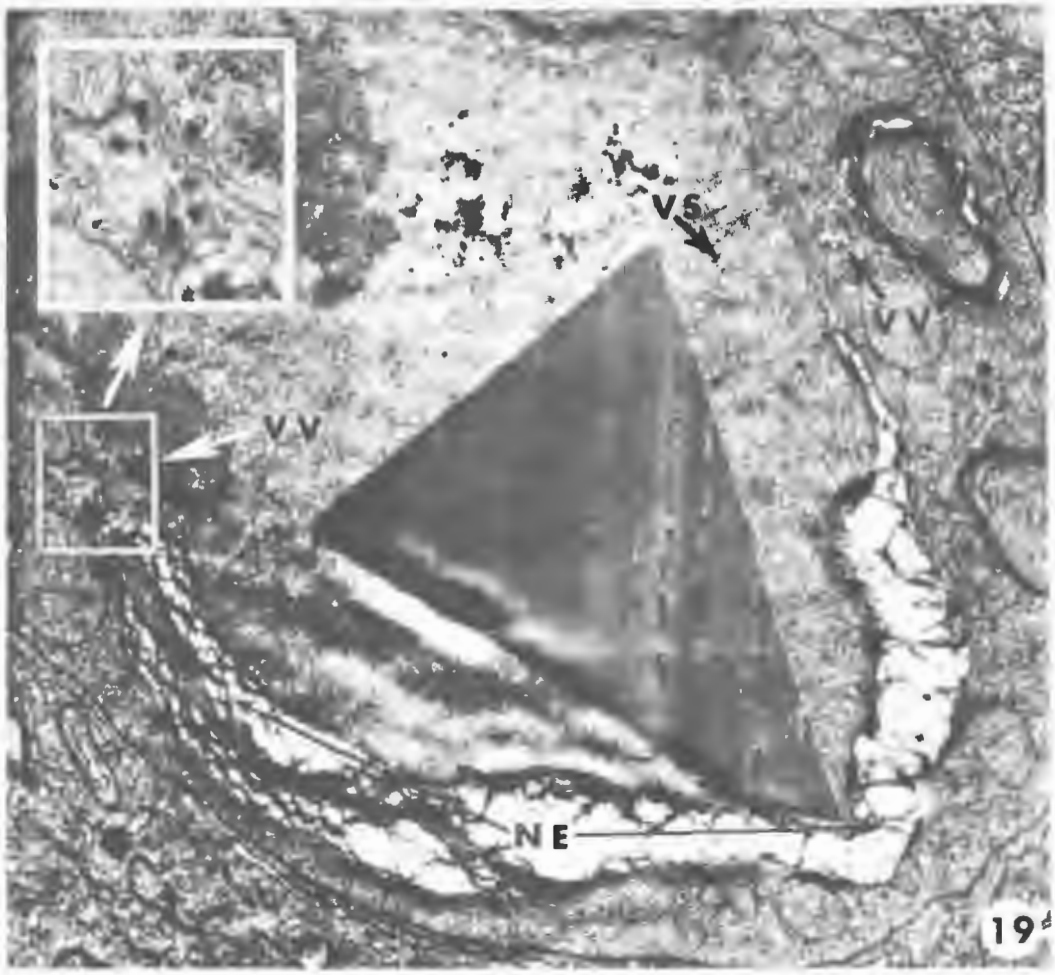
FIGS. 16 and 17. Different aspects of thin sections through PIB's.

FIG. 16. Section in plane of cubic face of lattice at right angle to linear lattice plane (see Fig. 15). ×70,000.

FIG. 17. Section through edge of PIB at an angle that reveals round subunits (S) and partially occluded virions; note U- and C-shaped membrane profiles in nucleoplasm adjacent to PIB (arrows). ×70,000.

15 and 17. Berghold (1963) described similar subunits in polyhedra of *Bombyx mori* nuclear polyhedrosis virus as "spherical protein molecules" which are arranged in a cubic system, but did not mention the presence of RNP in the subunits. The possibility that subunits in shrimp PIB's may be RNP bodies is suggested by their size (11–20 nm) and shape, which are close to those of presumed nucleolar RNP bodies and ribosomes in the cytoplasm of infected shrimp cells. Further evidence supporting the RNP nature of these subunits is the strong affinity of the whole PIB for pyronin





FIGS. 18 and 19

FIG. 18. Amorphous inclusion body that does not possess crystalline lattice substructure; note membrane profiles within body (arrows). ×70 000.

FIG. 19. Single large PIB in advanced infection of nucleus; note abnormal nuclear envelope (NE), virogenic stromata in nucleoplasm (VS), and virions in vesicles in cytoplasm near nucleus (VV). ×14,000. *Inset:* enlargement of virus particles in vesicle near nuclear envelope. ×28,000.

(methyl green-pyronin method), and for mercury bromophenol blue, indicating the presence of RNA and protein. Biochemical characterization, however, is needed to substantiate this possibility.

Rarely, profiles of amorphous dense bodies containing virions and membrane profiles are found in remains of nuclei (Fig. 18). These bodies do not show lattice and subunit substructure similar to those of PIB's. The origins and significance of the dense body are enigmatic, for there are no comparable structures reported in insect virus infections (Summers, personal communication).

Affected shrimp possess cells that are in different stages of the virus infective–reproductive cycle; thus a spectrum of virus-cell ultrastructural relationships can be observed. For convenience, three recognizable levels of cellular infections, characterized by morphological changes, will be described in the following order: (1) advanced or patent infections, (2) intermediate infections, and (3) early and eclipsed infections. Both stages (2) and (3) may be considered by some to be latent, cellular infections.

In advanced, patent infections the presence of a single, relatively large PIB in the nucleus is the usual cytological case (Figs. 11, 19). Advanced infections are further characterized by abnormal, bizarre forms of the nuclear envelopes of the affected cells (Fig. 19). Usually, the nuclear envelope membranes have proliferated, are multilaminate, and become widely separated, creating large cisternae. Further, the PIB may be large enough to completely distort the nuclear and cellular profile. Membrane-lined vesicles containing virions were found occasionally in the cytoplasm adjacent to nuclei with advanced infections (Fig. 19).

Intermediate infections may be characterized by hypertrophied nuclei that, in profile, contain from few to many nonoccluded virions (Fig. 20). Striking cytopathological changes occur within these cells. Nuclei are hypertrophied, usually 1.5–2 times the profile area of normal cell nuclei. Heterochromatin is lacking or reduced in amount and distribution (Figs. 19, 20). Aberrant stromatic patterns are present in the nucleoplasm, and nucleoli are absent or degenerate (Fig. 20). The most remarkable change, however, is the proliferation of nuclear envelope membranes, which begins in intermediate infections and increases to such an extent that, by the time advanced infections are reached, one is unable to recognize a normal nuclear envelope (Figs. 19, 20). The major result of the membrane proliferation is the production of a membranous labyrinth that has its origins in the nuclear envelope, but extends considerably into the cytoplasm (Fig. 20). The function of this labyrinth is unclear, but it resembles the network of reticulum membranes in cells of *Gyrinus natator* infected by a rod-shaped, nonoccluded virus described by Gouranton (1972), and that described by Summers (1971) for granulosis virus-infected cells of *Trichoplusia ni*.

The cytoplasms of cells with intermediate infections become filled with free ribosome and contain less endoplasmic reticulum and fewer mitochondria than normal (Fig. 20).

Early and eclipsed infections are characterized by nuclear hypertrophy, diminution of heterochromatin, and obvious segregation of the nucleoplasm into regions of granular and fibrillar stromata (Fig. 21). No virions are apparent in the eclipsed phase; however, during the early phase, a few virions and early virogenic stages are apparent (Fig. 21). The latter are represented by the U- and C-shaped membrane configurations associated with dense bodies resembling viral cores or nucleoids (Figs. 21, 22). These variable-shaped membranes (Figs. 14, 17, 19, 20, 22) in the nucleoplasm, associated with virions and nucleoids of virions at all stages of infection are strikingly similar to stages of virogenesis of *Rhabdionvirus oryctes* described by Huger (1966), and to virogenic figures reported in midgut cells of the whirligig beetle by Gouranton (1972).

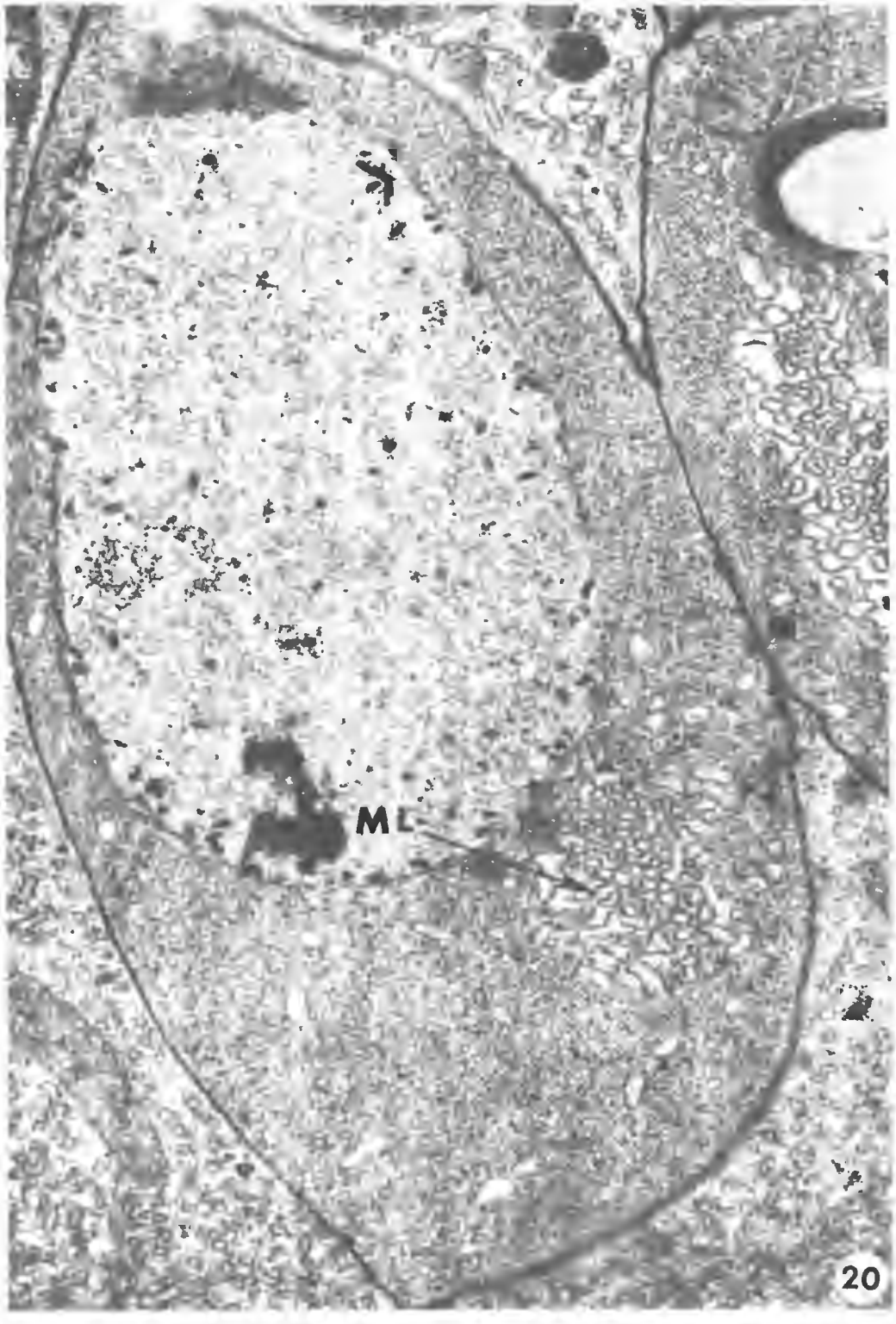
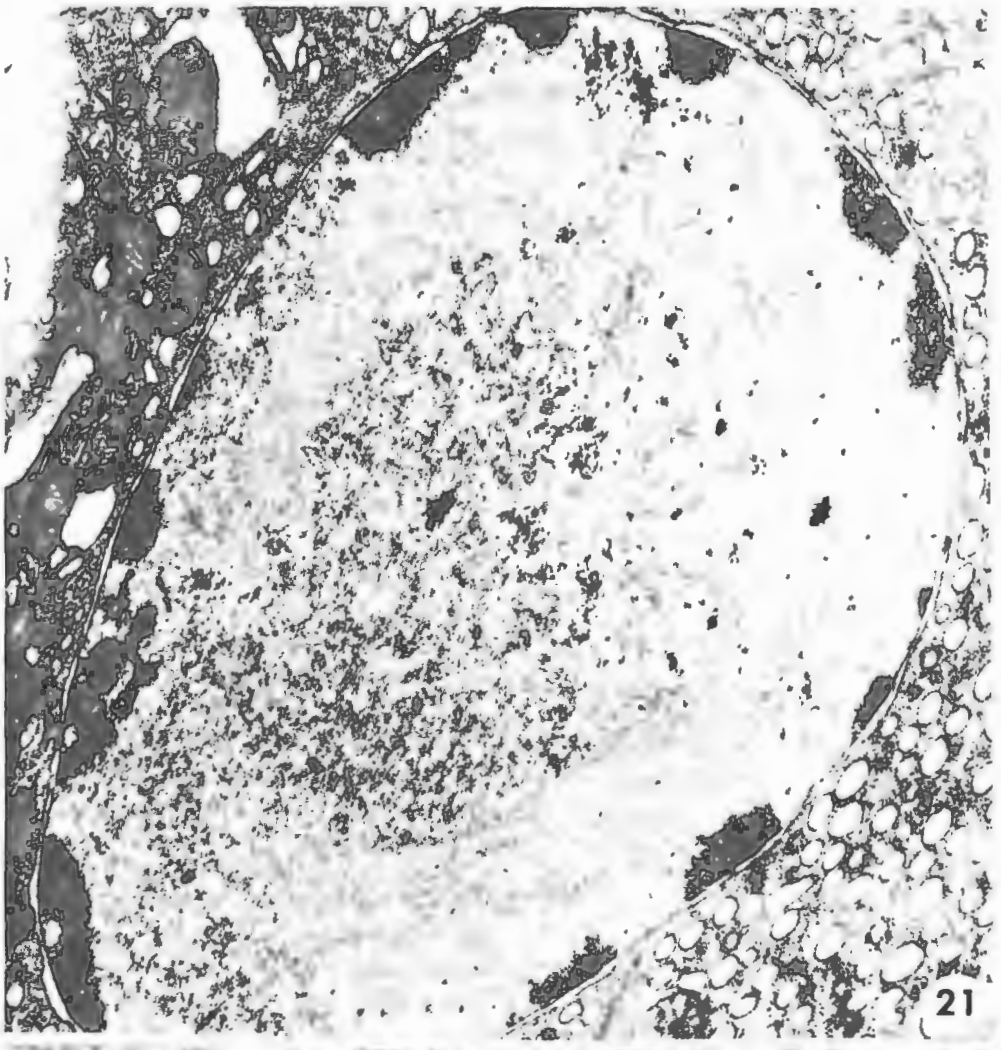
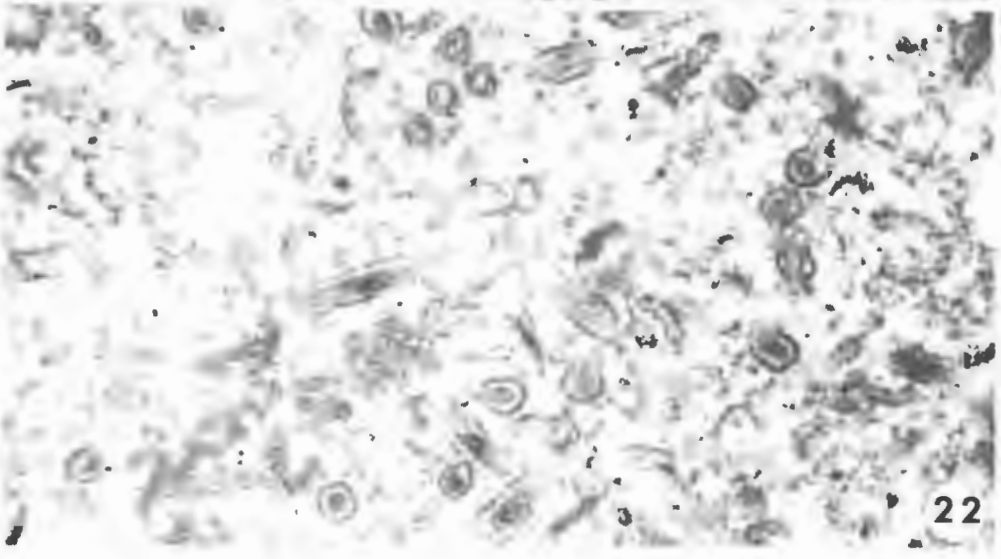


FIG. 20. Profile of hepatopancreatic cell at intermediate level of infection; note hypertrophied nucleus, loss of heterochomatin, membranous labyrinth (ML), and virions in nucleoplasm. Free ribosomes are abundant in cytoplasm. ×14,400.





FIGS. 21 and 22

FIG. 21. Nucleus at early stage of infection; note diminution of chromatin, nuclear hypertrophy and fibrillar and granular stromata in nucleoplasm. ×14,400.

FIG. 22. Numerous membrane profiles and early virogenic stages in nucleoplasms of infected cell. ×70,000.

TABLE 2

PREVALENCE OF PIB'S IN PINK SHRIMP FROM NORTHERN GULF OF MEXICO (1970–1974)

Year	Number examined	Number with PIB's	Month	Source (all in Florida)
1970	40	12	June	Keaton Beach
	1	1	August	Pensacola
1971	42	0	July	Pensacola
	14	7	August	Keaton Beach
	14	0	September	Keaton Beach
	10	0	October	Keaton Beach
1973	20	0	June	Pensacola
	20	0	August	Pensacola
	42	12	October	Keaton Beach
	40	6	November	Pensacola
	28	10	November	Port St. Joe
1974	30	0	January	Pensacola
	53	14	January	Keaton Beach
	20	4	February	Keaton Beach

TABLE 3

DIRECT-OBSERVATION ESTIMATES (FRESH SQUASH) AND ACTUAL HEMOCYTOMETER COUNTS OF PIB'S IN THREE PINK SHRIMP

Shrimp no.	Direct estimate (squash)	Hemocytometer counts[a]
1	Light	50–500 PIB's/mm^3
2	Moderate	550–850 PIB's/mm^3
3	Heavy	1100–1650 PIB's/mm^3

[a] Range of 12 counts from each animal.

Prevalence and Relative Concentrations of PIB's

The prevalence of polyhedral inclusion bodies, hence patent viral infections, in pink shrimp sampled from three points in the northern Gulf of Mexico is presented in Table 2.

To date, a regular seasonal pattern of PIB occurrence in pink shrimp has not been established. However, during 1973, PIB-containing shrimp were collected only during the fall and winter months, October through January. Shrimp samples from Apalachee Bay near Keaton Beach, Florida, had higher PIB prevalence than those taken near Pensacola, Florida.

More extensive sampling, presently underway in the northern Gulf of Mexico, should reveal more accurate and valid patterns of prevalence and distribution of PIB's in pink shrimp. However, the prevalence data presented in Table 2 demonstrates an enzootic occurrence of PIB's in feral shrimp from northeastern Gulf of Mexico waters.

Brown shrimp, *Penaeus aztecus,* white shrimp, *Penaeus setiferus,* and grass shrimp, *Paleomonetes pugio,* examined during the last three years, have not yet been found to possess PIB's or virions similar to those found in the pink shrimp. However, large samples of these species have not been examined thoroughly for PIB's.

Relative concentrations of PIB's in individual shrimp have been determined by hemocytometer counts. Counts for three shrimp that showed considerable difference in PIB concentrations are given in Table 3. These counts are compared to direct estimates of PIB concentrations (made a priori on fresh hepatopancreatic squashes) expressed as light, moderate, or heavy. The a priori direct estimates were based on the approximate numbers of PIB's per microscopical field (×430) in fresh squashes, and the general distribution of the PIB's throughout the squash.

Since each PIB represents, on the average, a single infected cell, it is possible to

estimate with LM the minimum number of cells/mm³ of tissue that are destroyed in the infection up to the time of examination. According to several counts, in heavily infected shrimp, from 1100–1600 cells/mm³ are infected patently, and are therefore destined to be lysed or destroyed by the growth of the PIB and associated cytopathic alterations. A quantitative relationship between cell death and organismic debilitation or death, however, has not been established in pink shrimp.

Enhancement and Transmission

Apparent increase in prevalence of patent virus infections occurs when samples of pink shrimp from natural populations with enzootic levels of virus (Table 2) are kept under crowded conditions in laboratory aquaria. Experiments were conducted in which samples of pink shrimp from Keaton Beach, Florida, showing no patent levels of infection, i.e., absence of PIB's in squash, but with probable latent infections, were kept for 40 days in aquaria. Figure 23 shows the occurrence and increased prevalence of PIB's in samples examined after 30 and 40 days under crowded aquarium conditions.

Shrimp are notoriously cannibalistic and in aquaria show no hesitation to feed on all organs of shrimp carcasses, including hepatopancreas. Therefore, one explanation for the 40–50% increase in PIB prevalence (Fig. 23) could be that transmission of the virus from shrimp to shrimp was facilitated via cannibalism, and individuals were exposed to levels of virus higher than normally encountered under less crowded conditions in nature, where other scavengers, i.e., fish, other invertebrates, quickly consume moribund or dead shrimp. Two other possible modes of enhancement of prevalence levels in aquaria are (1) physical stress of shrimp due to abnormal crowding, hence loss of resistance, and (2) gradual increase of infective PIB's in sediments in aquaria.

Five juvenile pink shrimp from a larger

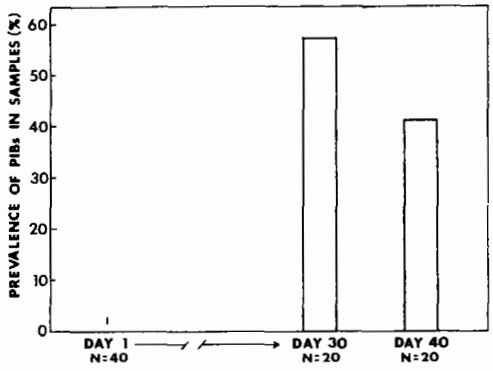


FIG. 23. Prevalence of polyhedral inclusion bodies (PIB's) in sample of pink shrimp held under abnormally crowded conditions in an aquarium for 40 days. The initial sample (N = 40) taken directly from nature showed no PIB's in any shrimp at onset of holding (Day 1).

sample of shrimp showing no patent virus infections were fed heavily infected hepatopancreatic tissues (1000–1600 PIB's/mm³). Four of the five died after 20 days feeding. Control shrimp from the same stock, fed frozen fish, did not die. Examination of the experimental shrimp after 20 days of feeding showed that one of five had a patent virus infection (PIB's present). None of the controls showed patent infections. Each of the experimental shrimp had some hypertrophied nuclei in hepatopancreatic cells, whereas none of the controls had hypertrophied nuclei. It is difficult to determine from this small experiment whether or not the deaths of the experimental shrimp were due to feeding heavily infected tissues, because it is possible that some experimental shrimp had latent virus infections initially. More intensive transmission feeding and inoculation experiments of virus and PIB's are presently underway.

In separate experiments pink shrimp were exposed to the polychlorinated biphenyl (PCB), Aroclor 1254, and to the commercial organchlorine pesticide, mirex. Prior to their exposure to the chemicals, these shrimp were not examined for PIB prevalence. Following exposure for 30 days to Aroclor 1254, 60% of the Aroclor-exposed shrimp had developed patent infections,

TABLE 4

PREVALENCE OF PATENT INFECTIONS (PIB's PRESENT) IN PINK SHRIMP AFTER EXPOSURE TO CHEMICALS FOR 30 DAYS IN FLOWING SEAWATER

	Patent infections	
	Exposed	Controls
Aroclor 1254 (PCB) (3 μg/liter)	12/20[a]	0/20
Mirex (0.01–0.23 μg/liter)	6/15	1/15

[a] Shrimp with PIB's/total number of shrimp exposed.

whereas controls for this exposure had none. (Table 4). Forty percent of the Mirex-exposed shrimp had patent infections, and only 6.6% of the controls from this group had patent infections (Table 4).

Results of these two experiments suggest that stress of shrimp by certain toxic chemicals may facilitate transmission or enhance the expression of latent viral infections. Further chemical exposure experiments, utilizing larger numbers of shrimp from populations with enzootic levels of virus, are underway.

CONCLUSIONS

The virus reported and described herein appears by virtue of its size, structure, site of reproduction, polyhedral body association, and cytopathic effects to be very similar to viruses in the genus *Baculovirus* (Subgroup A), that prior to 1974 were described only from insects and mites (Wildy, 1971). Therefore, the name *Baculovirus penaei* sp. n. is proposed for purposes of identification and classification of the nuclear polyhedrosis virus of pink shrimp, *Penaeus duorarum*. The group name, *Baculovirus*, is used here in the sense indicated by the Invertebrate Virus Subcommittee of the International Committee on Nomenclature of Viruses (ICNV) (Wildy, 1971). Further chemical and biological characterization of the shrimp virus is needed to confirm its relationship to *Baculovirus*. Presently, efforts are underway to identify the nucleic acid content of the shrimp virus. Since the ICNV has approved the rule that the law of priority shall not apply in virus nomenclature, future evidence showing lack of relationship of the shrimp virus to *Baculovirus* may be reason enough to reject or change the name at some later date.

It is not known at the present if *Baculovirus penaei* causes mortalities of shrimp in nature. Small-scale transmission-feeding experiments in the laboratory and maintenance of shrimp under abnormally crowded conditions, and under chemical stress appear to enhance and increase the prevalence of infection in shrimp samples. Dead and dying pink shrimp with heavily infected hepatopancreatic tissues have been found. However, the majority of shrimp found to be infected with PIB's and virus appear grossly to be healthy, and it is possible that under normal conditions the virus is enzootic and not prone to become epizootic. This appears to be the case for the similar, but nonoccluded rod-shaped virus in the whirligig beetle reported by Gouranton (1972).

Because mass mortalities of marine animals, particularly shrimp, are rarely detected and studied in nature, it is difficult at present to assess the role of the virus in shrimp ecology. Mass rearing of pink shrimp in aquacultural efforts may permit future assessments of roles of viral agents in shrimp health.

ACKNOWLEDGMENTS

Mr. Lee Courtney participated technically in many phases of the work and is gratefully acknowledged. Dr. Del Nimmo and Mr. Sam Tagatz, both of the Gulf Breeze EPA Laboratory, are thanked for their contributions of shrimp from their experimental, toxic exposure studies.

REFERENCES

BANG, F. B. 1971. Transmissible disease, probably viral in origin, affecting the amebocytes of the European shore crab, *Carcinus maenas*. *Infect. Immun.*, 3, 617–623.

BERGHOLD, G. H. 1963. The nature of nuclear-poly-

hedrosis viruses. *In "Insect Pathology An Advanced Treatise,"* Vol. 1. (E. A. Steinhaus, ed.) pp. 413–456. Academic Press, New York.

BONAMI, J. R., AND VAGO, C. 1971. A virus of a new type pathogenic to Crustacea. *Experientia,* **27,** 1363.

BONAMI, J. R., H. GRISEL, C. VAGO, AND J. L. DUTHOIT. 1972. Recherches sur une maladie epizotique de l'huitre pelate, *Ostrea edulis* Linné. *Rev. Trav. Inst. Peches Marit.*

BONAMI, J. R., C. VAGO, AND J. L. DUTHOIT. 1971. Une maladie virale chez les Curstaces decapodes due a un virus d'un type nouveau. *Comp. Rend. Seanc. l'Acad. Sci.,* **272,** 3087–3091.

COUCH, J. A. 1974. Free and occluded virus, similar to *Baculovirus,* in hepatopancreas of pink shrimp. *Nature (London),* **247,** 229–231.

COUCH, J. A., AND NIMMO, D. R. 1973. Cytopathology, ultrastructure, and virus infection in pink shrimp exposed to the PCB, Aroclor 1254. (Abstract) *Program of the Joint Meeting of The Society for Invertebrate Pathology and International Colloquium on Insect Pathology and Microbial Control.* p. 105. Oxford University, England.

DEVAUCHELLE, G., AND C. V[illegible]. 1971. Particules d'allure virale dans les cellules de l'estomac de la seache, *Sepia officinalis. Compt. Rend. Soc. Acad. Sci. Ser. D.,* **272,** 894–897.

FARLEY, C. A., W. G. BANFIELD, G. KASNIC, JR., AND W. S. FOSTER. 1972. Oyster herpestype virus. *Science,* **178,** 759–776.

GOURANTON, J. 1972. Development of an intranuclear nonoccluded rod-shaped virus in some midgut cells of an adult insect, *Gyrinus natator* L. (Coleoptera). *J. Ultr[illegible]act. Res.,* **39,** 281–294.

HAYAT, M. A., AND R. GIAQUINTA. 1970. Rapid fixation and embeding for electron microscopy. *Tissue and Cell,* **2,** 191–195.

HUGER, A. M. 1966. A virus disease of the Indian rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus), caused by a new type of insect virus, *Rhabdionvirus oryctes* gen.n., sp.n. *J. Invertebr. Pathol.,* **8,** 38–51.

KAZAMA, F. Y., AND SCHORNSTEIN, K. L. 1972. Herpestype virus particles associated with a fungus. *Science,* **177,** 696–697.

LUNA, L. G. (Ed.) 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed., pp. 134–135. McGraw–Hill, New York.

MAZIA, D., BREWER, P. A., AND ALFERT, M. 1953. The cytochemical staining and measurement of protein with mercuric bromophenol blue. *Biol. Bull.,* **104,** 57–67.

NIMMO, D. R., BLACKMAN, R. R., WILSON, A. J., AND FORESTER, J. 1971. Toxicity and distribution of Aroclor 1254 in the pink shrimp *Penaeus duorarum. Marine Biol.,* **11,** 191–197.

RUNGGER, D., M. RASTELLI, E. BRAENDLE, AND R. G. MALSBERGER. 1971. A virus-like particle associated with lesions in the muscles of *Octopus vulgaris. J. Invertebr. Pathol.,* **17,** 72–80.

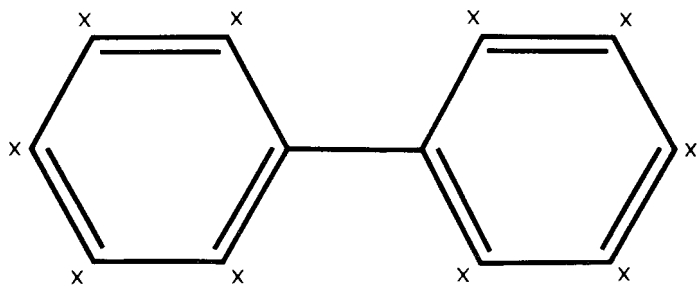
SUMMERS, M. D. 1971. Electron microscopic observations on granulosis virus, entry, uncoating and replication processes during infection of the midgut cells of *Trichoplusia ni. J. Ultrastruct. Res.,* **35,** 606–625.

VAGO, C. 1966. A virus disease in Crustacea. *Nature (London),* **209,** 1290.

WILDY, P. 1971. Classification and nomenclature of viruses. First report of the international committee on nomenclature of viruses. *Monogr. Virol.,* **5,** 1–81.

CONTRIBUTION NO. 216

MOLECULAR STRUCTURE OF PCBs



X - INDICATING THE POSSIBLE CHLORINE POSITIONS

From Reynolds L M. Bull. Environ. Contam. and Toxicol. 4 128 143 1969

Fig. 1. Polychlorinated biphenyl (Aroclor) molecule.

Ultrastructural Studies of Shrimp Exposed to the Pollutant Chemical Polychlorinated Biphenyl (Aroclor 1254)

John A. Couch, Ph.D., and Del Wayne R. Nimmo, Ph.D.
U.S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, FL 32561

Presented at the 63rd Annual Meeting of the International Academy of Pathology, March 13, 1974, San Francisco, CA

Gradually increasing signs of disease and toxicity in freshwater and marine ecosystems have increased the need for detailed aquatic animal pathology. Among others, there are two areas of investigation, utilizing pathobiological methods, that appear promising in toxicology of aquatic animals. These areas of inquiry are based on two questions:

1) Are there indicative sublethal cytopathic changes that occur in aquatic animals exposed to low levels of pollutant chemicals?,
and
2) Are there interactions between natural pathogens (e.g. parasites) and pollutant chemicals in aquatic animals that will produce harmful synergistic effects?

Answers to these questions should aid in determining long-term and short-term effects of pollutants on aquatic ecosystems.

Methods that we are using to attempt to answer these questions are: (1) experimental exposure of selected aquatic species to both sublethal and lethal concentrations of pollutants, followed by, or concomitant with, (2) histological, subcellular, and physiological investigations of exposed control and feral specimens.

One of the most useful marine species for our studies has been the pink shrimp (*Penaeus duorarum*). Pink shrimp are commercially valuable crustacea common in the South Atlantic and Gulf of Mexico. We have been using this species as a test animal in studies of effects of the widespread pollutant chemicals, the polychlorinated biphenyls (PCB's), (Figs. 1, 2).

In this study, hepatopancreatic tissue was selected as the tissue of choice for monitoring cellular effects because of the functional significance of the hepatopancreas to crustacea. The hepatopancreas is a complex, gland-like organ that functions in digestion, secretion, absorption, and storage of nutrients (Fig. 3). The hepatopancreas consists of masses of acini or digestive tubules which branch off of two hepatopancreatic ducts that have their origin in the wall of the pyloric stomach. The acinus, the functional unit of the hepatopancreas, consists of a tubule whose wall is epithelium. This epithelium is made up of several cell types which are distributed differentially along the length of the acinus from a point proximal to the hepatopancreatic duct to distal blind sacs at the end of the acinus. The major cell types are: (1) embryonic (distal tips of acini); (2) absorptive (adjacent and medial to the embryonic cells); (3) secretory (medial and proximal region of acinus relative to the hepatopancreatic ducts).

In several experiments, shrimp were exposed to 3-5 μg/l PCB (Aroclor 1254) in flowing seawater. After 20-30 days, 50% of these shrimp died, whereas control shrimp experienced little or no mortality. Exposed shrimp accumulated up to 500 mg/kg PCB in hepatopancreatic tissues according to results of gas chromatographic analyses (Fig. 4).

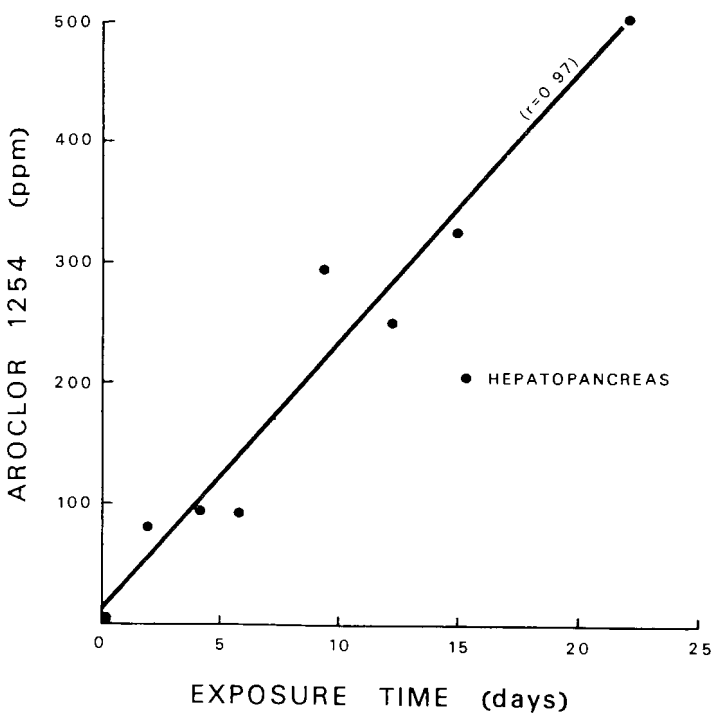


Fig. 4. Graph of uptake of Aroclor 1254 (PCB) by hepatopancreas over 25-day period.

Fig. 2. Pink shrimp with dorsal carapace of cephalothorax removed to expose hepatopancreas, *in situ* (arrow).

Fig. 3. Cross-section of hepatopancreas of pink shrimp showing complex relationships of ducts and acini (X 50).

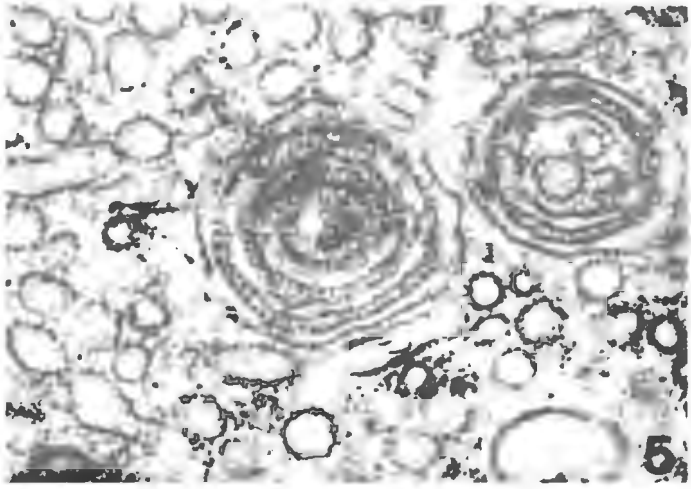


Fig. 5. Electron micrograph (EM) of early endoplasmic reticulum (ER) proliferation in shrimp cells exposed for 25 days to Aroclor 1254. Note both attached and free ribosomes and dilated cisternae of ER (X 28,500).

Fig. 6, 7. Advanced ER proliferation and whorls in hepatopancreatic cells of shrimp exposed to Aroclor 1254 (X 28,500).

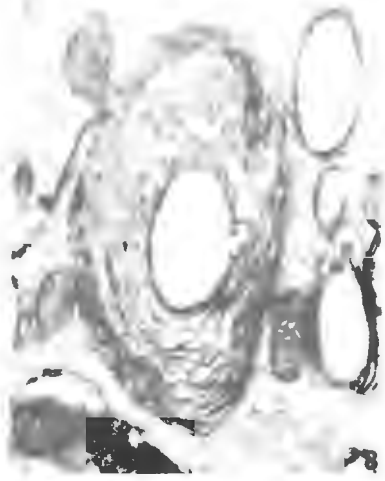


Fig. 8. ER membrane whorls surrounding lipid droplets in shrimp cells from same shrimp as in Figs. 6, 7. (X 84,000).

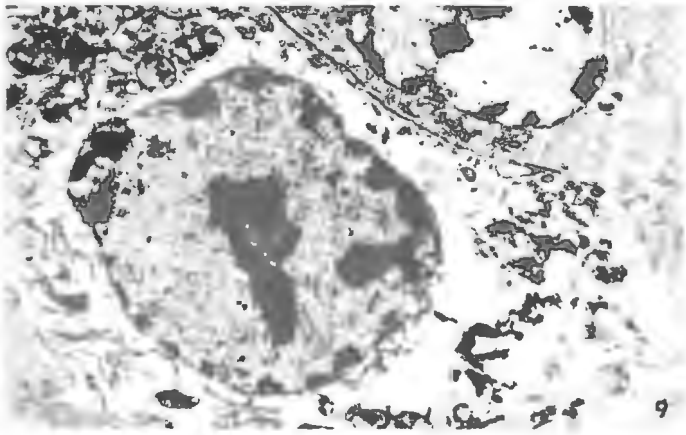


Fig. 9. Hepatopancreatic cell from shrimp exposed to 3 μg/l Aroclor 1254 for 30 days. Note small vesicles in nucleoplasm and loss of cytoplasmic integrity (X 14,400).

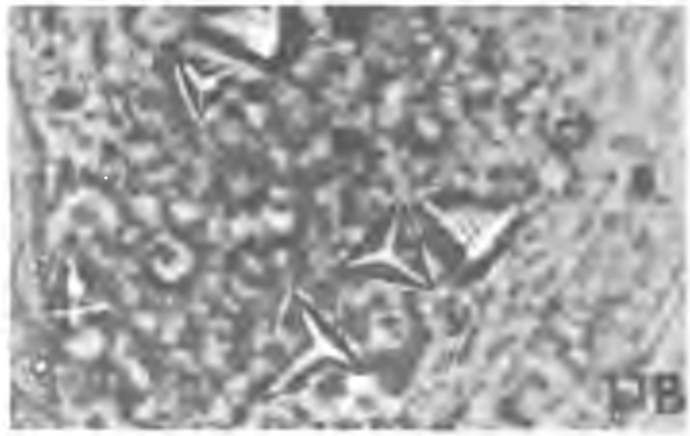


Fig. 11b. Fresh squash preparation of hepatopancreatic tissue containing numerous tetrahedral (3-D) inclusion bodies (X 3,000).

Fig. 10. Advanced nuclear degeneration in Aroclor-exposed shrimp. Note large vesicles in nucleoplasm surrounded by myelin-like sheaths. Note, also, the extreme modification of nuclear envelopes and myelin-like structures in envelope area (X 28,500).

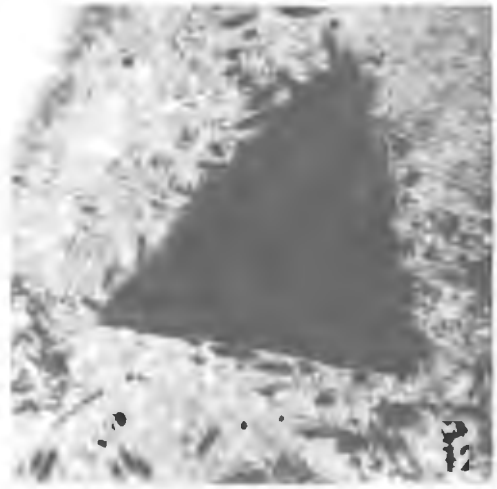


Fig. 12. Electron micrograph of inclusion body in hepatopancreatic cell nucleus from shrimp exposed to Aroclor 1254. Note rod-shaped virions, both free and occluded within body (X 28,500).

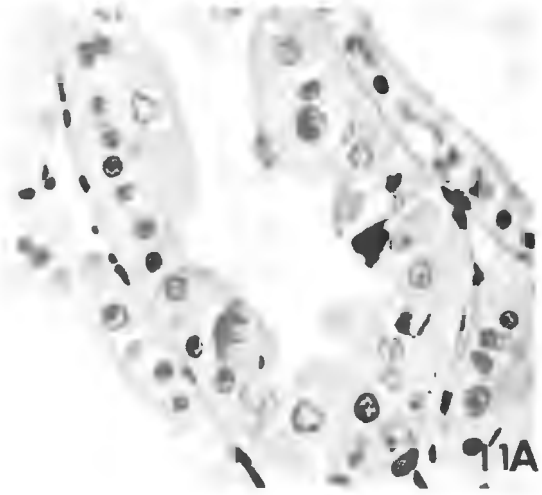


Fig. 11a. Cross-section (light micrograph) of hepatopancreas acinus from pink shrimp. Note triangular inclusion bodies in some nuclei, and hypertrophied nuclei as well as normal nuclei (X1,000).

Fig. 13. Electron micrograph of hepatopancreatic cell with infected nucleus. Note hypertrophy of nucleus, loss of heterochromatin, and cytoplasmic changes (X 14,400).

Hepatopancreatic absorptive cells from shrimp surviving 30 days' exposure revealed the following departures from normal:

1) 30 to 50% of cells had increased or proliferated endoplasmic reticulum associated with high numbers of attached and free ribosomes (Figs. 5, 6, 7).
2) Eventual production of "membrane whorls" or myelin bodies that often enclose lipid droplets (Fig. 8).
3) Nuclear degeneration characterized by the occurrence of vesicles in the nucleoplasm. These vesicles are of two classes: 20-50 nm in diameter, and 100-700 nm in diameter (Figs. 9, 10).

The proliferation of endoplasmic reticulum in certain cells (hepatocytes) of higher animals has been associated with toxic responses to phenobarbitol, dilantin (5), dieldrin (3), and carbon tetrachloride (4,5). This proliferation has been correlated with detoxification of poisons and can, if the poison persists, progress to the formation of membrane whorls, abnormal myelin bodies, and death of the cell (5). Since the hepatopancreas of shrimp performs some functions similar to those of the liver of higher animals, it is not unreasonable to suggest that the responses observed in the shrimp absorptive cells may indicate toxic responses to the PCB.

Another finding in the hepatopancreas of PCB-exposed shrimp was the discovery of a new virus associated with unique polyhedral inclusion bodies visible at the light microscope level (Fig. 11). This virus and its polyhedral inclusion body were found in higher prevalence in shrimp exposed to PCB than in shrimp taken directly from nature or held as controls (1). The virus is rod-shaped and may be free in the nucleus or occluded in the polyhedral inclusion body in nuclei of absorptive cells (Fig. 12). Therefore, it is very similar to *Baculovirus* that infects and causes diseases in insects. Cytopathic effects associated with the virus are shown in Fig. 13.

We are presently studying the interactions of the virus and PCB in shrimp in the laboratory to determine if there are harmful synergistic interactions between low levels of pollutants (pesticides) and virus infections. The concept of possible synergism between pollutants and natural pathogens introduces a novel field of research with aquatic animals, similar to that suggested by Friend and Trainer (2) for terrestrial vertebrates infected by viruses.

References

1. Couch, J.: Free and occluded virus, similar to *Baculovirus*, in hepatopancreas of pink shrimp. *Nature, 247*: 229-231, 1974.

2. Friend, M., and Trainer, D. O.: Polychlorinated biphenyl: Interaction with duck hepatitis virus. *Science, 170*: 1314-1316, 1970.

3. Hutterer, F., Schaffner, F., Klion, F., and Popper, H.: Hypertrophic, hypoactive, smooth endoplasmic reticulum: A sensitive indicator of hepatotoxicity exemplified by dieldrin. *Science, 161,* 1968.

4. Smuckler, E. A., and Arcasoy, M.: Structural and functional changes of the endoplasmic reticulum of hepatic parenchymal cells, *in: Inter. Rev. Exper. Path. 7*: 305-418. Academic Press, New York and London, 1969.

5. Trump, B. F., and Arstila, A. V.: Cell injury and cell death, *in*: Principles of Pathobiology. Oxford University Press. New York, pp. 9-95, 1971.

CONTRIBUTION NO. 219

In: 1974 Proc. Gulf Coast Regional Symposium on Diseases of Aquatic Animals. LSU-SG-74-05. Baton Rouge, La. 70803

DETECTION OF INTERACTIONS BETWEEN NATURAL PATHOGENS AND POLLUTANTS IN AQUATIC ANIMALS[1]

John A. Couch and D. R. Nimmo
U. S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island
Gulf Breeze, Florida 32561
(Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon)

Hopefully, it is now generally accepted that the "germ theory" of disease applies to aquatic vertebrates and invertebrates as well as to terrestrial forms. That this has not always been the case is best illustrated by the fact that in the past when mass mortalities of aquatic animals occurred, the general ecologist often overlooked the possibility that infectious pathogens might have been the etiologic agent. More often than not, every other possible avenue of cause and effect was explored before a search for infectious disease agents was launched.

Presently, in this age of environmental consciousness when mass mortalities of aquatic animals occur, one of the first causes to be searched for is pollution. This is justified, of course, based on documented evidences that water quality and aquatic ecosystem stability have been lowered significantly in the last three decades by increasing industrialization, population growth, and water misuse

Thus, those of us who must consider health of aquatic species have, on the one hand, infectious diseases and, on the other, pollution of waters, coming together as one complex of environmental factors that affect health in aquatic ecosystems. Impinging upon

---

[1] Contribution No. 219, Gulf Breeze Environmental Research Lab.

the complex of aquatic disease and pollution are, of course, other environmental factors such as temperature, pH, salinity, oxygen content, available nutrients, and agents of mechanical trauma. These too must be considered in evaluating the health of an aquatic species.

Only recently has the possible interaction of infectious diseases and pollutants, as environmental complexes, been seriously consided as threats to aquatic life. Snieszko (1972) has recently reviewed fish diseases that are heavily dependent upon environmental interactions. His general summation could be applied to any group of interacting environmental complexes, particularly infectious diseases and pollution:

> Modern epidemiology is based on the premise that epidemic outbreaks are caused by an imbalance between the host, the pathogens or other disease agents and the environment. Aquatic cold-blooded animals are much more affected by the environment than are the terrestrial homeotherms. Therefore, outbreaks of various diseases of fish are strongly affected by ecologic factors.

The purpose of this paper is to give two examples of the detection of possible interactions between natural pathogens and chemical pollutants in selected Gulf of Mexico, estuarine animals. These will include results of both experimental laboratory work and field observations in the vicinity of Pensacola, Florida.

Nimmo et al. (1971) have used pink shrimp (Penaeus duroarum) (Fig. 1) as test animals in toxicity studies for several years at the Gulf Breeze EPA laboratory. Recently, Couch (1974a) described a new virus in pink shrimp. It was observed during light and electron microscope studies of the hepatopancreas of toxicant exposed, control and feral shrimp (Figs. 2,3). The virus was found in hepatopancreatic epithelial cell nuclei (Fig. 4), is rod-shaped, and is either free in the nucleus or occluded in patent infections in crystalline inclusion bodies that range from 0.5 μm to 20 μm in size (Figs. 5,6).

The shrimp virus, named Baculovirus penaei by Couch (1974b), is considered to be a nuclear polyhedrosis virus because it shares many characteristics with the NPV or Baculoviruses (subgroup A) of insects (Wildy, 1971). It is the first Baculovirus reported in a host other than insects. The virus occurs in feral pink shrimp and is enzootic in populations of shrimp from Apalachee Bay, Florida and Santa Rosa Sound, Florida. It has been found most prevalent during the fall and winter months, occurring in from 0-30 percent of shrimp in given samples.

Pink shrimp from natural populations with enzootic levels of the virus have been used as subjects in toxicity tests of the PCB,

Aroclor^{R} 1254, and the organochlorine insecticide, Mirex. Shrimp exposed to 3 µg Aroclor/1 in flowing seawater for from 30 to 50 days accumulated up to 500 mg Aroclor/kg in their hepatopancreatic tissues. After approximately 30 days exposure, 50 percent of these shrimp died (Nimmo et al., 1971). From these series of tests, surviving, exposed shrimp were examined histologically and 60 percent were found to be lightly to heavily infected with the nuclear polyhedrosis virus. Control shrimp were free of patent virus infections. In another exposure test, conducted by Tagatz (personal communication), 38 percent of shrimp exposed to 0.01 to 0.23 µg commercial Mirex/1 of flowing sea water for 30 days demonstrated patent virus infections. Only 6.6 percent of control shrimp for this experiment showed patent infection. Mortality of shrimp in the Mirex exposure experiment was 81 percent, whereas the controls had only a 9 percent mortality. Thus, the prevalence of virus infections in the chemically exposed and control shrimp corresponded to the level of mortalities in those respective groups.

Another interesting finding has been that samples of pink shrimp kept under abnormally crowded conditions for 30-40 days in aquaria have shown 40-50 percent virus prevalence as compared to initial prevalence of 0-10 percent at onset of captivity (day one of holding period). This strongly suggests that the stress and proximity of crowding for a period of time enhances or facilitates the virus infection. Transmission of the virus from individual to individual via cannibalism in densely crowded aquaria or culture containers should be expected. In nature, even on fertile fishing grounds, shrimp are rarely as densely found as under aquacultural or aquarium conditions. Further, in nature dilution of infective stages of the virus by several factors (water volume, predation of infected shrimp by non-shrimp predators, etc.) would be greater than in closed less voluminous, artificial systems.

Therefore, as an example of a model system, we have the apparent enhancement of the prevalence of a Baculovirus by certain potential environmental factors, including chemical pollutant stress (PCB's and Mirex) and crowding. A similar model system of interactions between a toxicant chemical and a virus for higher vertebrates was presented by Friend and Trainer (1970), whose research demonstrated enhancement of duck hepatitis virus by polychlorinated biphenyls.

Numerous casual and careful observations of natural, aquatic ecosystems have led us to believe that where one finds chronic low level pollution (both natural nutrients and synthetic chemicals), one also observes increasingly frequent epizootics of certain diseases and a gradual increase in prevalence of certain pathogens in stressed hosts. This is not true, however, for every pathogen since some parasites may be adversely affected by the pollution as much as, or more than, their hosts. Each disease agent-pollutant

complex must be considered separately as well as part of more complicated, larger systems.

Over the last 10 years, we have observed in Escambia Bay, Florida, high prevalence of fin rot syndrome associated with mortalities in croakers (Micropogon undulatus) and spot (Leiostomus xanthurus) during periods of warm weather and oxygen depletion. Escambia Bay has been contaminated with the PCB, Aroclor 1254, for several years (Duke et al., 1970) and is a rapidly eutrophying system. Although we have not attempted to isolate pathogens from healthy or moribund fish, the patterns of disease and their seasonal occurrences strongly suggest a bacterial etiology. In this regard, Schwartz (1974) found that the bacteria Aeromonas and Pseudomonas, representative well-known fish pathogens, were found in higher prevalences in fish from Clear Lake, Iowa, in warmer seasons than in cooler seasons. In the laboratory during warming months of the year (April through June), we have been able to induce fin rot syndrome, identical to that in fish from Escambia Bay, in up to 90 percent of spot exposed to 3-5 µg/l of Aroclor 1254 (Couch, 1974c). This fin rot was associated with high mortality (80 percent), but again, no attempts were made to isolate a bacterial pathogen.

Thus, there is strong empirical and circumstantial evidence which suggests that interactions between natural pathogens and pollutants probably occur (Snieszko, 1974). Further research must determine the extent of the threat of such interactions to aquatic life and ecosystems. The Gulf of Mexico and its northern and eastern estuaries provide numerous natural sites for study of such interactions.

LITERATURE CITED

Couch, J. 1974a. Free and occluded virus, similar to Baculovirus, in hepatopancreas of pink shrimp. Nature 247:229-31.

Couch, J. 1974b. An enzootic nuclear polyhedrosis virus of pink shrimp. I. Ultrastructure, prevalence, and enhancement. J. Invert. Path. (in press).

Couch, J. 1974c. Histopathologic effects of pesticides and related chemicals on the livers of fishes. In The Pathology of Fishes. Univ. of Wisconsin Press (in press).

Duke, T., J. I. Lowe, and A. J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor^R 1254) in the water, sediment, and biota of Escambia Bay. Florida Bull. Environ. Contam. and Toxic. 5:171-80.

Friend, M., and D. O. Trainer. 1970. Polychlorinated biphenyl: interaction with duck hepatitis virus. Science 170:1314-16.

Nimmo, D. R., R. R. Blackman, A. J. Wilson, and J. Forester. 1971. Toxicity and distribution of Aroclor 1254 in the pink shrimp Penaeus duorarum. Marine Biology 11:191-97.

Schwartz, J. J. 1974. Prevalence of pathogenic pseudomonad bacteria isolated from fish in a warmwater lake. Trans. Amer. Fish. Soc. Vol. No. 114-16.

Snieszko, S. F. 1972. Progress in fish pathology in this century. Symp. Zool. Soc., London 30:1-15.

Snieszko, S. F. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. J. Fish Biol. 6:197-208.

Wildy, P. 1971. Classification and nomenclature of viruses. First report of the international committee on nomenclature of viruses. Monographs in Virology 5:1-81.

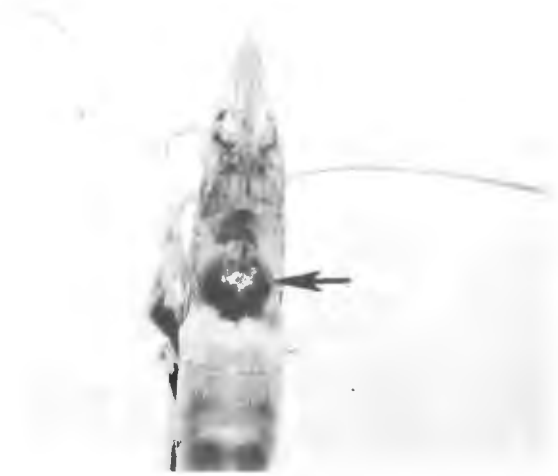


Figure 1. Dorsal view of pink shrimp (Penaeus duorarum) with dorsal cuticle removed to show hepatopancreas in situ (arrow).

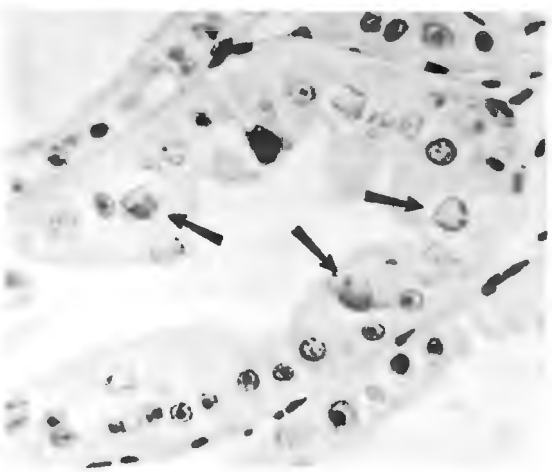


Figure 2. Cross section of shrimp hepatopancreatic tubule or acinus showing epithelial cell nuclei containing triangular baculovirus inclusion bodies (usually one per nucleus if cell is infected; arrows); normal nuclei are small with prominent nucleoli (820X). Feulgen, picromethyl blue stain.

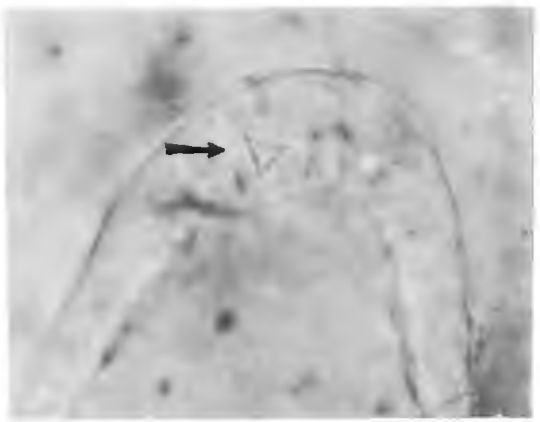


Figure 3. Fresh squash preparation of end of hepatopancreatic acinus; note single patently infected cell with large inclusion body that has the form of a tetrahedron (820X). No stain.

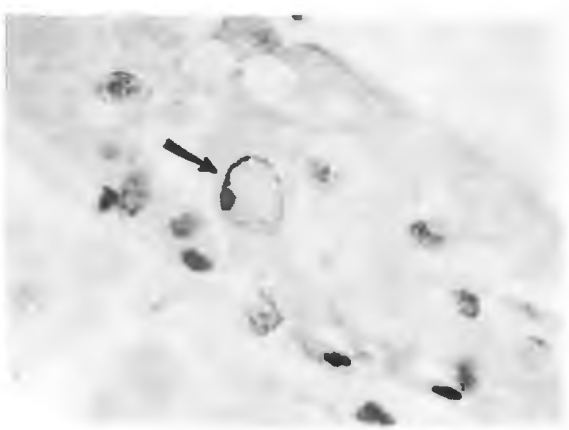


Figure 4. Section of hepatopancreatic epithelium showing cell with viral inclusion body in nucleus (arrow). (1230X)

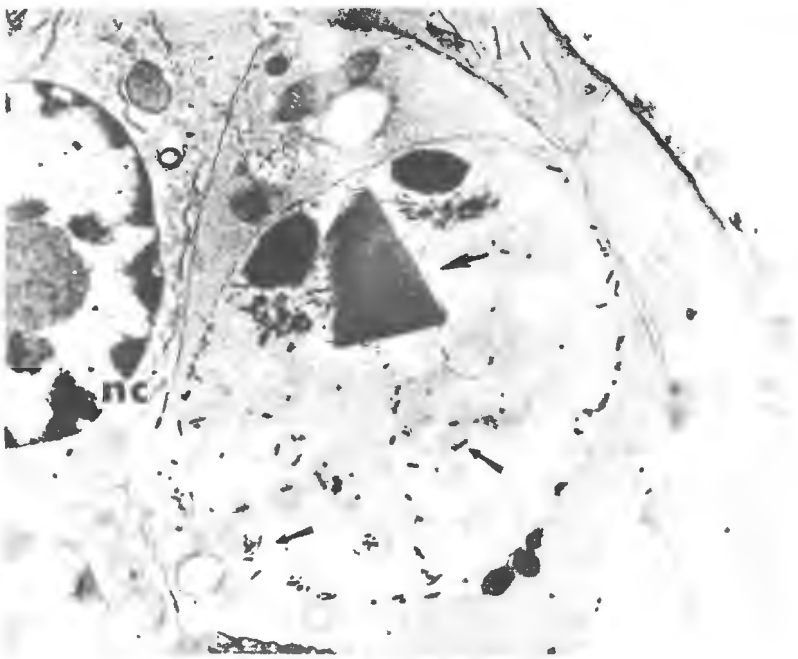


Figure 5. Electronmicrograph of virus infected hepato-pancreatic epithelial cell from PCB-exposed shrimp; note triangular section of virion-containing inclusion body in infected cell; note virions free in nucleoplasm (arrows). Compare normal cell (nc) with infected cell (7800X).

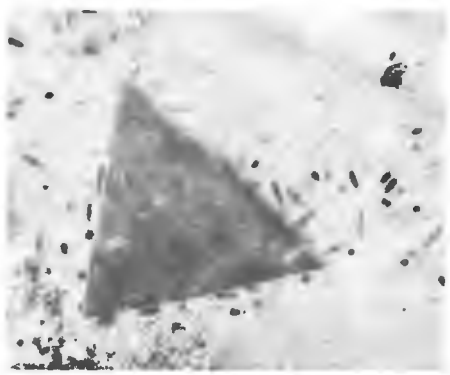


Figure 6. Higher magnification of inclusion body in shrimp cell. Note virions in longitudinal and in cross section embedded within matrix of characteristic triangular inclusion body (15,400X).

CONTRIBUTION NO. 220

Ultrastructural and Protargol Studies of *Lagenophrys callinectes* (Ciliophora: Peritrichida)

JOHN A. COUCH

Reprinted from THE JOURNAL OF PROTOZOOLOGY.
J. PROTOZOOL., 20(5), 638–647 (1973).
Made in United States of America

J. PROTOZOOL. **20**(5), 638-647 (1973).

Ultrastructural and Protargol Studies of *Lagenophrys callinectes** (Ciliophora: Peritrichida)

JOHN A. COUCH‡

U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory,§ Gulf Breeze, Florida, U.S.A. 32561

SYNOPSIS. Ultrastructural and protargol studies reveal that the trophont of *Lagenophrys callinectes*, though highly specialized, generally conforms to the basic peritrich structural pattern.

Features described for *L. callinectes* trophonts which are unique for the genus are the fine structure and arrangement of the lorica and lips, the attachment organelle of the peristomial cytoplasm, its attachment to the loricastome walls, and the arrangement of the aboral kinetosomes of the trophont. Lack of a distinct scopularized region, and of a ventral lorica wall also characterize *L. callinectes* trophonts.

The 4-row terminal peniculus, as revealed by protargol staining, differs from the 6-row terminal peniculus of *L. nassa* suggesting that the patterns of infundibular structure, as revealed by protargol, should be useful in future taxonomic studies of *Lagenophrys* species.

Index Key Words: *Lagenophrys callinectes*; ultrastructure; protargol; peritrich; morphology; infraciliature; lorica; kineties.

RESULTS of electron microscope (EM) studies have been published on the trophonts of the following genera of sessiline, stalked peritrichs: *Campanella* (7); *Opercularia* and *Vorticella* (7, 16); *Zoothamnium* (7, 8); *Epistylis* (8, 16); and *Carchesium* (8, 16, 23). Work on members of the stalkless, but sessiline genus *Scyphidia* was recently completed (13), having been preceded by work on a possible scyphidian species in the genus *Termitrophrya* (14). Nonsessiline, free-swimming peritrichs studied by EM have been *Opisthonecta henneguyi* (1) and *Telotrochidium* sp. (14).

Protargol-silver staining studies of representative species of peritrichs have been reported by earlier workers (4-6, 12, 23, 24). To date, however, transmission EM studies of the highly specialized, loricate, peritrich genus *Lagenophrys* have not been published while the protargol studies of this genus have been brief (4, 5, 12).

The purpose of the present paper is to describe aspects of the ultrastructure of *Lagenophrys callinectes* Couch, 1967, a commensal on the marine blue crab, and to give results of further protargol studies on the trophont of this species.

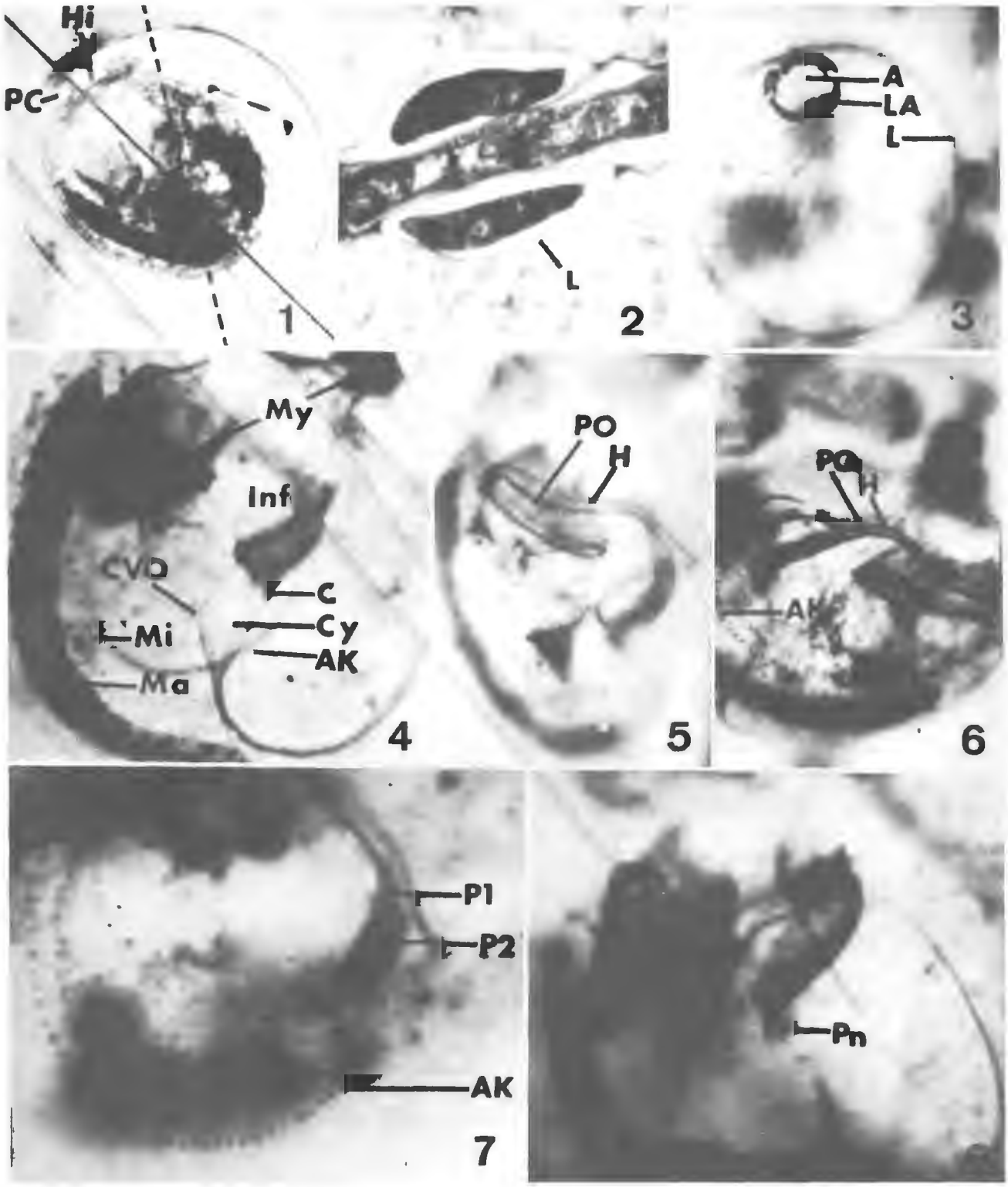
MATERIALS AND METHODS

The blue crab (*Callinectes sapidus* Rathbun, 1896), host of *Lagenophrys callinectes*, was collected by means of traps, nets, and trawls. Blue crabs are euryhaline and are usually found in estuarine and coastal waters ranging from fresh-water to oceanic salinities (22). I have found *L. callinectes* on the gills of blue crabs from Chesapeake and Chincoteague Bays in Maryland and Virginia, coastal estuaries of North and South Carolina,

* Much of this work was done while the author was employed with the National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Biological Laboratory, Oxford, Maryland.

‡ Great appreciation is here expressed to Dr. Frank Perkins for his generous advice and guidance in the use of the electron microscope. Drs. R. B. Short, John Corliss, and Eugene Small read and constructively criticized portions of the paper.

§ Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon.



All figures are of *Lagenophrys callinectes*.

Figs. 1-8. [Hematoxylin (Figs. 1, 2) and protargol (Figs. 3-8) preparations of trophonts.] **1.** Solid line represents long axis of organism; broken line represents plane of asexual division. Hi, hiatus; PC, peristomial cytoplasm. × 1000. **2.** Longitudinal section on both surfaces of gill lamella of blue crab. L, lorica. × 1000. **3.** Lips and lorica outline. A, lorica aperture; L, lorica; LA, lips around lorical aperture. × 1000. **4.** Trophont with important cytoplasmic structures. AK, aboral kinety; C, cytostome; CVO, contractile vacuole orifice; Cy, cytopharynx; Inf, infundibulum; Ma, macronucleus; Mi, micronucleus; My, myoneme. × 1600. **5, 6.** Mirror image views of adoral kineties of trophont. AK, aboral kinety; H, haplokinety; PO, oral polykinety. × 1400. **7.** Polykineties (P1, P2) and aboral kinety (AK). × 2000. **8.** Peniculus (Pn); note 4 terminal kineties. × 2000.

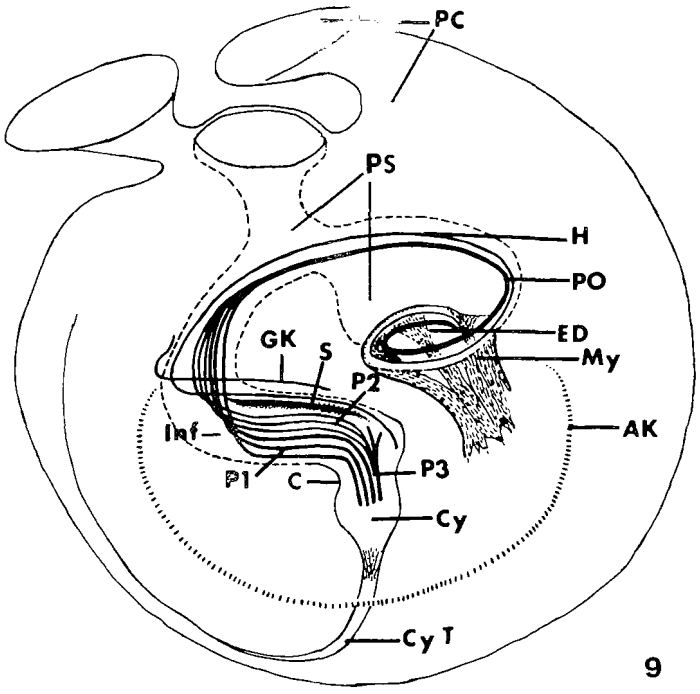


Fig. 9. Scheme of *L. callinectes infraciliature*. Mirror image of Figs. 1, 4, 5, 7, 8. Same orientation as Fig. 6. Protargol. AK, aboral kinety; C, cytostome; Cy, cytopharynx; CyT, cytopharyngeal tube; ED, epistomial; GK, germinal kinety; H, haplokinety; Inf, infundibulum; My, myoneme; P1, P2, P3, polykineties one, two, and three; PC, peristomial cytoplasm; PO, oral polykinety; PS, peristomial space; S, "S-belt" of Lom.

Georgia, and Florida; and the Gulf of Mexico near New Orleans, Louisiana and Pensacola, Florida. Salinities of collection sites ranged from 10‰ to 35‰.

To prepare *L. callinectes* for microscope studies, the dorsal carapace of the host was removed; branchiae were removed and individual lamellae cut from them. For short-term study, lamellae were placed in drops of seawater on slides with No. 1 coverslips directly applied. For long-term observation the lamellae were placed in seawater under No. 1 coverslips mounted on fragments of coverslips to prevent crushing the ciliate.

Infested gill material was fixed in Bouin's or Davidson's (20) fixative for treatment with protargol silver, modified method (10), and subsequent study of the morphologic and morphogenetic aspects of the infraciliature of different stages of *L. callinectes*. Lom (12) reported that the protargol method is far superior to any other (including Klein, Chatton-Lwoff) for the difficult job of impregnating the well protected infundibular infraciliature of peritrichs. Materials fixed as above were also stained with Heidenhain's iron hematoxylin for general cytology. Infested gill lamellae fixed in Schaudinn's fluid were Feulgen stained.

For EM studies heavily infested pieces of lamellae were teased apart into portions less than 1 mm^2. These pieces were fixed in 2.5% (v/v) glutaraldehyde for 1 hr, washed in 0.2 M Millonig's phosphate buffer for 45 min (3 changes) and postfixed for 4 hr in 1% (w/v) OsO_4 buffered in 0.4 M Millonig's phosphate buffer. The lamellar fragments were then dehydrated in alcohol and embedded in Durcupan ACM. Sections were cut with an LKB ultramicrotome, mounted on Formvar-coated grids, and stained in Reynolds' lead citrate (17) for 15 min and 2% (w/v) aqueous uranyl acetate for 4-5 min. The stained sections were examined and photographed in a Hitachi HU-11B electron microscope.

Many terms used in this work concerning ciliate taxonomy, biology, and structure are defined by Corliss (2) and Kane (11).

All measurements are in micrometers unless otherwise stated.

RESULTS

General Form.—Lagenophrys callinectes lives in a colorless, transparent, hemispherical lorica attached to the gill lamellar surface of the blue crab. The lorica wall is ~0.2-0.4 thick and 48-57 wide (Figs. 1, 2). The aperture of the lorica is 9.1-11.4 wide and is surrounded by 2 anterior and 2 posterior lip elements; the anterior ones are very unequal in size, whereas the posterior ones are equal in size (Fig. 3). These lips are easily seen since they are opaque in the living as well as in the fixed, stained animal. The form and relative size of the lip elements comprise the most important features available for identification of *L. callinectes*. The living animal is able to flex its peristomial cytoplasm, opening and closing the aperture by pulling the posterior lips together and then apart.

The alimentary complex (Figs. 4-9) of *L. callinectes* in contracted specimens begins in the peristomial space, which as used here is the region surrounded by the contracted peristomial cytoplasm of fixed specimens. This space includes what some would call the buccal cavity, but it does not include the infundibulum (Figs. 9, 15). The floor of the peristomial space is made up of the epistomial disc surrounded by the oral kineties (Figs. 5, 6). These kineties lead to the infundibulum (Fig. 9) which spirals down to the cytostome, which opens into the ampulla-like cytopharynx.

The relatively long cilia that surround the protrusible epistomial disc are visible in the living animal both when it is feeding with an extruded adoral apparatus and when the disc is retracted with the aperture closed. Individual feeding trophonts have been observed in salinities of 17‰, forming food vacuoles in the region of their cytopharynx at the rate of one every 2 min for up to 8-10 min, after which feeding may cease for long periods.

The cytoplasm of living trophonts may contain a few or many food vacuoles. In trophonts stained with iron hematoxylin, food vacuoles often appear basophilic and when examined closely appear to be filled with small basophilic rods. These are probably bacteria upon which the peritrich feeds.

The contractile vacuole is associated with the side of the cytopharynx immediately beneath the base of the epistomial disc in both living and fixed, stained specimens. The vacuole empties into the ampulla of the cytopharynx (Fig. 4).

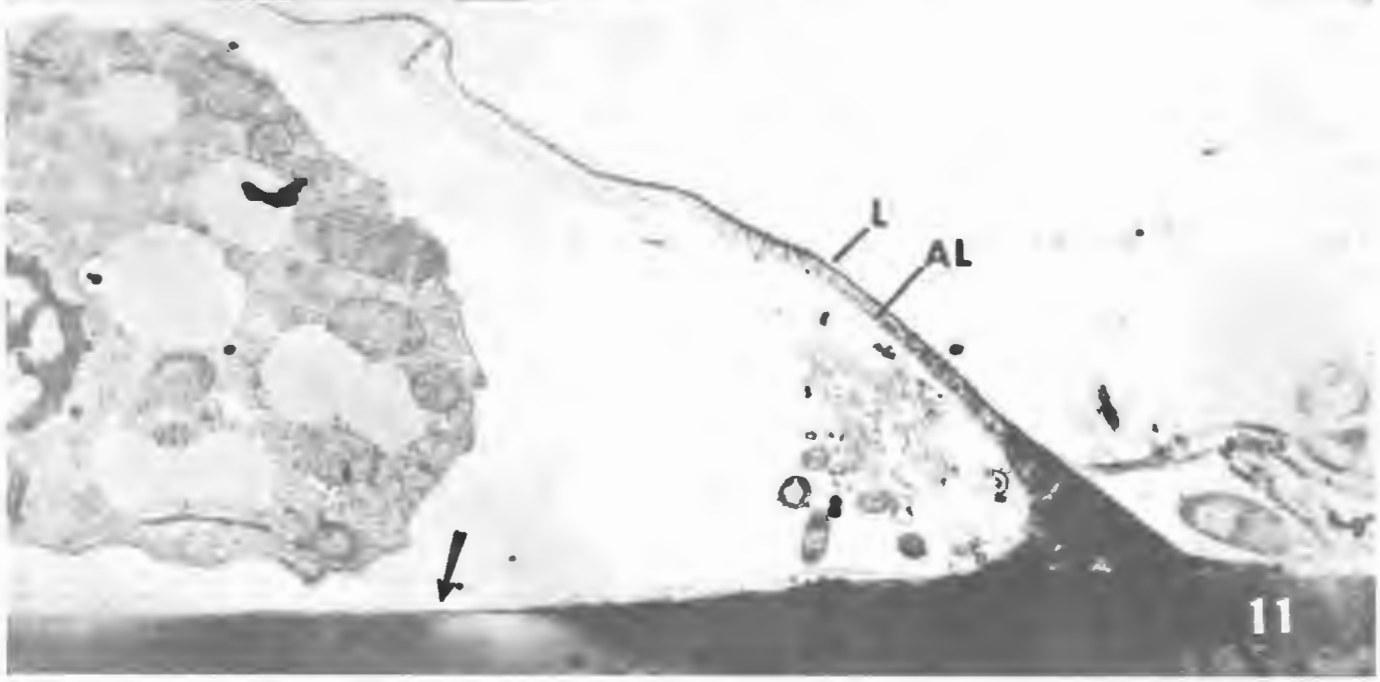
Portions (rarely all) of the macronucleus are visible in living

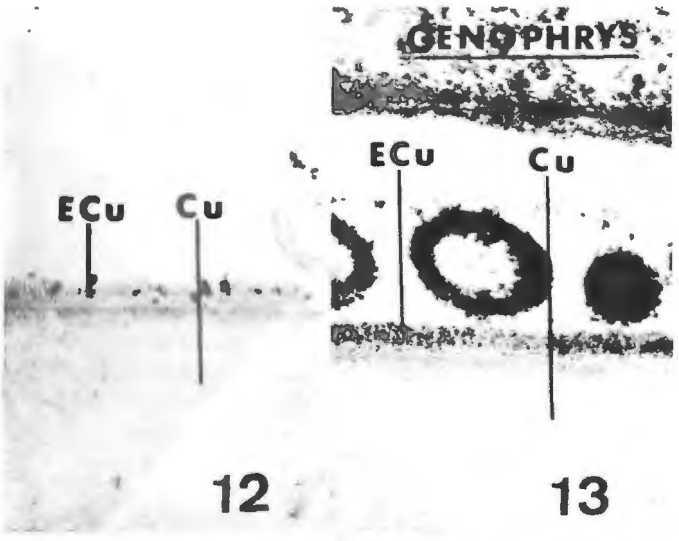
→

Fig. 10. Anterior and slightly lateral section of trophont; note the myonemal mass that makes up one of the lateral shoulders of peristomial cytoplasm. ALsW; anterior loricastome wall; LA, lips around lorical aperture; Ls, loricastome; Mt, mitochondria; My, myoneme; PC, peristomial cytoplasm; PLSW, posterior loricastome wall. × 11,250.

Fig. 11. Posterior or aboral region of lorica; arrow indicates point where lorica material disappears; note remnants of prokaryotic organisms (bacteria?) within lorica (L). AL, amorphous layer. × 12,500.


LA
Ls
ALsW
PLsW
PC
My
10


L
AL
11



Figs. **12**, **13**. [Cuticle of uninfested (Fig. 12) and infested (Fig. 13) crab gill lamellae. Cu, crab gill cuticle; ECu, crab gill epicuticle.] **12**. Note absence of lorica above epicuticle layer. × 47,500. **13**. Lorica is absent above epicuticle layer and beneath trophont. Dark bodies above epicuticle are remnants of bacteria. × 64,750.

trophonts. The micronucleus usually cannot be observed with bright field microscopy in living animals. In iron-hematoxylin-stained specimens macronuclei vary from short rodlike and reniform to L, U, or C shapes. The small micronucleus is usually round to ellipsoid, from 1.7-3.4 in diameter, and usually rests along the concavity of the curved macronucleus (Fig. 4).

A dense basophilic meshwork is distributed evenly throughout the macronucleus, interspersed with lacunae that contain round basophilic inclusions. In Feulgen preparations the evenly distributed material, corresponding to the basophilic meshwork, is positive for DNA, whereas the basophilic inclusions are Feulgen negative and not visible.

Lorica.—Ultrathin sections were cut parallel to the long axis of the soft body and lorica of *L. callinectes* (see Figs. 1, 2, 10, 11 for orientation). The lorica wall is slightly variable in thickness (0.1-0.4), being thickest mid-dorsally and at its point of attachment to the cuticle of the host (Fig. 11). The lorica does not appear to extend beneath the soft body of the ciliate to form a complete ventral wall, as described in light microscope studies for certain species of *Lagenophrys*, like *L. metapauliadis* (3). Instead, the inner portion of the lorica wall where it meets the host cuticle, appears to extend inward over the host cuticle for a few μm, gradually decreasing in thickness, until it becomes imperceptible (see arrows Figs. 11, 19). The rigid portion of the laminated gill cuticle of the crab host is divided into 2 major layers: a very thin epicuticle (0.1-0.3, Fig. 12) and a much thicker layer (beneath the epicuticle) that may range 1-1.5 (Fig. 12). These layers are illustrated from sections of gill cuticle both free of and infested by *L. callinectes* (Figs. 12, 13). Therefore, the nature of the gill epicuticle layer with and without *L. callinectes* can be determined. It appears that the area of host cuticle midventral to attached *L. callinectes* has no discernible layer on the epicuticle that could be ciliate-contributed (Fig. 13).

An amorphous, granular layer of material was found beneath the lorica wall (Fig. 11). Toward the point of attachment of the lorica, where the wall thickens considerably, there appears to be an increase in density of the amorphous layer and a gradual integration of the granular material into the thickened peripheral buttress of the wall where it attaches to the host cuticle (Figs. 10, 11).

The borders of the aperture of the lorica are formed by the specialized lorica extensions previously called lips (Figs. 3, 10). Uneven folds in the lorica are thrown up in the regions anterior, lateral, and posterior to the lips (Fig. 10). From the region of these folds, the lorica wall is expanded upward around the aperture to reach electron-opaque crests (the lips) that are apparent in thin sections (Fig. 10). The wall of the lorica plunges down from the apex of the crests to form the inner tube or loricastome of Kane (10) (Fig. 10). The walls of the loricastome extend downward to contact the peristomial cytoplasm of the trophont. Electronmicrographs of sections of the lips obtained in this study reveal little about the actual topography (Fig. 3) of the different lip elements.

The relationship of the trophont of *L. callinectes* to its lorica is schematized in Figure 19.

Peristomial region and pellicle.—The posterior loricastome wall is folded immediately before making contact with a specialized dorsal rim of peristomial pellicle and cytoplasm of the trophont (Figs. 15, 16, 19). This specialized pellicular-cytoplasmic organelle appears to be of paramount importance in the attachment of the trophont to its lorica. I can find no comparable peritrich organelle described in the literature; therefore it is designated here as the *attachment organelle*. The structure is 1 μm or less wide (in section) and extends outward and upward as a semi-V form of folded pellicle filled with cytoplasm that is continuous with the peristomial cytoplasm (Fig. 16). Partial reconstruction (composite) of the posterior loricastome-peristomial complex from available serial sections suggests that the extremity of the posterior loricastome wall is attached to the organelle along the entire dorsal width of the peristomial region of the trophont (Fig. 15), forming a firm but elastic relationship between the trophont and its lorica.

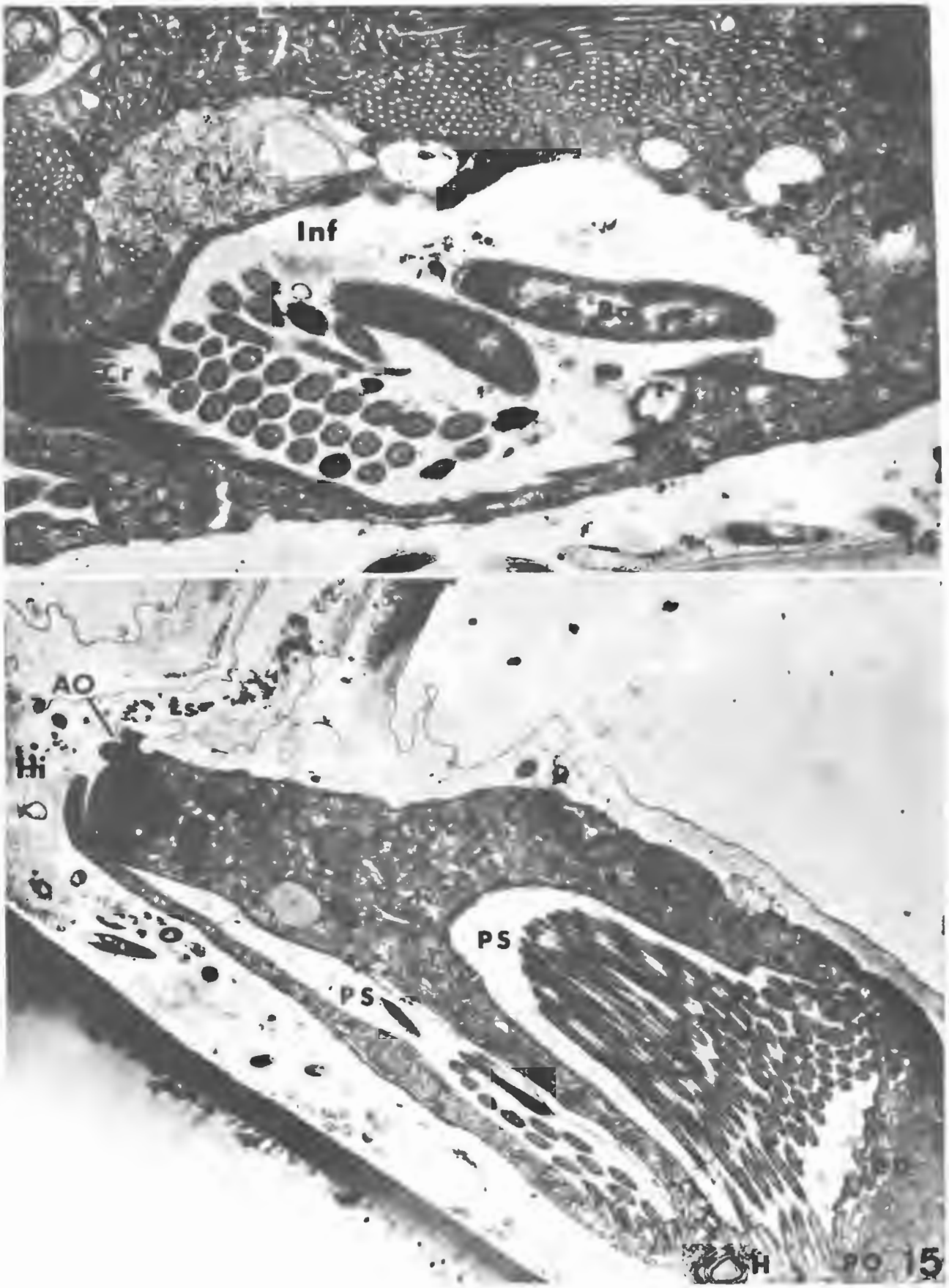
The anterior loricastome wall attaches to the left and right shoulders of the peristomial cytoplasm (Figs. 10, 19), but only with its extreme left and right edges. Thus a small anterior hiatus (Figs. 1, 15, 19) exists between the left and right shoulders of the peristomial region, anterior to the frontal portion of the anterior loricastome wall edge, and the oral opening of the trophont. This hiatus is apparent with light microscopy (Fig. 1) and provides the telotroch an escape hatch to the lorica aperture.

In the peristomial cytoplasmic region is a mass of muscle-like fibers that correspond in their fine structure to the contractile myofibrillar system or "myoïde" described for other peritrichs (1, 7, 13). These protargol-positive myonemes are concentrated mostly in the right or left shoulders of peristomial cytoplasm (Fig. 10). However, they extend across the whole dorsal width of the trophont as a band, subtend-

→

Fig. **14**. Section through infundibulum (Inf) and nephridial region or spongiome; note bacteria (Ba) in infundibulum, and projecting crests (Cr) of infundibular wall. CV, contractile vacuole; NS, nephridial system. × 19,375.

Fig. **15**. Longitudinal section of aboral region, peristomial space (PS) attachment organelle (AO), and epistomial disc (ED). H, haplokinety; Hi, hiatus; Ls, loricastome; PO, oral polykinety. × 9,350.


CV
Inf
Er
AO
Ls
Hi
PS
PS
H
PO
15

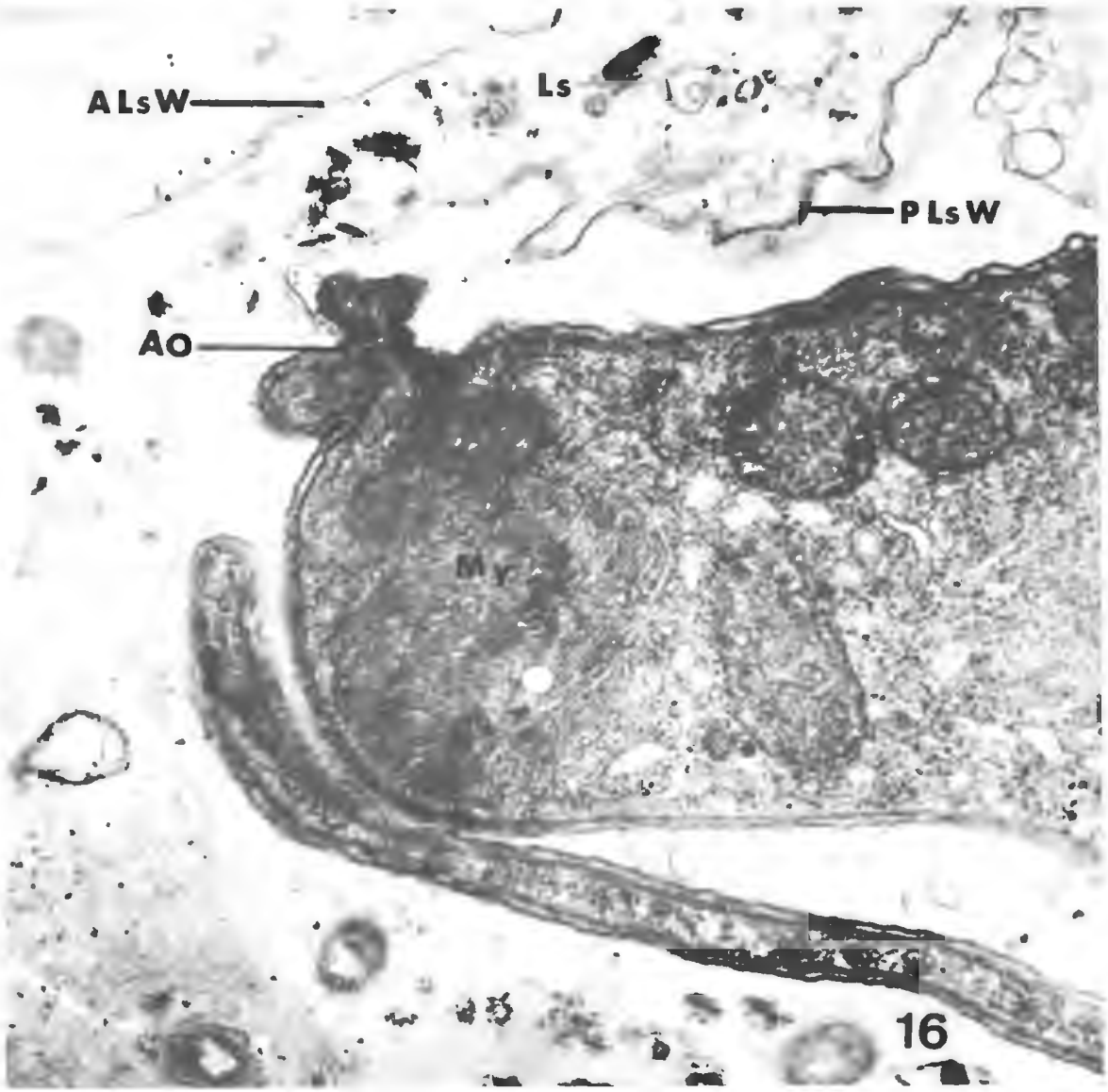


Fig. 16. Higher magnification of adoral region and attachment organelle. Compare relatively small region of myonemes (my) subtending attachment organelle (AO) in this figure to larger myonemal structure in lateral shoulder of peristomial cytoplasm in Fig. 10. ALsW, anterior loricastome wall; Ls, loricastome; PLsW, posterior loricastome wall. × 38,250.

ing but not entering the attachment organelle (Fig. 16). The myonemes have a fine structure and system of canals ("lacunes") similar to that possessed by the "myoïdes endoplasmiques" described for the sphincter collar of *Vorticella campanula* (7). The peristomial myonemes of *L. callinectes* are organized into such a specialized subpellicular organelle, and probably function as a muscle to open and close the aperture of the lorica as well as a sphincter to close the oral opening of the trophont. This myonemal organelle corresponds topographically to the contractile portion of the sphincter collar described with light microscopy for *Pyxicola nolandi* (9).

The oral opening of the contracted trophont through which adoral cilia are protruded with the epistomial disc when the animal is feeding, lies at the bottom of the tube formed by the loricastome walls (Figs. 15, 16, 19).

The pellicle of the trophont of *L. callinectes* consists of 3 unit membranes ("3-ply" pellicle, Refs. 13, 15). The arrangement of the pellicle layers of *L. callinectes* differs from that of *Scyphidia ubiquita* (13), but somewhat resembles the arrangement reported for *S. inclinata* (13). The 3 membranes of the pellicle of *L. callinectes* overlie one another closely so that no well developed, rigid, pellicular alveoli are apparent between the outer membranes and the inner one and its thin underlying epiplasm (Fig. 17). Thin spaces do exist between the outer and the inner membranes, but these spaces are apparent only along certain regions of the trophont's body surface.

→

Fig. 18. Higher magnification of adoral region. Note myonemes (My) beneath epistomial disc (ED). H, haplokinety; PO, oral polykinety. × 14,375.

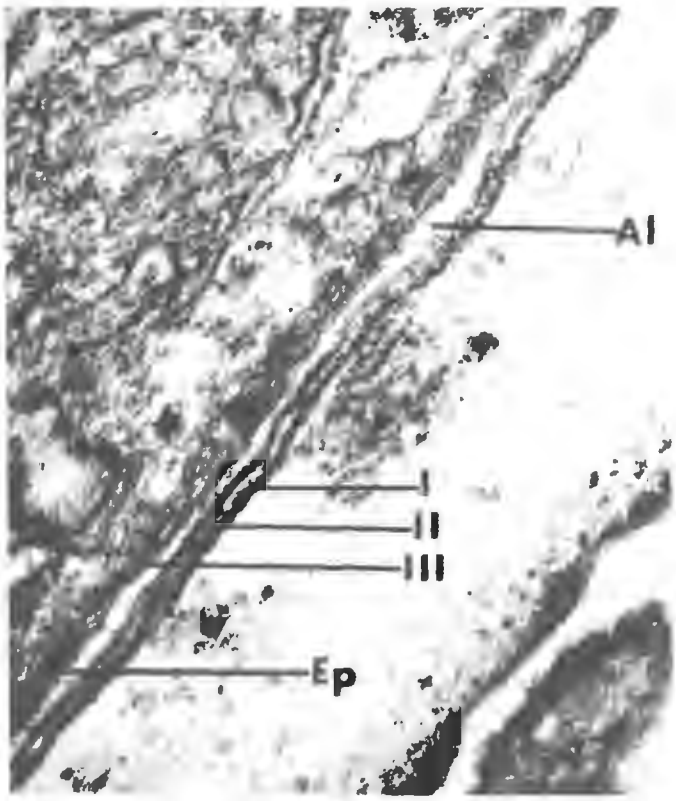


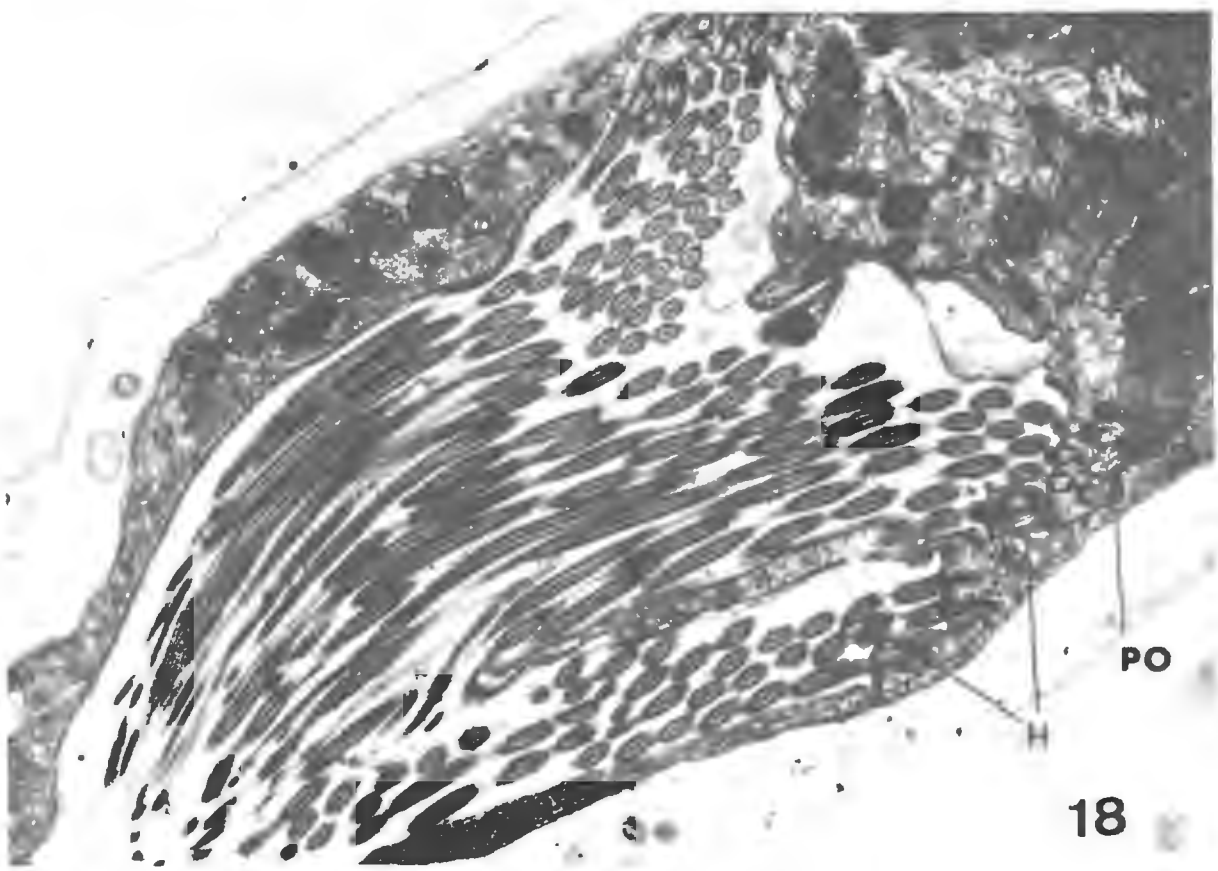
Fig. 17. Dorsal surface with 3 unit membranes (I, II, III) of pellicle, the epiplasm (Ep) underlying membrane III, and the alveolar space (Al) between membranes II and III. × 152,500.

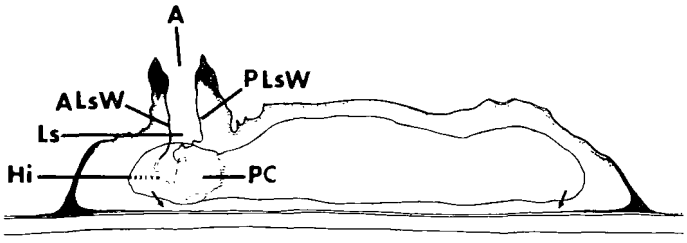
There are no kinetosomes directly and intimately associated with the outer body (nonoral) pellicle of *L. callinectes* trophonts, nor is there an obvious arrangement of the pellicle into specialized scopular areas as reported for *Scyphidia* (13, 14), and stalk forming peritrichs (6). A single row of barren kinetosomes does exist, however, in an aboral ventral subpellicular position corresponding to the infraciliature of a trochal band or pectinellar fringe in other peritrichs (1, 13). Lacking are subpellicular myonemes that have been commonly reported in other works on peritrich ultrastructure (7, 8).

Peristomial space, infundibulum and cytopharynx.—The outer pellicle of the trophont's body continues over and around the peristomial cytoplasm down into the peristomial space and infundibulum (Fig. 16), thus lining much of the alimentary complex. Figures 15 and 18 are from a longitudinal section of the trophont, and show the nature of the peristomial space and adjacent structures. At the base of the peristomial space lies the epistomial disc surrounded by the haplokinety and polykinety (Fig. 18).

Underlying the epistomial disc is a large but discrete mass of myonemes (Fig. 18). Their ultrastructural detail is similar to the "myoïde endoplasmique retracteur" of the epistomial disc of *Opercularia articulata* (7). The disc myonemes appear to be associated closely with ciliary rootlets of the oral ciliature (Fig. 18). It is probable that these myonemes function to extrude or retract the epistomial disc and buccal ciliature in *L. callinectes* as they were thought to do in *O. articulata* (7). This epistomial myoneme organelle appears to surpass in development any comparable structure in *Scyphidia*, *Epistylis* or *Opisthonecta.*

The haplokinety and polykinety are visible in section on either side of the epistomial disc as they make their complete





19

Fig. 19. Scheme of relationships of lorica, trophont, and blue crab gill cuticle. Arrows indicate approximate points where ventral lorica material ends. A, lorica aperture; ALsW, anterior loricastome wall; Hi, hiatus; Ls, loricastome; PC, peristomial cytoplasm; PLsW, posterior loricastome wall.

turns (360°) around the disc before descending into the infundibulum. They run parallel at the base of the peristomial space (Figs. 9, 18). The haplokinety is composed of 2 intimately associated rows of kinetosomes external to the polykinety. The inner row of the haplokinety is always barren. Thus, the ultrastructure of the adoral haplokinety of *L. callinectes* resemble closely that described in *Termitophrya* (14) and *Opisthonecta* (1). The adoral polykinety consists of 3 closely associated rows of kinetosomes (Fig. 18), thus also fitting the general peritrichan plan. A row of "germinal" kinetosomes running parallel to the haplokinety in the infundibular regions of other peritrichs (12), was observed in *L. callinectes* with light microscopy but was not precisely identified in the present ultrastructural study.

At the infundibular entrance the paths of the adoral haplokinety and polykinety separate (Fig. 9), the former running down the wall of the infundibulum 100-180° away from the latter until the cytostome is reached. This was determined from light microscope studies of protargol-treated specimens in conjunction with EM observations. The continuity of these infundibular kineties is best discerned by examining figures of both protargol-treated specimens (Figs. 5-8) and electronmicrographs (Figs. 15, 18). The adoral polykinety (P1), consisting of 3 rows of kinetosomes at the entrance of the infundibulum, is joined by parallel polykinety (P2) of 3 kineties just within the infundibulum (Figs. 7, 9). These 2 sets form the middle peniculus. Unfortunately, no electronmicrographs of the terminal portion of the peniculus were obtained. Therefore, the following description of its structure comes from the study of protargol-treated specimens with the light microscope. Near the cytostome, in the lower quarter of the infundibulum, P2 terminates and P1 is joined by a very condensed polykinety (P3) to form the end of the peniculus. The exact number of rows of kinetosomes making up P3 is unknown. However, P3 appears, at its origin (with light microscopy) to consist only of 2 rows of kinetosomes. At the very end of the peniculus, P3 gives the appearance of a single row (Figs. 8, 9). Thus, the number of distinguishable rows of kinetosomes making up the end of the peniculus for *L. callinectes* appears to be 4.

The infundibular haplokinety terminates at the cytostome at a point close to, but separate from the end of the peniculus (Fig. 9). Sometimes visible is a protargol-positive beltlike structure that runs downward, through the infundibulum, parallel to the course of the haplokinety (Fig. 9). This corresponds to the structure described for *L. nassa* which Lom termed the "impregnable structure" or "S-belt" (12).

Higher magnifications of sections of the infundibulum reveal folds of the wall projected inward toward the lumen (Fig. 14). These folds correspond to the infundibular "crests" described for *Epistylis* (8) and *Opisthonecta* (1). Often subtending these crests are masses of fibers that appear identical to the fibers of the "filamentous reticulum" described first in the peritrich *Campanella unbellaria* (18), and subsequently found in *Epistylis*, *Vorticella*, *Termitophrya*, and *Opisthonecta*. According to other investigators (1, 15) the reticulated fibers beneath the infundibular crests of peritrichs served to strengthen or reinforce the infundibular wall. The crests and associated fibers probably correspond to the impregnable structure of the "S-belt" of Lom (as revealed by protargol, Fig. 9).

No electronmicrographs of the cytopharynx and the cytopharyngeal tube of *L. callinectes* were obtained. However, these organelles can be observed in some detail in good protargol preparations in the light microscope. The cytostome proper is at the approximate level of the end of the peniculus (Figs. 4, 9). The cytopharynx begins there as an ampulla-shaped vesicle whose walls are heavily protargol-positive (Fig. 4). In certain protargol-treated specimens the ampulla region is greatly distended, probably as a result of food vacuole formation at the time of fixation. From the ampulla a long narrow cytopharyngeal tube extends posterior and to the left (dorsal view) and then curves anteriorly forming a U shape. This tube narrows gradually until it disappears in the cytoplasm beneath the peristomial region of the trophont, ending presumably in a cytoproct or cytopyge.

Bradbury (1) illustrated a section through the cytopharynx (tube) of *Opisthonecta henneguyi* in her ultrastructural study. She observed that the wall of the tube was made up of, or surrounded by, a bundle of fibers, which were not silver-positive. As reported above, an analogous structure (the wall of the cytopharyngeal tube) was found to be heavily protargol-positive in *L. callinectes* (Fig. 4). The difference here probably resulted from the different silver methods used. Bradbury used the Chatton-Lwoff method, whereas I used the protargol method which stains the deep alimentary structures of peritrichs (12).

The presence of rod-shaped bacteria in the deep infundibulum and cytopharynx of some *L. callinectes* trophonts was observed (Fig. 14). The bacteria within the cytopharynx were consistent in size (averaging 3 × 0.5) and larger than other prokaryotes often observed within the lorica cavity of both living and fixed trophonts (Fig. 14). The most probable explanation for the presence of these bacteria in the alimentary system of *L. callinectes* is that the trophont feeds on bacteria and was preparing to ingest those at the time of fixation.

Cytoplasmic organelles.—The very granular cytoplasm of *L. callinectes* contains many mitochondria. Mitochondria are most abundant in the peripherial cytoplasm of the trophont, except in the peristomial region where large numbers are found close to the myonemal mass (Fig. 10).

A complex system of tubules and canals is found adjacent to the infundibulum (Fig. 14). The smaller tubules of this system range 19-20 nm diameter and run into larger canals that range 70-80 nm in diameter (Fig. 14). There is little doubt that the complex is identical to the spongiome or nephridial (excretory) system described in *Epistylis anastatica* (8), *Paramecium caudatum* (19), and *P. aurelia* (19). The tubules and canals of the nephridial system of *L. callinectes* empty directly into the lumen of the contractile vacuole (Fig. 14).

Aboral infraciliature.—An aboral kinetal structure was found in all protargol-treated trophonts of *L. callinectes*. In the vegetative trophont (nondividing) this structure appeared as a curved row of protargol-positive bodies, each of which is 1.0

× .05 (Figs. 7, 9). These bodies appear, in the light and electron microscopes, to be in a ventral, subpellicular position, extending (dorsal view) in a single row from the upper infundibular region posterior to the macronuclear-micronuclear region and then curving anterior to terminate just below the myonemal mass of the epistomial disc (Fig. 9). The bodies that make up the structure are here designated as barren kinetosomes of the aboral kinety (= infraciliature anlage of trochal band or aboral ciliary girdle). Though homologous aboral kineties have been reported for other peritrich trophonts (1, 6, 13) they have not been reported for any species of *Lagenophrys* to date. The evidence that these aboral bodies in the trophont are indeed barren kinetosomes of a trochal band anlage comes from protargol, and morphogenetic studies (5). The role that these aboral kinetosomes play in the morphogenesis of *L. callinectes* is of paramount importance, particularly in the formation of the locomotor girdles of the telotroch and microconjugant stages (5).

DISCUSSION

The trophont of *Lagenophrys callinectes*, though highly specialized, essentially conforms to known peritrich form and ultrastructure. However, the present study has revealed interesting variations on the peritrich plan as well as several cytoplasmic structural modifications heretofore not reported.

Organelles possessed by *L. callinectes* and found in other well-studied peritrichs are: 3-unit-membrane pellicle; peristomial sphincter collar; myonemes of the peristomial region plus retractor myonemes beneath the epistomial disc; haplokinety, consisting of an inner barren row of kinetosome and an outer ciliated row; polykinety, consisting of 3 parallel rows of ciliated kinetosomes all within the outer haplokinety; infundibular crests subtended by a filamentous reticulum; and nephridial system or "spongiome" associated with the contractile vacuole.

The ultrastructure and arrangement of the lips of the lorica aperture, the attachment organelle, the loricastome walls, and the arrangement of aboral kinetosomes are unique for the genus *Lagenophrys* as revealed in the trophont of *L. callinectes*.

The lorica of *L. callinectes* appears to be a hemispherical, elastic, protective house for the trophont. It does not have a complete ventral shelf, and the soft body of the trophont lies directly above the gill lamellar cuticle of the host. The chemical composition of the lorica is unknown. The necessity for elasticity of the aperture region of the lorica is demonstrated by the ultrastructural relationships of the lips of the lorica aperture, the loricastome walls, the attachment organelle, and the fact that the lips of the aperture are movable in living animals (opening and closing motions). The attachment organelle of the dorsal peristomial cytoplasm appears to be a unique modification of the *Lagenophrys* pellicle region for the attachment of the trophont to its lorica.

The number of rows of kinetosomes making up the terminal peniculus varies among different peritrichs. Although Lom (12) stated that the uniformity of the buccal apparatus of peritrichs is strong among closely related species, it is apparent from the present study that the 6-row terminal peniculus (P1 and P3) of *L. nassa*, as described by Lom, differs from the 4-row terminal portion of the peniculus of *L. callinectes*. However, the 4-row terminal peniculus of *L. callinectes* appears almost identical to the 4-row terminal peniculus of *Carchesium polypinum* as described by Zagon & Small (24). Lom (12) further described up to 9 rows of kinetosomes in the terminal peniculus (P1, P2, P3) of *Telotrochidium* sp. This suggests that protargol comparisons of other species in the large genus *Lagenophrys* [52 species reported (21)] would be useful in further species identification and characterization.

The lack of distinct scopularized regions in *L. callinectes* trophonts probably reflects the high degree of specialization of members of the genus *Lagenophrys*, including their existence within loricae, and attachment to their loricae by adoral cytoplasm rather than by aboral poles of the trophonts.

REFERENCES

1. Bradbury, P. C. 1965. The infraciliature and argyrome of *Opisthonecta henneguyi* Fauré-Fremiet. *J. Protozool.* **12**, 345-63.
2. Corliss, J. 1959. An illustrated key to the higher groups of the ciliated Protozoa, with definition of terms. *J. Protozool.* **6**, 265-84.
3. ——— & Brough, I. M. 1965. A new species of *Lagenophrys* (Ciliata: Peritrichida) from the Jamaican crab *Metopaulias depressus*. *Trans. Am. Micros. Soc.* **84**, 73-80.
4. Couch, J. A. 1967. A new species of *Lagenophrys* (Ciliatea: Peritrichida: Lagenophryidae) from a marine crab, *Callinectes sapidus*. *Trans. Am. Micros. Soc.* **86**, 204-11.
5. ——— 1971. Aspects of morphogenesis in *Lagenophrys callinectes* (Ciliatea: Peritrichida). *J. Protozool.* **18** (Suppl.), 22.
6. Davidson, A. L. & Finley, T. E. 1972. A comparative study of argentophilic structures in three peritrich ciliates. *Trans. Am. Micros. Soc.* **91**, 8-23.
7. Fauré-Fremiet, E., Rouiller, C. & Gauchery, M. 1965. Les structures myoïdes chez les ciliés. Étude au microscope électronique. *Arch. Anat. Micros.* **45**, 139-61.
8. ———, Favard, P. & Carasso, N. 1962. Etude au microscope électronique des ultrastructures d'*Epistylis anastatica* (Cilié, Péritriche). *J. Micros.* **1**, 287-312.
9. Finley, H. E. & Bacon, A. L. 1965. The morphology and biology of *Pyxicola nolandi* n. sp. (Ciliata, Peritrichida, Vaginicolidae). *J. Protozool.* **12**, 123-31.
10. Honigberg, B. M. 1947. The characteristics of the flagellate *Monocercomonas verrens* sp. n. from *Tapirus malayanus*. *Univ. Calif. Publ. Zool.* **53**, 227-36.
11. Kane, J. R. 1965. The genus *Lagenophrys* Stein, 1852 (Ciliata, Peritricha) on Australasian Parastacidae. *J. Protozool.* **12**, 109-22.
12. Lom, J. 1964. The morphology and morphogenesis of the buccal ciliary organelles in some peritrichous ciliates. *Arch. Protistenk.* **107**, 131-62.
13. ——— & Corliss, J. O. 1968. Observations on the fine structure of two species of the peritrich ciliate Genus *Scyphidia* and on their mode of attachment to their host. *Trans. Am. Micros. Soc.* **87**, 493-509.
14. Noirot-Timothée, C. & Lom, J. 1965. L'ultrastructure de l'haplocinétie des ciliés péritriches. Comparaison avec la membrane ondulante des hyménostomes. *Protistologica* **1**, 33-40.
15. Pitelka, D. R. 1969. Fibrillar systems in flagellates and ciliates, *in* Chen. T. T., ed., *Research in Protozoology*. Pergamon Press, London and New York **3**, 279-388.
16. Randall, J. & Hopkins, J. M. 1962. On the stalks of certain peritrichs. *Phil. Trans. Roy. Soc. Lond.* **245**, 59-79.
17. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208-17.
18. Rouiller, C. & Fauré-Fremiet, E. 1957. Ultrastructure réticulée d'une fibre squelettique chez un cilié. *J. Ultrastruct. Res.* **1**, 1-13.
19. Schneider, L. 1960. Elektronenmikroskopische Untersuchungen über das Nephridialsystem von *Paramecium*. *J. Protozool.* **7**, 75-90.
20. Shaw, B. L. & Battle, H. I. 1957. The gross and microscopic anatomy of the digestive tract of the oyster. *Crassostrea virginica* (Gmelin). *Can. J. Zool.* **35**, 325-47.
21. Sprague, V. & Couch, J. A. 1971. An annotated list of protozoan parasites, hyperparasites, and commensals of decapod crustaceans. *J. Protozool.* **18**, 526-37.
22. Williams, A. B. 1965. Marine decapod crustaceans of the Carolinas. *U. S. Fishery Bull.* **65**, 1-298.
23. Zagon, I. S. 1970. *Carchesium polypinum*: cytostructure after protargol silver deposition. *Trans. Am. Micros. Soc.* **89**, 450-68.
24. ——— & Small, E. B. 1970. *Carchesium polypinum*: somatic and buccal structure analysis after protargol staining. *Trans. Am. Micros. Soc.* **89**, 443-9.

CONTRIBUTION NO. 238

A Clinical Centrifuge Tube for Small Blood Samples

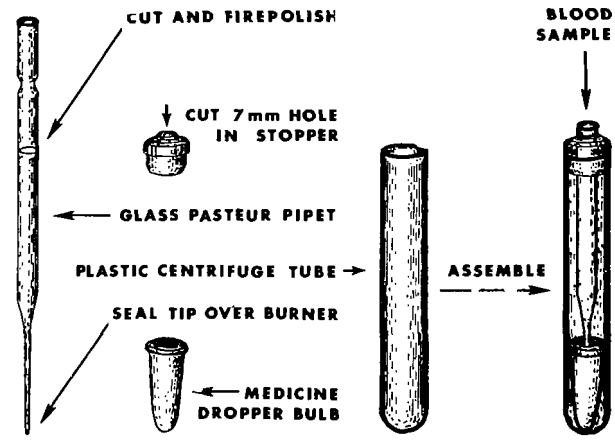
Occasionally, large amounts of whole blood are difficult to obtain for physiological studies. Little blood is available when the animal is small (e.g. some fish and crustaceans) or when blood samples are taken periodically without sacrificing the animal. It is often difficult to obtain clear serum or plasma from small blood samples. In many microanalytical procedures (e.g. electrophoresis) only a few microliters of serum are needed, but the serum must be free of other blood components. Because of this, a simple and inexpensive device for separating components of small amounts of blood in a clinical-type centrifuge is described.

To obtain separation of cells from serum in a small sample, (e.g. one drop of blood) a long centrifuge tube of small diameter is needed. Usually a microcentrifuge or hematocrit centrifuge is necessary to achieve separation. However, the cost of these instruments makes them impractical for most students and some researchers.

The device illustrated permits routine centrifugation of small blood samples in a clinical-type centrifuge. Materials used to modify the plastic centrifuge tube are inexpensive and readily available.

The centrifuge tube [1] serves as a holder for the sample tube made from a disposable Pasteur capillary pipet. The pipet is heat-sealed at the tip, cut to an appropriate length, and the end fire-polished. A rubber medicine dropper bulb, inverted in the bottom, cushions the tip of the sample tube. The top of the sample tube is

[1] International Autoclear® Plastic Centrifuge Tube I. E, No. 1649 and corresponding polypropylene stopper, International Equipment Co., Needham Heights, Massachusetts 02194.



Clinical centrifuge tube adapted for small blood samples.

retained by a polypropylene centrifuge tube stopper [1] with a 7-millimeter hole at the center. Other materials could be utilized to construct a similar apparatus (e.g. an adapter for heparinized hematocrit tubes).

Approximately 50 microliters of blood is inserted into the sample tube with a syringe or pipet. After centrifugation for 3 minutes at 1,800 *g*'s one drop of blood is distinctly separated into its components at the tip of the sample tube. Several microliters of serum can be removed for analysis using a capillary pipet or a microliter syringe.

—PATRICK W. BORTHWICK, *Department of Biology and Marine Sciences, The University of West Florida, Pensacola, Fla. 32504.*

The author's present address is U.S. Environmental Protection Agency, Gulf Breeze, Fla. 32561, an affiliated laboratory of the National Environmental Research Center, Corvallis, Oreg. 97330.

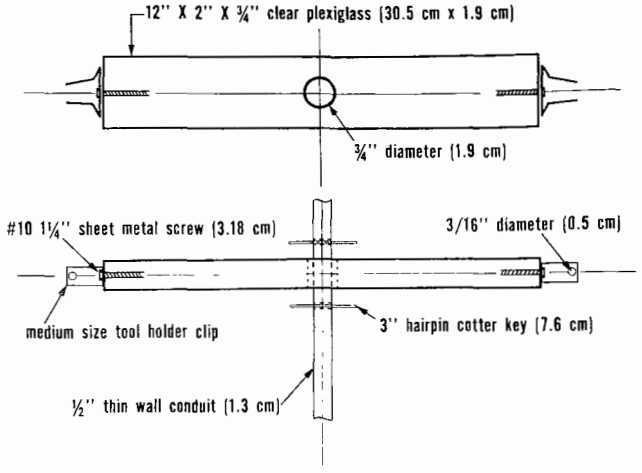
GB 238

Bottle Rack for Primary Productivity Studies

Limnological studies on large midwestern reservoirs have required primary productivity measurements utilizing the method originally developed by Steemann-Nielson in 1952 (The Use of Radioactive Carbon for Measurements of Organic Productivity in the Sea. *Journal du Conseil,* vol. 28, p. 117-140). For these studies we needed a compact bottle rack sturdy enough to withstand rough water and inexpensive to construct. The rack was designed to hold 300-milliliter BOD bottles by the neck.

A single rack consisted of a 12- by 2- by ¾-inch (30.5- by 5.1- by 1.9-cm) block of clear plexiglass with a ¾-inch (1.9-cm) diameter hole drilled on center (see diagram). On either end of the rack medium size tool holder clips have been attached using No. 10, 1¼-inch (3.18-cm) sheet metal screws. The ends of the clips have been bored to 3/16-inch (0.5-cm) diameter to accept 3-inch (7.6-cm) hairpin cotter keys which secure the bottles in the clip. We have found it desirable to sheath the clips with short pieces of ¼-inch (0.6-cm) ID Tygon tubing when using aluminum-foil-wrapped dark bottles to prevent accidental tearing of the foil.

Metal conduit ½-inch (1.3-cm) ID which slides through the center of the rack is the support. A series of holes have been bored through the conduit so that the racks may be held at a desired depth with the use of a 3-inch (7.6 cm) hairpin cotter key beneath the rack. To increase the depth capability beyond 10 feet (3 meters), additional conduit may be joined using smaller diameter metal tubing for a ferrule. The joint is made by connecting these pieces with hairpin cotter keys placed in holes drilled through the tubing and ferrule. The rack may be assembled or disassembled in minutes which eliminates having to leave the rack in the water overnight and discourages vandalism. Flotation for the assembled rack can be accomplished by using buoys or styrofoam logs attached to another permanently anchored float or dock. We have found that a cylindrical marker buoy with a hole through the center makes a satisfactory float and does not shade the racks to any appreciable degree.



Primary productivity bottle rack for 300-milliliter BOD bottles.

We have been using the racks all summer for nearly 700 sets without a broken or lost bottle. Lake conditions ranged from calm to extremely rough during exposure periods. Use of a small float and the length of conduit extending perpendicular in the water makes this device extremely stable regardless of weather and lake conditions.

Total cost of four complete racks assembled on a 10-foot conduit was under $8, excluding labor.

—STEPHEN L. BUGBEE, THOMAS F. LORENZ, and LEOTIS MOSBY, *Surveillance and Analysis Division, U.S. Environmental Protection Agency, 25 Funston Road, Kansas City, Kans. 66115.*

CONTRIBUTION NO. 242

Cytopathology, Ultrastructure, and Virus Infection in Pink Shrimp Exposed to the PCB, Aroclor® 1254

by

JOHN A. COUCH and DELWAYNE R. NIMMO

U.S. Environmental Protection Agency
Gulf Breeze Environmental Research Laboratory
Sabine Island, Gulf Breeze, Florida 32561
(Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon)

Little information is available concerning the effects of pollutant chemicals on the fine structure of tissues in aquatic invertebrates. Even less is known concerning possible interactions of pollutant chemicals and natural pathogens in commercially valuable invertebrate species. In experiments at the Gulf Breeze EPA Laboratory we have exposed pink shrimp (Penaeus duorarum) to 3 µg/ℓ Aroclor 1254 in flowing seawater from 30 to 52 days. During these exposures, up to 50% or more of the animals died. Both living and dead shrimp were analyzed for Aroclor residues and, after 30 days exposure, were found to accumulate from 33 mg/kg to 40 mg/kg in their hepatopancreatic tissues.

Hepatopancreatic tissues from experimental shrimp (surviving the exposures) and control and feral shrimp were prepared for histology and electron microscopy. Light microscopic examination revealed that approximately 30% of the exposed shrimp possessed intranuclear crystalloid inclusions in hepatopancreatic epithelial cells. Electron microscopy revealed that shrimp possessing the crystalloid inclusions were infected by an intranuclear, rod-shaped, free, and occluded virus similar morphologically to the nuclear polyhedrosis viruses (Baculovirus group) of insects. To date, control and feral shrimp have not shown this infection.

Cytopathologic changes in hepatopancreatic cells of exposed shrimp consisted of: (1) proliferation and hypertrophy of rough and smooth endoplasmic reticulum in large numbers of cells; (2) loss of cytoplasmic density and structural integrity; and (3) formation of small vesicles in the nucleoplasm of degenerating nuclei of cells showing the above cytoplasmic changes. These changes were not directly associated with virus infection because infected cells demonstrated several alterations apparently directly attributable to the influence of the virus. These changes were: (1) hypertrophy of the infected nucleus; (2) loss of chromatin; (3) proliferation of nuclear membranes, and (4) production of crystalline inclusion bodies containing virus rods. Further work concerning the possible interactions between organochlorines and the shrimp virus is presently underway.

Gulf Breeze Contribution No. 242.

(Abstract). Program of the Joint Meeting of The Society for Invertebrate Pathology and International Colloquium on Insect Pathology and Microbial Control. p. 105. Oxford University, England. (1973)

CONTRIBUTION NO. 245

In: Guidelines on sampling and statistical methodologies for ambient pesticide monitoring. Federal Working Group on Pest Management: Washington, D. C., Oct. (1974). Chapter V, pp: V1-V5.

CHAPTER V - ESTUARIES

Philip A. Butler, Ph.D.*

The decision to monitor an estuary for pesticides may derive from any one or several specific needs. These needs or objectives will largely determine the character and *modus operandi* of the program. Obviously, two pesticide monitoring programs in the same estuary might be entirely different because of the kinds of information sought. Estuarine monitoring objectives may be for the purpose of determining:

1. Background levels of an array of persistent waterborne pesticides by randomized sampling of estuaries in a particular geographical area.

2. The escapement of pesticides in surface run-off from specific use areas in the drainage basin by sampling deltaic sediments.

3. The cause of increased faunal mortalities or lack of species diversity in an otherwise normal appearing estuary.

4. Tissue residue levels of persistent pesticides to ensure that they are within legal tolerance levels for edible fish and shell fish or their products.

5. Pesticide residues in food chain organisms to alert resource management agencies of possible mortalities resulting from trophic magnification.

6. Pesticide residues in pre-spawning gonads of commercially valuable species to identify causes of change in productivity.

The choice of which physical or biological elements are to be monitored in an estuary will be determined by specific program objectives.

Water samples taken at infrequent intervals or at limited points in an estuarine system will usually be of limited value. If the monitoring program objective requires specific knowledge of pesticide residues in the water, the guidelines enumerated in Chapter IV should be followed for such samples as well as for sediment samples.

Sediment samples are useful in detecting persistent pesticides in the estuary. However, interpretation of their analyses requires knowledge of particle size, organic/inorganic composition, station location with reference to current flow and similar data that frequently are not readily available. Sediments in shallow estuaries can be

*Contribution No. 245, Gulf Breeze Environmental Research Laboratory, EPA

disturbed by storm conditions and their pollution burden may change drastically in time of flood or drought without reference to pesticide usage in the area. Analysis of stratified estuarine sediments may reveal unusual patterns of pesticide residue accumulation, but our lack of information on aerobic and anaerobic degradation of persistent pesticides complicates the interpretation of such sediment samples. Carefully collected samples at the sediment-water interface along the geographic axis of an estuary may be useful, however, in pinpointing up-stream sources of pesticide pollution.

The choice of a biological sample for monitoring the estuary is determined largely by two factors: is the form to be sampled migratory, and what is its position in the trophic web? Sessile or non-migratory species, representing entire communities present obvious advantages since they reflect pollution levels at specific locations. Their life style usually facilitates age determination and permits some degree of bracketing of the occurrence of the pesticide pollution.

Many non-mobile species, e.g., molluscs and barnacles, are detritus and filter-feeders. Pesticide residues may be biologically retained and magnified in their tissues and reflect the introduction of pollutants into the lowest levels of the food web. Such residues suggest direct contact with pollution sources. Residues in vertebrate carnivores, on the other hand, are more likely to reflect trophic magnification of persistent pesticides. Fish-eating birds or mammals not only concentrate but store for long periods compounds that contaminate their diets.

Plankton offers several advantages as a tool for estimating levels of pesticide pollution. Its small particle size presents a relatively large sorptive surface. The cells are usually high in lipid content and readily take up organochlorine compounds. However, the rapid cell division and growth rates favor dilution of tissue residues. Also the interpretation of data on pesticide residues in plankton may be confused by the facts that plankton is a composite of living and dead materials that contains varying amounts of silt and other inorganic materials, and its moisture content varies widely depending on the components. Plankton samples containing pesticide residues are probably indicative of fairly recent contamination. When samples are taken at frequent intervals, they may be especially useful in identifying pollution sources.

Crustacea, such as commercial shrimp, are generally one of the least satisfactory groups to monitor because of their extreme sensitivity to insecticides. Both organophosphorus and organochlorine insecticides

will kill crustaceans at concentrations in the parts-per-trillion range. Carbamates are somewhat less toxic to them and herbicides generally are not toxic at concentrations likely to be encountered. The net result is that pesticide effects on crustaceans are likely to be an all-or-none affair and crustacean samples may reveal little about relative levels of pesticide pollution in the estuarine environment.

Molluscs (oysters, clams, and mussels) have special merit as bioassay tools because of their sensitivity to synthetic organochlorine pollutants present in the ambient water. They detect and accumulate these persistent pesticides to an astounding extent without being themselves markedly affected by the pollution levels generally encountered in estuaries. Not all molluscs are equally sensitive and, as with other bioassay animals, care must be exercised in comparing pesticide residue levels between individuals and species. Salt-water mussels are especially useful as monitor species because of their wide geographic distribution and their ubiquity over a broad range of salinity regimes.

The chief objection to the use of molluscs lies in their rapid metabolism of pesticide residues. Although they can concentrate pollutants in their tissues by a factor of 50,000 or more, these residues are lost in a matter of days when the ambient water becomes free of contamination. Thus, oysters and mussels are monitor animals of choice when sampling can be done on a monthly or more frequent basis. They are relatively useless in reflecting trends in environment pollution when sampling can be done only once a year.

Fish are often the most convenient group to monitor because of their availability from the commercial catch. They are sometimes sensitive to high pesticide residues in their environment as evidenced by massive fish-kills, but may accumulate relatively large residues and become pesticide-resistent when the concentration of a pollutant is low. Experimental work has shown that they accumulate pesticides directly from the ambient water as well as from their food supply. The interpretation of pesticide residue data in fish is difficult unless their life history is known.

Fish are highly selective in their diet and may accumulate small or large pesticide residues in polluted estuaries, depending on their food supply. Plankton-feeding or herbivorous fish tend to have significantly lower pesticide residues, for example, than carnivorous species that feed on small fish in the same estuary. Populations of even the same species of fish may have quite different diets in two different estuaries. Comparison of pesticide residues in these two populations could be very misleading if their food habits are not known.

Fish store organochlorine residues primarily in tissues having a high lipid content. Such residues accumulate as the fish age but may diminish sharply at spawning or in starvation periods when stored body fats are mobilized. Wide variations in pesticide residues may occur also in fish having presumably similar backgrounds. In one series of analyses of 15 "similar" specimens collected simultaneously from a school of estuarine fish, for example, DDT residues varied by two orders of magnitude.

Fish are most useful as monitors when they are small enough to permit whole body analysis of at least 15 specimens, thus averaging individual variations. If the fish are about 1 year in age or sampled prior to their first spawning, the residues reflect pollution exposure during a known time period.

Sample Preservation - The handling of estuarine samples for pesticide monitoring poses the same types of problems as samples from any other medium. Increased knowledge of the dangers of sample contamination through contact with various kinds of synthetic wraps and containers has demonstrated the necessity for glass and perhaps aluminum foil containers to preserve the integrity of wet samples. Immediate freezing and maintenance of frozen sample until analysis is still the best way to protect samples and prevent degradation or loss of pesticide residues. Freezing, because of its simplicity, is also perhaps the best method for use by unskilled personnel.

The preservation of tissue samples for pesticide analysis at room temperature by the use of desiccants has been used with marked success in estuarine monitoring. Although this method requires a modest amount of personnel training as well as special chemicals, it avoids the loss of frozen samples because of missed airmail schedules and power failure. It has proven especially useful for samples collected long distances from the analytical laboratory. In practice, field samples of plants or animals are chilled, homogenized, and blended with a combination of two desiccants sodium sulfate and powered silica. The resulting mixture is a dry, free-flowing powder in which pesticide residues remain intact for 15 days or more at room temperature. This desiccant mixture is compatible with chromatographic procedures for organochlorine, phosphorus, and phenoxy pesticide compounds.

Representative Samples - The collection of representative field samples requries careful planning and pre-supposes a good understanding of estuarine ecology. The introduction of stratification into the selection of sample type, collection frequency, and station location is advisable if the most knowledge is to be gained from samples that are necessarily limited in number and areal coverage.

It has been found in past monitoring programs, that apparent pesticide pollution levels have been grossly altered or misinterpreted because of drought, windstorms, substitution of one species of clam for another, collection of samples on different tidal stages, and by assuming that the diet of a particular species of fish being monitored was the same in two different estuaries. Ultimately, the understanding of field sample analyses is determined by knowledge of the response of similar samples, either organic or inorganic, to pesticide pollution under controlled conditions. Too often, money and effort in environmental monitoring have been largely wasted because of the failure to select samples whose analyses could be meaningfully related to environmental conditions.

CONTRIBUTION NO. 247

EPA-660/3-74-024
DECEMBER 1974

A CONCEPTUAL MODEL FOR THE MOVEMENT OF PESTICIDES THROUGH THE ENVIRONMENT:

A contribution of the EPA
Alternative Chemicals Program

By

James W. Gillett
James Hill IV
Alfred W. Jarvinen
W. Peter Schoor

National Ecological Research Laboratory
Gulf Breeze Environmental Research Laboratory
National Water Quality Laboratory
Southeast Environmental Research Laboratory
National Environmental Research Center
Corvallis, Oregon 97330

Project Element 1EA487
ROAP 21BCL, Task 03

NATIONAL ENVIRONMENTAL RESEARCH CENTER
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS, OREGON 97330

ABSTRACT

This report presents a conceptual model of the movement and disposition of pesticides in the environment. A multi-media model is built up from simple modules representing basic processes and components of air, soil, and water. More specific models are exposited for the atmospheric/terrestrial, freshwater aquatic, and estuarine/marine environments. Through iterative operations of expansion and systematic reduction of the components and processes these models of segments of the environment can be joined to provide a holistic view of the disposition of a chemical and its attendant effects. Ultimately systems analysis and mathematical simulation techniques can be employed to evaluate the fate of a specific chemical in a particular environment. The conceptual model is thus a first step in organizing facts, assumptions, and hypotheses into a graphic and logical array capable of exploitation in further experimentation of pesticide disposition and effects.

While rejecting formulation of a model with global validity, the authors emphasize the commonalities of the basic processes and components in the various environments. Thus, a multi-media approach to disposition studies is made explicit even in the absence of a single, all-media global model.

This report was submitted in fulfillment of Project Element 1EA487, ROAP 21BCL, Task No. 10 by the National Ecological Research Laboratory under the sponsorship of the Environmental Protection Agency. Work was completed as of September 1974.

CONTENTS

FIGURES

FIGURES (Cont'd)

ACKNOWLEDGEMENTS

The authors gratefully and most humbly acknowledge the contributions of their colleagues Ray R. Lassiter and Edward J. Rykiel, Jr. (SERL); Patrick W. Borthwick, Marlin E. Tagatz, and Gerald J. Walsh (GBERL); and Eugene Elzy, F. T. Lindstrom, Marvin L. Montgomery, and Rizanul Haque (Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon). Helpful and constructive comment was received from N. R. Glass and A. S. Lefohn (NERL), J. Eaton (NWQL), T. W. Duke (GBERL), and D. W. Duttweiler, W. M. Sanders, and G. L. Baughman (SERL). Timely preparation would not have been possible without the dedicated assistance of Program Support Center (NERC-Corvallis).

FOREWORD

This report is a product of the Environmental Protection Agency's Substitute Chemicals Research Program, which seeks chemical alternatives to certain pesticides. The report provides an overall view of these chemicals regarding their pathways through and possible effects on the environment. Since the substitute chemicals to be investigated may exhibit properties similar to conventional pesticides, such as bio-concentration and bio-degradation, this program was initiated to study the environmental routes and rates of transport, metabolic fate, and sinks for a variety of these substances.

Many chemicals, including the substitute chemicals, move throughout all of the environment, and their total impact cannot be evaluated by a research program dealing with only one part of the environment. Experiments designed to provide data for regulatory function must include as many parts of the environment as possible. For this reason, the whole ecosystem approach has been adopted in this program.

We have thus presented an overall conceptual scheme from which scientists, administrators, management executives, and other interested persons with a concern for pesticide-related problems can obtain an overview.

SECTION I
CONCLUSIONS

Pesticides are applied to the ecosystem of the pest and not to the pest alone. An ecosystem by definition is a causally closed system in which each process is influenced by overall system structure. The concept of the ecosystem represented simply in thought or language is of little operational use until translated into more functional diagrams. Each of the many forms of system diagrams has strengths and weaknesses depending upon their application. An iterative process of expansion and systematic reduction of components to achieve an optimal balance between resolution and effort can be employed to join various segments of the environment.

Placing the pesticide problem in the control diagram format forces the investigator explicitly to define and delimit a complex hypothesis. Further, systems analysis and simulation techniques may be applied to mathematical approximation of the hypothesis stated in the control diagram. When applied to a preliminary system diagram, these analyses allow systematic reduction to a less complex form. As a preliminary to an experimental study, these techniques can provide answers to many questions concerning the variables to be measured, the accuracy required of the measurement, and the frequency of sampling. Thus, these methods of modeling and techniques of analysis enable investigators to develop models for the behavior of a specific pesticide in a specific ecosystem yielding an approach to optimum information re resource expenditure.

Ultimately, mathematical modeling and analysis could precede introduction of chemical which might be potentially hazardous in the environment. By identifying those properties of the agent and the

systems and by quantifying interactions of components, mathematical simulation can direct critical experiments to verify hypotheses of disposition and effect. The conceptual model is the first step in a rigorous scientific treatment of the fate and effects of agents and their alternatives in pest control.

SECTION II
RECOMMENDATIONS

The conceptual model necessarily will benefit from criticism, experimentation, and utilization in research. The process of improving and updating the conceptual relationships should be a continuing function of this program.

Analysis of the disposition of pesticides in particular segments of the environment and of the effects accompanying their distribution and fate should employ the conceptual models in developing more explicit hypotheses and as an operational framework. Research in laboratory microcosm and in field validation of laboratory studies of processes, effects, etc., should be correlated through appropriate models derived from this conceptual base.

In relation to the Substitute Pesticides Program, this conceptual model should be employed in referencing the probable disposition of an alternative chemical to that of the de-registered or suspect agent that the substitute might replace.

SECTION III
INTRODUCTION

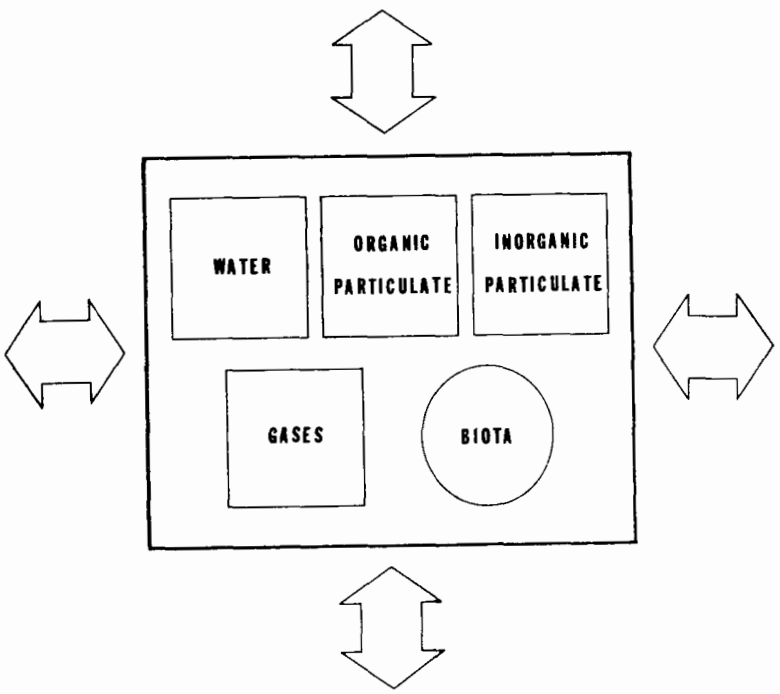
Literally millions of chemicals and combinations of chemicals are now manufactured and isolated, formulated, used, and ultimately disposed of in the environment. Management of the resources of regulatory agencies, supporting scientific institutions and manufacturers of the chemicals demands effective and reliable shortcuts in evaluating the potential hazard involved in such chemicals. The purpose of this conceptual model is to elucidate the disposition of an agent in the environment to permit judicious collection and evaluation of data that indicate the critical points in that disposition. From the conceptual model one could develop a more explicit model for the behavior and disposition of a specific chemical in a particular environment--a model that includes realistic parameters and by computer simulation provides realistic estimates of the concentration of that chemical in space and time.

A number of models have been proposed for the movement of specific agents or classes of chemicals in various environments. Some attempt to represent the global distribution of agents; others relate to smaller portions of the whole environment or to generalized segments (e.g., within man). Highly significant contributions to this effort are the works of BISCHOFF AND BROWN (1966), WOODWELL et al., (1967, 1971), HARRISON et al., (1970), NISBET AND SAROFIM (1972), KENAGA (1972), LINDSTROM et al., (1974), and ELZY et al., (1974).

In setting forth this particular set of models encompassing the atmospheric/terrestrial, freshwater aquatic, and estuarine/marine

environments, this report has established limits of validity and relevance focused locally rather than globally. The utility of the conceptual model rests in its conversion and evolution into an explicit mathematical statement capable of evaluation as a hypothesis. Current and near-future capabilities for extrinsic control of environments will limit such testing to laboratory microcosms, such as those of METCALF et al., (1971), and to small external sites, both characterizable as limited within the concepts of the model. Extension of the model conceptually in space and time can be made to the extent that the elements of the models can be grouped, subsected or interconnected.

Figure 1: Variable-form Module: chemical.
A chemical may exist "free" or "bound" in one of the states shown, all of which can interact within a region (inside box) or interact with adjacent modules of other environments (indicated by arrows).



The regional models can be considered as amplified aspects of a basic variable-form module (Figure 1) within which a chemical may exist in a "free" or "bound" form. Since any chemical may be used as a pesticide, a term describing its function, the fate and movement of any agent (and effects consequent to that disposition) can be described and displayed without regard to that extrinsic function. Thus, the model should serve not only for pesticidal chemicals, but also for other natural and man-made agents that are being evaluated. The subcompartments of modules may exist in varying proportions and with diverse relationships in different environments. Specification and elaboration of this basic chemical module are employed to relate it more specifically to a region or zone within the environment, and interrelating and interfacing such subsystems generates models of broader relevance. Subsequently, iteration of models can occur longitudinally (to represent stream flow, geographical or climatic regions, or atmospheric processes), vertically (to represent water depths, soil horizons, or meteorologic events), or horizontally (to represent distances from interfaces) to develop multi-media models.

At the interfaces of the regions explicit representation becomes most difficult. Although the models exposited cannot be viewed as globally valid, the iteration and conjunction of subsystems generate a global array (Figure 2) that serves conceptually as an overall model. As shown in Figure 2, some elements are "shared" in a more or less regular manner through seasonal, circadian, or shorter cycles and in an irregular manner through meteorologic and geologic changes. The tidelands, flood plains, and marshes are not fully represented by either aquatic or terrestrial models exposited herein, but both provide sufficient elements for subsequent elaboration as knowledge of the physical structure, physicochemical relationships, and alteration rates of these interfaces is improved. These interactions cannot be ignored simply because the mean flux appears to be zero,

because the rates of change are so slow or so fast as to lie outside apparent rate-limiting processes, or because events do not appear to affect disposition or effects of pesticides directly.

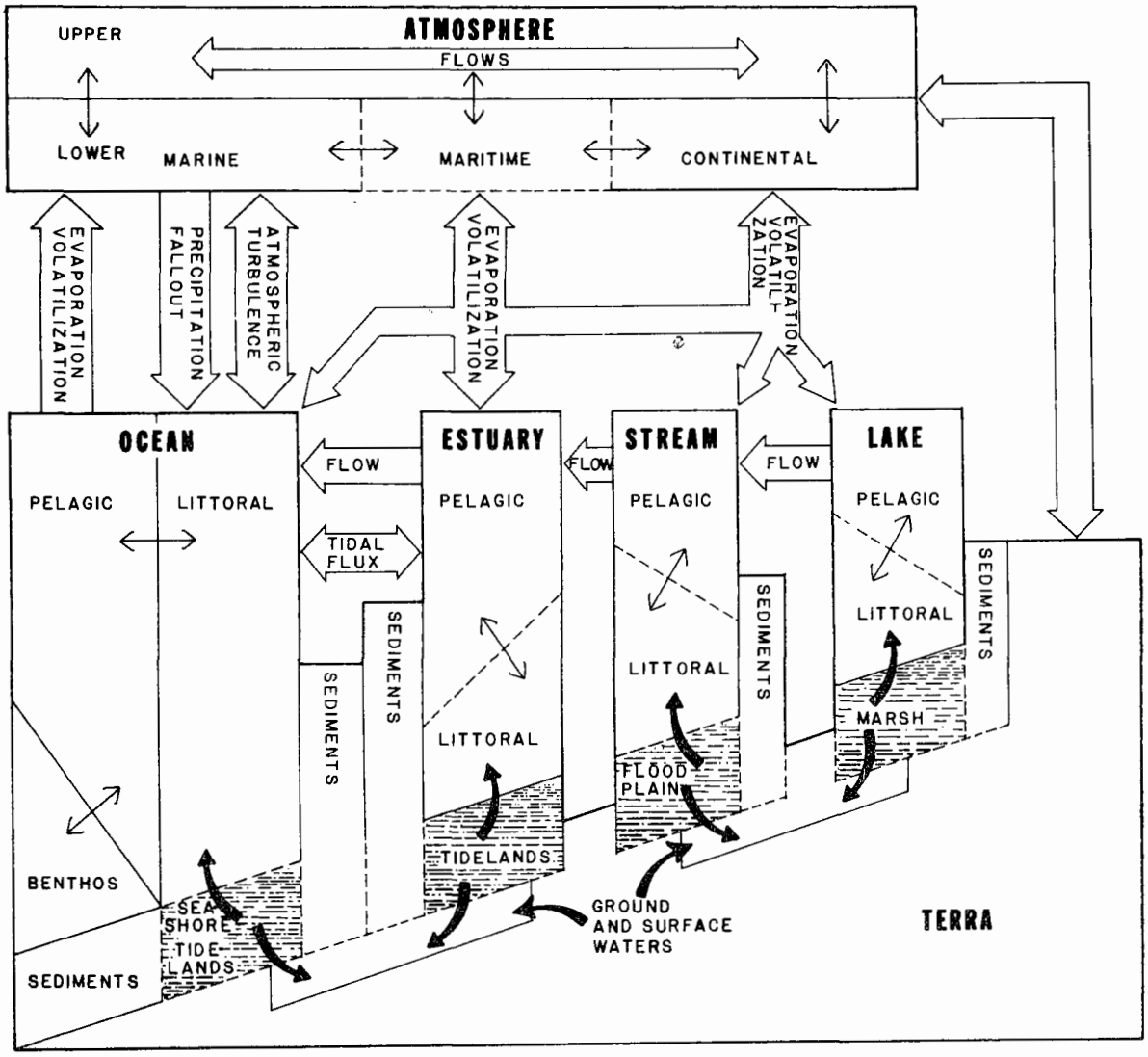


Figure 2: Global array of environmental regions. Modules can be arrayed as representing environmental regions interacting by flow (open arrows) or other transport and transfer phenomena (solid arrows) so as to represent global disposition.

A second major unifying thread of shared elements are the plants and animals designated as "Biota" in Figure 1. The interfaces of the physical environmental regions provide for considerable crossover of an agent via the biota, yet explicit representation is difficult. The phenomena of predation, migration, and vectorial transport (associated with compartmental flows) are indicated in Figure 3. Similar to the variable-form module of an environment, iteration of biological transfer and storage modules provides extension and expansion of these routes of disposition. Unlike interactions with the physical components of the environment, however, the biocidal and physiologic activities of pesticides can have pronounced direct and indirect effects on the disposition of a given agent. Determination of such effects within ecosystems would be vital to development of realistic simulation models.

Chemicals are altered by both physical and biological systems in the environment, so that site and rate of such change are highly significant aspects of the disposition. Representing these changes in a single model is difficult, especially when the agent (or its products) may alter the rate of biotransformation. Where an agent is altered chemically, we are assuming that the disposition of the product can be considered to be into a model parallel to that of the parent compound. The particulars of interaction may be describable for a given relationship, so that defined systems can be set forth for a specific chemical. In tracing the movements of an agent through this conceptual model, the products of photolysis, chemical alteration, and biotransformation can be visualized as leaving the global array (Figure 2) and entering a similar point on a model for each product. There might be many points interacting between the model of the parent agent and models of products.

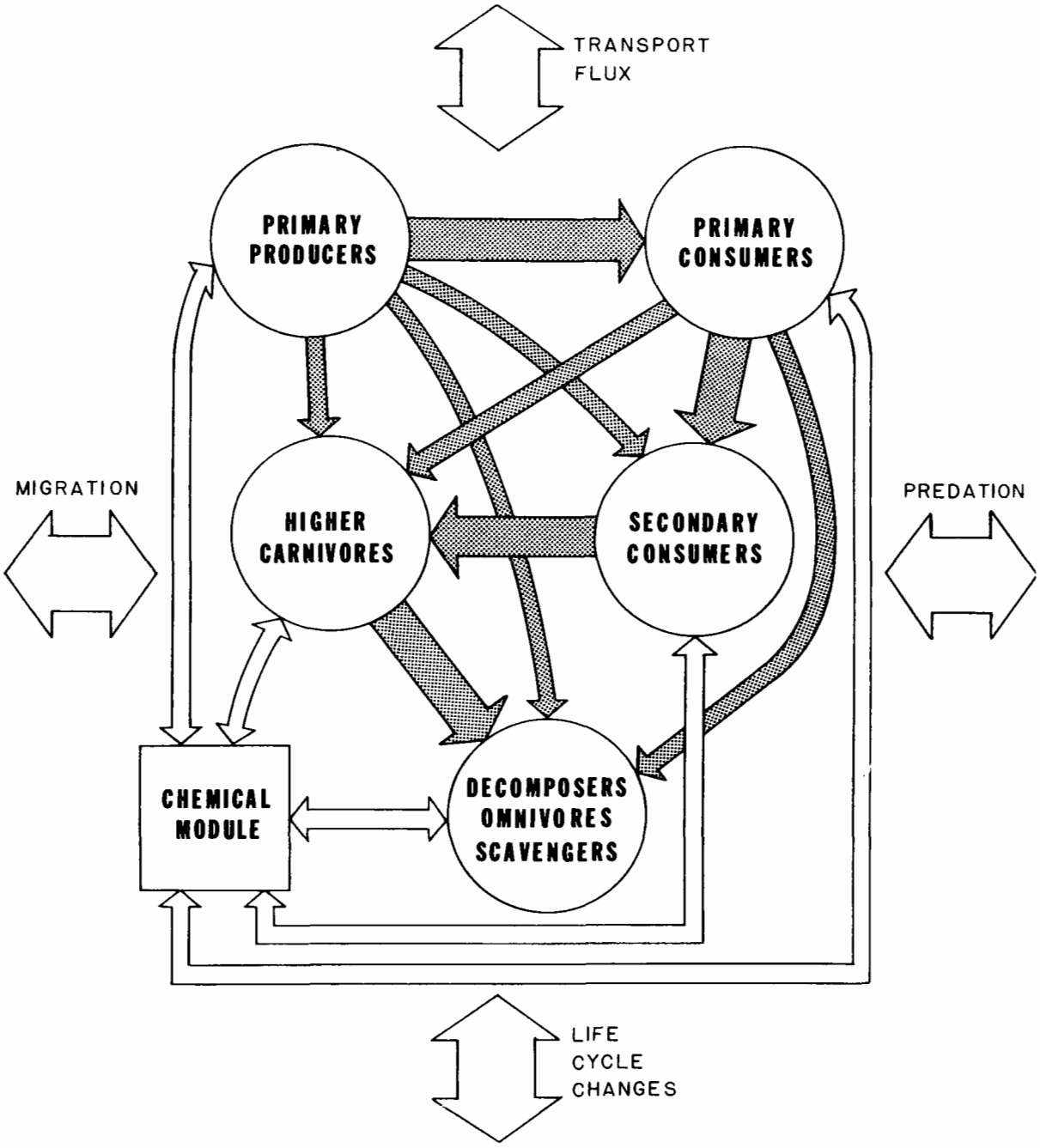


Figure 3: Food web module.
Solid arrows indicate intra-web flux by predation and feeding; open arrows indicate other transport and transfer within the food web or between food webs of different regions or zones.

Another major concept utilized in these models is that components can be represented as compartments equivalent to a well-stirred chemical reactor in a processing plant. Definition of what constitutes a compartment or component is part and parcel of the process of bringing the conceptual model into specific focus with a particular agent in a given segment or region of the environment. The extent of correspondence between (a) the definition of a "compartment" of the model, and (b) the characteristics of an environmental component determines how well a given variable-form module represents reality. Redefinition of compartments serves to make the model more sophisticated or less complicated, as knowledge is gained about the component and its functions.

The conceptual model for the transport of pesticides in the environment has been devised from three units: atmospheric/terrestrial, freshwater aquatic, and estuarine/marine. The nature of the presentations differ somewhat as expected for diverse points of reference, but the basic components and chemico-physical and biological flows are compellingly similar. This report will attempt to synthesize these components and processes further into an overall concept, then consider representations for the three major areas.

SECTION IV
OVERALL CONCEPT OF THE MODEL

An explicit, overall conceptual model derived from the principles of chemistry, physics, and biology and valid for all pesticides and environments, would over-reach the bounds of current knowledge. For practical translation into a quantitative model, the common threads of these principles and of the constituents of the environmental regions must be woven into a fabric or matrix of systems capable of analysis. Practically, we are forced to examine experimentally relatively small regions which can be characterized and/or controlled, or we must generalize these models by summation (see HARRISON et al., 1970; WOODWELL et al., 1971). Iterative simulation of the models over all environmental regions would require an unachievable data base, but much can be learned about the whole even from the parts. These will tell us where sampling and monitoring will be valid and helpful. Attention could thus be focused on the processes and mechanisms affording (and on these factors affecting) disposition.

PRELIMINARY SYSTEMS ANALYSIS FOR REDUCTION AND EXPERIMENTAL DESIGN

A diagrammatic representation of a system is usually of value to a scientific investigation even if the potential applications of the system representation are not realized. The trial-and-error expansion and reduction of compartments forces the investigators explicitly to acknowledge the boundaries and the level of definition of the system.

Deciding upon alternative representations of flow and control paths promotes consideration of even the most remote possibilities. Finally, many of the assumptions necessary to represent the system are explicit in the diagram.

The system diagram is a complex, qualitative hypothesis which must be tested by experiment. The hypothesis cannot be realistically tested in the graphical form of the conceptual models given so far (Figures 1 and 2). A more exact representation of the relationship between storages and flow rates is needed. The many possible mathematical forms for these relationships may be classified as linear or nonlinear and as recipient-controlled, donor-controlled, or mixed.

The linear, donor-controlled form (PATTEN, 1971) is probably the most elementary (CHILD and SHUGART, 1972). It can be represented mathematically as

$$\frac{dX}{dt} = AX + BZ \qquad (1)$$

in which X is a storage level vector, dX/dt is a flow rate vector, Z is an input vector, and A and B are coefficient matrices.

Donor-control implies that flow rate depends only upon the storage level from which the flow originates. Although this assumption may be unrealistic, the use of a linear, donor-control approximation of the system representation appears to be justified for these preliminary analyses. Often, linear approximations are less sensitive to parameter estimation errors than nonlinear representations. Also several expedient techniques of analysis may be applied to the linear, donor-control approximations. The following analysis techniques can yield alternative statements of the system diagram hypothesis that can be interpreted in terms of reduction and experimental design.

1. Topological analysis is currently being developed by a group of Dr. B. C. Patten's graduate students at the University of Georgia (PATTEN et al., In Press). This technique is intended to allow determination of the

influence of the topological structure on system behavior. Such information is useful in evaluating alternative system structures and particularly in determining the effects of reduction or aggregation of components.

2. Flow analysis (HANNON, 1973) or input-output analysis (LEONTIEF, 1966) is based upon the manipulation of the A coefficent matrix in the linear, donor-control approximation. Briefly, a matrix $\underline{G}$ is generated by

$$G = \sum_{i=1}^{\infty} A^i \qquad (2)$$

in which each element (G_{ij}) is a relative measure of the fraction of flow out of storage $\underline{j}$ that appears as input to storage $\underline{i}$ under steady state conditions. This information may be used to identify important processes or flow paths in the system.

3. Sensitivity analysis (TOMOVIC and VUKOBRATOVIC, 1972; PATTEN, 1973), may be used to evaluate the effect of a perturbation, $\underline{v(t)}$, upon the storage levels in the system. The measure of sensitivity, $\underline{S}$, is useful in determining which parameters have a prominent effect upon system behavior. A linear approximation of $\underline{S(t)}$ is determined from

$$\frac{d\,S(t)}{dt} \approx \left[\frac{d\left(\frac{dX_i}{dt}\right)}{d\,X_i}\right] S(t) + \left[\frac{d\left(\frac{dX_i}{dt}\right)}{d\,A_{ij}}\right] V(t) \qquad (3)$$

where the terms in brackets are Jacobian matrices. With a unit perturbation of each parameter, A_{ij}, the steady-state values of $\underline{S}$ for each storage variable may be used as a relative measure of system sensitivity to each parameter.

4. Frequency response analysis (CHILD and SHUGART, 1972; WEBSTER et al., In Press) provides frequency-related measures of system behavior. Both the referenced papers and current studies indicate that the sampling ratio (ξ) and the undamped natural frequency (ω_n) are well described by a second-order control system approximation of the system (KUO, 1962; DERUSSO et al., 1965). When the system is overdamped (most ecosystems appear to be so), then the undamped natural frequency becomes a measure of the maximum required sampling rate for system variables.

5. Component analysis (HILL, 1973) allows numerical determination of a limited number of coefficient values from the A matrix of the linear, donor-controlled representation and the system transfer function as determined from experimental input-output data.

Topological analysis can be used as an aid in evaluating the influence of connectivity upon process rates in the system. Flow analysis can provide a measure of steady-state distribution of flow through the process pathways. A preliminary sensitivity analysis can determine the effect of an error in parameter estimation upon storage levels and hence upon flows. These three evaluations of process-system interaction provide criteria for elimination of components that have the least effect on system behavior, thus systematically reducing the graphic representation. This results in information that may be used as a first approximation in choosing measurement methods and sampling rates for evaluation of system hypotheses.

MECHANISMS OF DISPOSITION

Much of the movement and fate of a given agent is dependent on the rate and nature of certain mechanisms or processes which do not differ

in character or principle between the various compartments. Explicit in this dependency are (a) the physical, chemical, and biological principles of behavior of the chemical and environmental component and (b) the organization of the constituents as described in the diagram.

It is convenient to divide these processes into two major groups: transport processes, where the agent is moved vectorially in association with an environmental component or by mass flow and diffusion; and kinetic processes, where the movement can be described by kinetic rate constants related more specifically and pointedly to the agent. When considering distributions of a chemical with respect to time, these diverse processes may play significant roles in determining whether a given disposition is flow-limited (by transport processes), compartmentalized (by kinetic processes), or some combination of both. The reference time frame, not specified for the conceptual model, is a highly significant parameter vital in translating the conceptual model to realistic simulations. Similarly, the spacial reference point (volume, location) has purposely been left vague to permit the general case to be stated with the understanding that specification of spacial and geographic localization will be carried out in translation and elaboration of the modules (Figures 1 and 3) into models.

Examples of transport processes can be seen in dispositions primarily dependent on stream flow, surface-to-ground water flow (leaching), blood circulation, xylem transport, and precipitation from air. Kinetic process-dependent dispositions may involve high or practically irreversible sorption or binding, differential rates of sorption or desorption between compartments of a major subsystem, or differential chemical alteration. The following are offered as the principle processes limiting or affording disposition of an agent in the environment. More than one process may be occurring simultaneously along the same route, so that the factors controlling the process determine the proportion going by a particular pathway, which in turn may alter that of another route.

Transport Processes

Convective Mass Transport ("leaching," "drift") -

This physical process operates in all environments, in both the gaseous and liquid phases, usually along the direction of mass flux. In SOILS it would depend strongly on the degree of soil saturation, in the ATMOSPHERE on the micrometeorological air flows, and in the AQUATIC environment on hydrodynamics.

Inter-particle Diffusion (linear, eddy, etc.) -

This process operates where chemical gradients or local turbulence exist. Viscous solvent drag effects (included in the commonly used term "dispersion coefficient") also operate.

Intra-particle Diffusion (absorption/de-absorption) -

Fickian chemical gradients act as driving forces causing chemical mass to enter and diffuse into or out of particulate matter itself. The structure of the particle, its degree of internal saturation with water, the size and diffusivity of the chemical, and the chemical's structure are important factors. Included in this category would be "exclusion-type" processes, where pore size of inorganic particulates may be large under one set of environmental conditions (pH, degree of saturation), permitting entry of chemical to sites unexposed under other conditions, and the subsequent trapping or binding of the agent therein when the conditions change.

Co-distillation -

Volatilization in association with water evaporation takes place at the soil/air and water/air interfaces and is highly dependent on the temperature, degree of soil moisture or amount of water surface exposed, and the chemical vapor pressure.

Sublimation from a Surface -

This might be regarded as a compartment with the barrier consisting of the heat of vaporization of the component and is significant for the outer portions of multi-layered chemical adsorbed or held on a surface exposed to the atmosphere.

Ingestion (includes feeding, drinking, imbibing, inhalation, pinocytosis, etc.) -

The mass of the compartment ingested moves into biota at rates highly dependent on age, physiological and nutritional status, species, season, temperature, availability of alternative foods or sources of water, etc. Several physicochemical and biological processes may be involved with the intimate uptake (absorption, facilitated or active transport, etc.).

Kinetic Processes

Adsorption-Desorption Phenomena (phase-surface interactions) -

The principle parameters of this movement are the enthalpy of sorption of the chemical and the activation energy of the surface. Hence the structure and properties of the agent and the total surface chemistry of the interface are critical. The nature and type of surface (composition of soil, tissue of animal, type of particle) and the surface area presented to the phase containing the "free" agent are important. This process is regarded as being represented by a pair of kinetic equations, the ratio of which rate constants is the measure of the equilibrium attainable between the surface and the medium. The residence time of the medium (rate of change in compartment contact), if small in relation to the rates of these reactions, may limit disposition. Where the rate of binding exceeds very greatly the rate

of desorption, the material may appear to be irreversibly bound. Where these rates are both substantially slower than the rate of media movement, the surface interaction will characterize the disposition. Moisture level, pH, and temperature, as they affect the chemical and the surface, will play major roles in this phenomenon.

Chemical Transformation -

The non-biological alteration of a chemical introduced into any part of the environment is dependent on the moisture, pH, and temperature of that environment, on the nature of reactive groups on the agent, and on the presence of catalytic sites (on particles, etc.). The nature and intensity of illumination additionally determines photochemical reactions. At very high temperatures (pyrolysis) both physical and chemical structure may be broken down to yield material in the vapor state. In biota, soils, and water, and to a much less extent in air, cation and anion exchange capacity coupled with eletrolyte levels determines ionic interactions which may alter the structure or availability of a chemical, such as by the formation of insoluble complexes. In some instances the chemical reaction phenomena are closely associated with adsorption-desorption processes, related nonlinearly to the extent of coverage by, say, soil or air moisture of the catalytic binding site where the reaction might be hydrolysis. To the extent that the media are suitable for reaction or provide a necessary reactant (e.g., ozone) these processes can appear to be compartmentalized in rate of disposition.

Biological Alteration (includes activation, degradation and conjugation) -

These processes are assumed to be catalyzed by enzymes, although similar or identical chemical or photochemical reactions may be taking

place at the same time (at reduced rates) in the same compartment or others. The great increase in rate of the enzyme-catalyzed reaction provides opportunity for compartmental differentiation of disposition. These reactions are highly dependent on species, status (physiological, nutritional, and previous chemical history), and route of exposure. They may: provide for an agent becoming more or less biologically active; for binding or conjugation in a form more or less available to other organisms, compartments, etc., without altering the potential biological activity; or for the covalent interaction of the agent with an enzyme, thus altering the capacity of the system subsequently to carry out alterations at the same rate (inhibition).

The biological effects of an agent are difficult to separate from disposition, inasmuch as one potential effect is to alter disposition routes and/or rates. Known pesticide-induced enzymatic reactions in both vertebrates and invertebrates include oxidation-reduction, hydrolysis, conjugation, and carbon-carbon bond cleavage. The enzyme activities induced may represent de novo synthesis of theretofore unexpressed genomes (microbial) or amplification of the rate of genome expression (higher animals). Biochemical alteration of environmental contaminants and agents can be viewed as a function of the expression of genetic material in coordination with the ability of the environment and the biological species to provide for synthesis of enzyme and cofactors to support the reactions. Changes in the course of this expression may be one of the biological effects interacting strongly on the disposition of a particular chemical.

Factors Affecting Disposition

As noted in the foregoing discussion of mechanisms, the disposition of chemicals in the environment is governed by physicochemical, physical, and biologic processes which can be related to properties of the chemical

Table I. FACTORS AFFECTING DISPOSITION OF CHEMICALS IN THE ENVIRONMENT

Mechanism, Pathway, or Process	Properties of Agent	Properties of Environmental Component	Environmental Control
Convective mass transport; Inter-particle diffusion	General-association with compartmental component	Vectorial flux; degree of saturation of immobile matrix by movement	Physical (water or air flow, soil movement); temperature and gross energy distribution
Co-distillation; volatilization/ Condensation	Size, diffusion coefficient in media; vapor pressure, latent heat of vaporization; interaction with media; intra-molecular interactions	Water evaporation rate, surface area, interaction with agent, degree of soil moisture, extent of saturation of air	Temperature, energy flux
Intra-particle diffusion	Size, diffusion coefficient in particle, chemical gradient	Structure (micro), degree of water saturation, alterations of structure by temperature, pH, ionic strength	Temperature, pH, humidity or soil moisture
Ingestion	General-association with compartmental component	Nutritional value to feeder, attractiveness (chemical or physical), availability of alternative foods, degree of competition with other feeders, nutrietional and physiologic status of feeder	General-biological structure of ecosystem, physical conditions affecting rate or choice of foods (temperature, season, light)
Adsorption/desorption	Structure, enthalpy of sorption (mono-layered); enthalpy of fusion (multi-layered)	Macro- and microstructure, surface area, activation energy of surface	Temperature, humidity or soil moisture, pH

Table I (cont)

Mechanism, Pathway, or Process	Properties of Agent	Properties of Environmental Component	Environmental Control
Chemical reaction phenomena	Structure (reactive groups); energy of activation, free energy of reaction, nature of mechanism	Structure (catalytic sites), energy of activation, reactive sites, degree of coupling to other systems providing reactants or removing products	Temperature, humidity or soil moisture, pH nature and quantity of light
Biological alteration (activation, degradation, conjugation)	Structure (reactive groups), energy of activation, free energy of reaction, nature of reaction mechanism, binding constant to enzymes acting on it	Genetic capacity for eliciting appropriate enzyme, nature of enzyme, species status (physiological, nutritional, psychological), sensitivity to agent (inhibition, synergism, toxicity), degree of coupling to other systems providing reactants or removing products, presence or absence of cofactors	Temperature, pH, humidity or soil moisture, biological structure of ecosystem, previous chemical history

and environmental components. Table 1 summarizes these to indicate those factors which should be known or determined in making judgments as to the probable disposition of the chemical. Obviously, all properties play some role in that disposition in the complex, real world. As modeling proceeds from the conceptual level to mathematical simulation, these values become the critical inputs, especially as the disposition is related over time.

SOURCES OF CHEMICALS RELEASED INTO THE ENVIRONMENT

Each of the major compartments of the model can receive direct input of certain chemicals as a result of the action of man. These inputs are derived from "sources," which can be defined as the places and activities leading to the release of a particular agent. A source may result in a variety of inputs into major compartments and subcompartments, and more than one source may have very similar input into a model of pesticide behavior. For example, if methoxychlor were sprayed on a forest in a diesel oil medium, this application ("source") would have inputs into the atmosphere (both gases and aerosols), on to the cuticular or dermal surfaces of biota, and on to the surfaces of soil and water. A source may be deliberate, accidental, or adventitous, but the inputs have been handled uniformly in the models.

The sources can be grouped generally according to the major compartments to which inputs are directed and according to the time frame in the history of a particular agent that it may enter a model from a source. The latter might be divided into preconsumption (synthesis and manufacture), distribution (transport, storage, consumption, application, or use), and disposal (dumping, release). A chemical plant might serve as a source of atmospheric release of a pesticide during manufacture, a site of accidental spills during storage and transportation, and then have to dispose of waste materials containing the agent in

sanitary landfill, so that it would be a source having several inputs. Other typical sources are shown in Table 2. For the purposes of models, we then should consider the specific nature of sources providing inputs into the environment.

Atmosphere

Considerable atmospheric input occurs upon application of the large class of organic chemicals used as pesticides; i.e., insecticides, fungicides, herbicides, and rodenticides. For example, DDT is commonly applied by spraying a liquid suspension or solution by aircraft or mobile ground equipment. WOODWELL et al., (1971) report that in aerial applications of DDT to forests in the northeastern United States 50 percent or less of the amount applied was deposited in the forest. The rest was dispersed in the air either in the gaseous form or as small droplets. While much of the airborne liquid droplet fraction settles to the ground nearby, a significant amount remains aloft, to become associated with other particles and distributed in the environment at distances far from the point of initial application.

Table 2. SOURCES OF CHEMICALS FOR THE TERRESTRIAL ENVIRONMENT

Phase of History of Chemical	Examples
Preconsumption	Manufacture, food processing, mining, refining
Distribution	Application of chemical in pest control, agriculture, or for public health purposes; unintentional release resulting from the use of products containing or made of chemicals which are not totally confined or immobilized; accidental spills in transport or storage
Disposal	Release of wastes in air or industrial and domestic waste water; landfill operations; incineration; dumping and discarding

The chemical input may be in the gaseous state or adsorbed onto particulates released into the atmosphere. Accidental discharge resulting from explosions, containment vessel failure, human error, or other accidents involving vehicles or devices for transporting chemicals can cause major problems in a local geographic area, but are probably minor when considered on a global scale.

Chemical input into the atmosphere through routine use of products made of chemicals not totally immobilized, either intentially or unintentionally, is of major concern. For their model, NISBET and SAROFIM (1972) had to estimate the amount of PCBs lost to the atmosphere by evaporation of hydraulic fluids, lubricants, dielectric fluids used in transformers, and various plastics which are manufactured using PCBs.

Flora

Except for the direct application of plant growth regulators and chemicals used in pest control and for other agricultural purposes, sources are generally separated from flora by atmosphere, soil, and water of the environment. With direct application, input may occur on the foliage and/or fruiting body; alternatively, soil or water applications are sources of indirect inputs.

Fauna

As with flora, few sources directly input into these compartments. Medical and veterinary application of drugs and medicines, cosmetic and hygienic dermal applications, and consumption of food and non-food items constitute typical types of deliberate exposure from sources. In occupational use and, to a lesser extent, the general public, exposure can occur by direct inhalation of vapors or absorption through the skin. Hence, concern has been evidenced for

workers breathing or otherwise coming into contact with chemicals present at relatively low concentrations for long periods of time or at relatively high concentrations intermittently for short periods of time. Direct or indirect application of chemicals to flora or fauna can constitute a significant input for animals higher in the food chain if residues of the chemical or its alteration products are retained in the food.

Inadvertant and accidental release or even purposeful misuse or abuse of various chemicals and chemical products can also be a serious and significant direct source of agents to fauna. While some such sources are moderated through the atmosphere, soil, water, or flora, opportunities arise for direct inputs to fauna under some such circumstances.

Soil and Water

Many of the direct introductions of pesticides into the environment are sources closely connected to the soil and water regions. Application of pesticides and fertilizers by spraying a solution, liquid suspension, or granular formulation are important inputs to the soil surface, subsurface and the aquatic surfaces on both a local and global scale. In addition to adventitious contamination by accidental spills, other usages, and leakage from sources, a local region becomes a source by dumping or discarding material or by creation of sanitary landfills. Since the latter are generally of greater scope, the subsequent infiltration by rainfall and movement of surface or ground water can be major inputs throughout the soil, as detailed by ELZY et al., (1974).

Once a chemical is introduced into the soil and/or water environments, those compartments may continue to act as a reservoir for long periods of time, leading to transfer of an agent to flora and fauna. Depending

on the time rate of change of the concentration of a chemical at a site of localization, the compartment may act as a "sink" (where an agent is effectively withheld from participation in the system) or as a "reservoir" (where flows and transfer permit participation). From the point of reference of a given species, a compartment may be either a reservoir (and thus a "source" of an input) or a sink. A breakdown product (such as DDE from DDT or methylmercury from mercury) may arise in the soil and biota and subsequently appear broadly in the environment, even though it was not manufactured or synthesized as such. Thus, as the ultimate repository of waste, unwanted materials, and the products for which the chemicals were manufactured or prepared, the SOIL and WATER have pervasive major inputs into other segments of the environment.

SECTION V
THE ATMOSPHERIC/TERRESTRIAL MODEL

The model consists of a set of assumptions derived from experiment, experience, and physical law that are set forth graphically to illustrate the principal components ("compartments") of the system, the means by which the chemical itself or the components bearing the chemical being modeled move or change in the environment, and the relationships between compartments vis-a-vis this movement. Also enumerated and elucidated are the factors affecting these routes, such as the characteristics of the chemical and compartment.

ASSUMPTIONS

1. Elements or components of the terrestrial environment considered are confined in a geographic and geophysical sense to a local environment consisting of "ATMOSPHERE," "SOIL and WATER," "FLORA," and "FAUNA."

2. These elements and their constituent aspects can be set forth as compartments, representing chemical reactors.

3. The model is directly applicable only to the agent; its breakdown products or metabolites are representable as parallel models following identical conceptual functions of disposition.

4. The interrelationships of compartments and the movement of chemicals can be represented by a chemical process flow sheet.

DEFINITIONS

1. ATMOSPHERE. The gaseous phase containing suspended aerosols and particulates above the earth and its biota.

    a. Troposphere. The portion of the atmosphere in direct contact with soil, water, and the biota.

(1) Suspended particulates. Solid matter, including certain microscopic biota, suspended in the atmosphere. Each particle has a surface subcompartment.

(2) Aerosols. Microscopic matter (solid or liquid) dispersed in the atmosphere, each with a surface subcompartment.

(3) Gases. The gases and vapor-phase components of the atmosphere.

b. Stratosphere. A compartment above the troposphere and below the mesosphere having components as in (1-a), but not interacting directly with the earth and its biota.

c. Mesosphere. A compartment below the mesopause and ionosphere and above the stratosphere having compartments as in (1-a and b), but not interacting directly with the earth except through the troposphere and stratosphere.

2. FAUNA. Biota excluding plants and microorganisms (except protozoans) and including not only the terrestrial surface species but also those living predominantly in the atmosphere and in the soil.

a. Man. Human beings representable by a subsystem of several compartments based on anatomical and physiological characteristics.

b. Higher carnivores. Those creatures feeding on primary carnivores (and perhaps in some instances on forms lower in the food web).

c. Primary carnivores. Those species feeding predominantly on herbivores and (to a lesser extent) primary producers.

d. Herbivores. Those species feeding on primary producers, usually plants and related microorganisms.

e. Soil-organisms. Those primary producers dwelling predominantly in soil and the scavengers of the plant and animal matter constituting the organic matter of soils.

3. FLORA. The biota including largely the photosynthetic primary producers consumed by man, herbivores, and soil organisms. Generally the plant is represented as having a subsurface portion (consisting of subcompartments for the root tissues and potential storage or fruiting bodies) and a surface portion (consisting of foliage and fruiting body), all surrounded by a cuticular compartment.

4. SOILS AND WATER. In addition to soil organisms (2-e), this compartment is separated into two regions containing the same components

a. Surface. The top portion of the soil, capable of interacting with the air directly.

(1) Surface water. The result of precipitation, ground water springs, etc., but distinct from streams, ponds, lakes, etc. (parts of the AQUATIC model); includes "free" water associated with soils and all solutes.

(2) Organic particulates. Colloidal materials in suspension including organic matter and decaying material derived from biota.

(3) Inorganic particulates. Inorganic soil structural materials (clay, silicates, minerals, etc.) and those insoluble materials of non-biological origin.

b. Subsurface. Similar to (4-a) but containing the ground water and associated soil water. Actually there exists a series of parallel plates or zones through the soil profile which will differ in composition, environmental condition, etc. The subsurface region indicated in this model is considered to be all that below the immediate surface in contact with the atmosphere.

PATHWAYS

The foregoing compartments are displayed in Figure 4. A compound introduced into the ATMOSPHERE (Figure 4A) may be in the vapor phase, as an aerosol, or in the form of a large particle. Chemicals in the vapor phase would be expected to adsorb reversibly to the surface of aerosols and other particulates, where the potential for alteration by chemical or photolytic means (due to catalytic sites thereon) is much greater than in the vapor phase. The particles might condense or break down, and chemicals would be redistributed. A chemical on the surface of a particle or aerosol could absorb reversibly into the particle, where photolysis would be very much less likely. All of these interactions would be taking place in the Mesosphere and Stratosphere as well as the Troposphere, which are mixed by diffusive and meterological conditions and events. Photolysis would be expected to

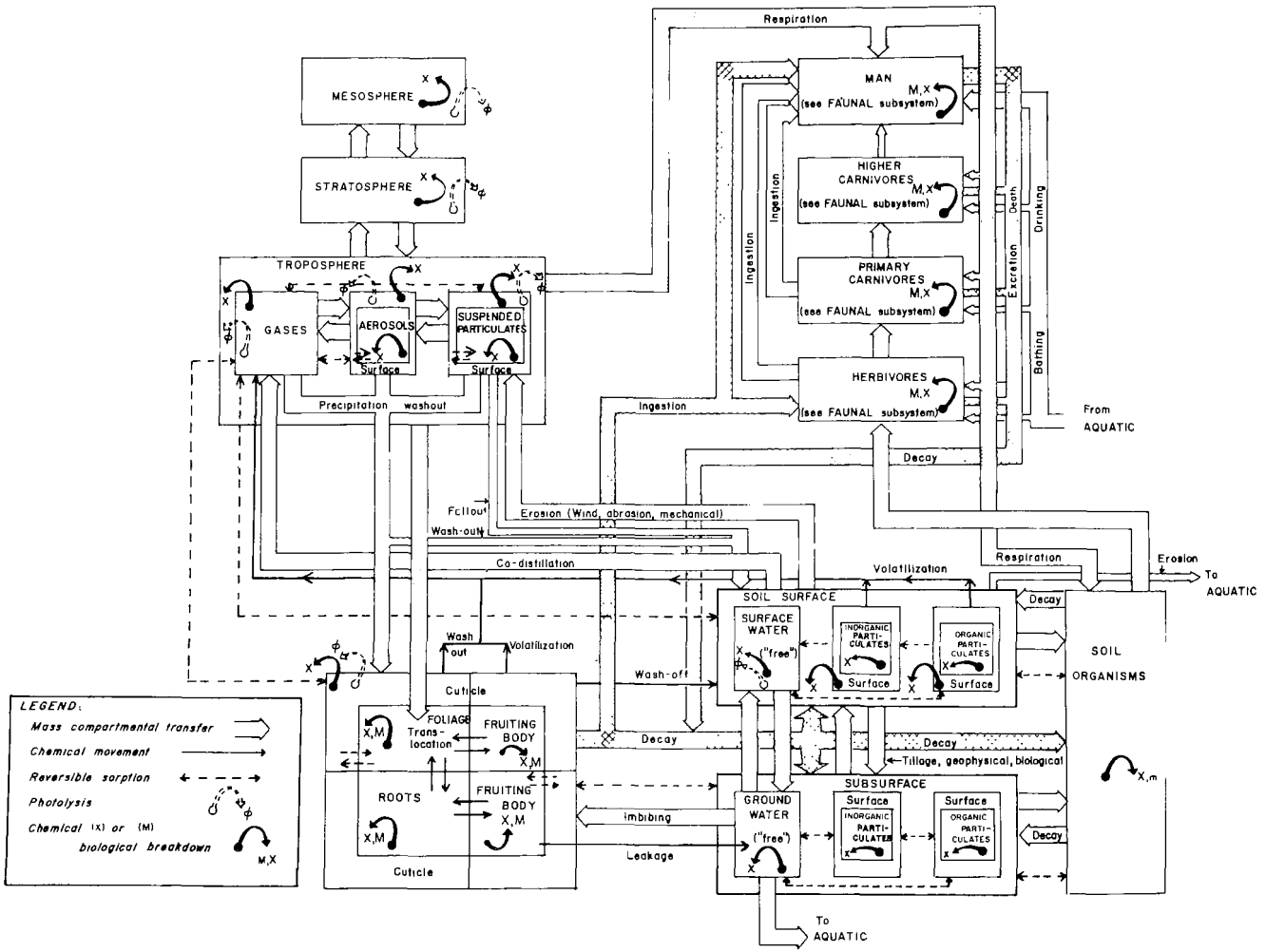


Figure 4: Diagram of the Atmospheric/Terrestrial Model. Flow and transfer functions are indicated by the legend. Focus on segments or major compartments of the model is provided by expanded views in Figures 4A-D.

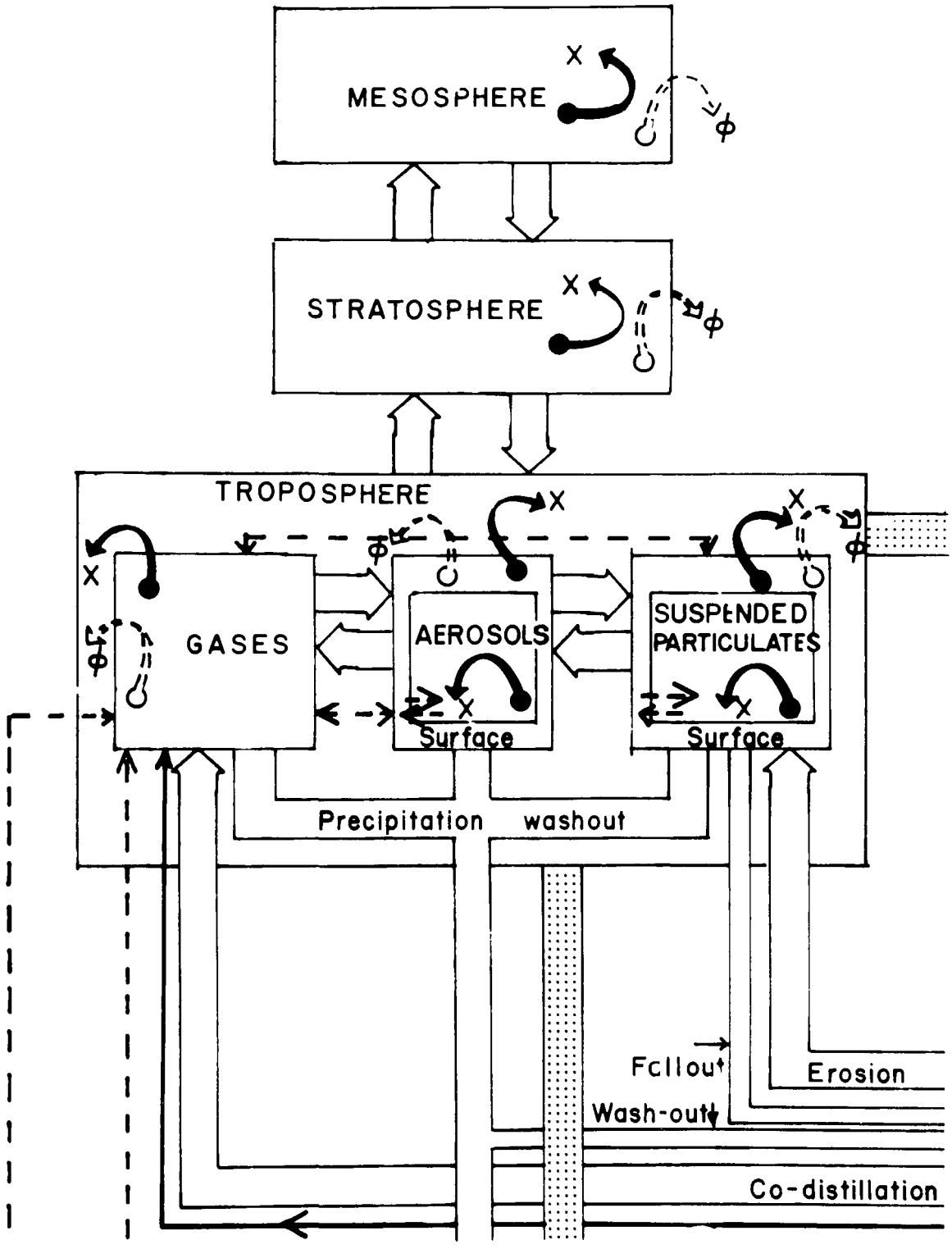


Figure 4A: ATMOSPHERE

play a progressively great role in the upper atmospheric compartments and, conversely, chemical reaction (except for ozonolysis) would be expected to be of less importance in those upper compartments. Iteration of the basic Troposphere model through the upper atmospheric compartments is easily accomplished.

Materials can enter or leave the atmospheric compartments by reversible sorption, interacting especially with SOIL and FLORA surfaces, or by volatilization/condensation from these surfaces. Particulate matter would settle out onto surfaces or be washed out by precipitation. Winds, mechanical action (such as abrasion), various modes of direct introduction (application, emission sources), and meteorologic aerosol formation in association with codistillation would result in particulate aerosol introductions into the ATMOSPHERE.

FAUNA, and to a lesser extent FLORA, would be subject to ingestion of portions of the tropospheric compartment by respiration, while sorption would provide dermal exposure. Inhaled particles not trapped in lungs, spiracles, etc., and particles or aerosols trapped on skin, hair, etc., and thereby subjected to grooming (e.g., fur licking), may be ingested with mucous. Air is also present in soils, in equilibrium with the soil surfaces re any component chemical; it likely plays little role per se in exposure of Soil Organisms and is therefore ignored. Depending on atmospheric mixing and soil movement, the exposure of Soil Organisms may be qualitatively and quantitatively different than the exposure of surface FAUNA via the air.

A schematic faunal subsystem (Figure 5) illustrates the probable inputs and outputs of the several compartments in Figure 4B (compare to Figure 3). Depending upon the food source, material from one or more of the other major compartments may be ingested, exposing the lumen tissue to the chemical in the food, air, or water. It may be sorbed, broken down within the lumen, and/or passed out with the

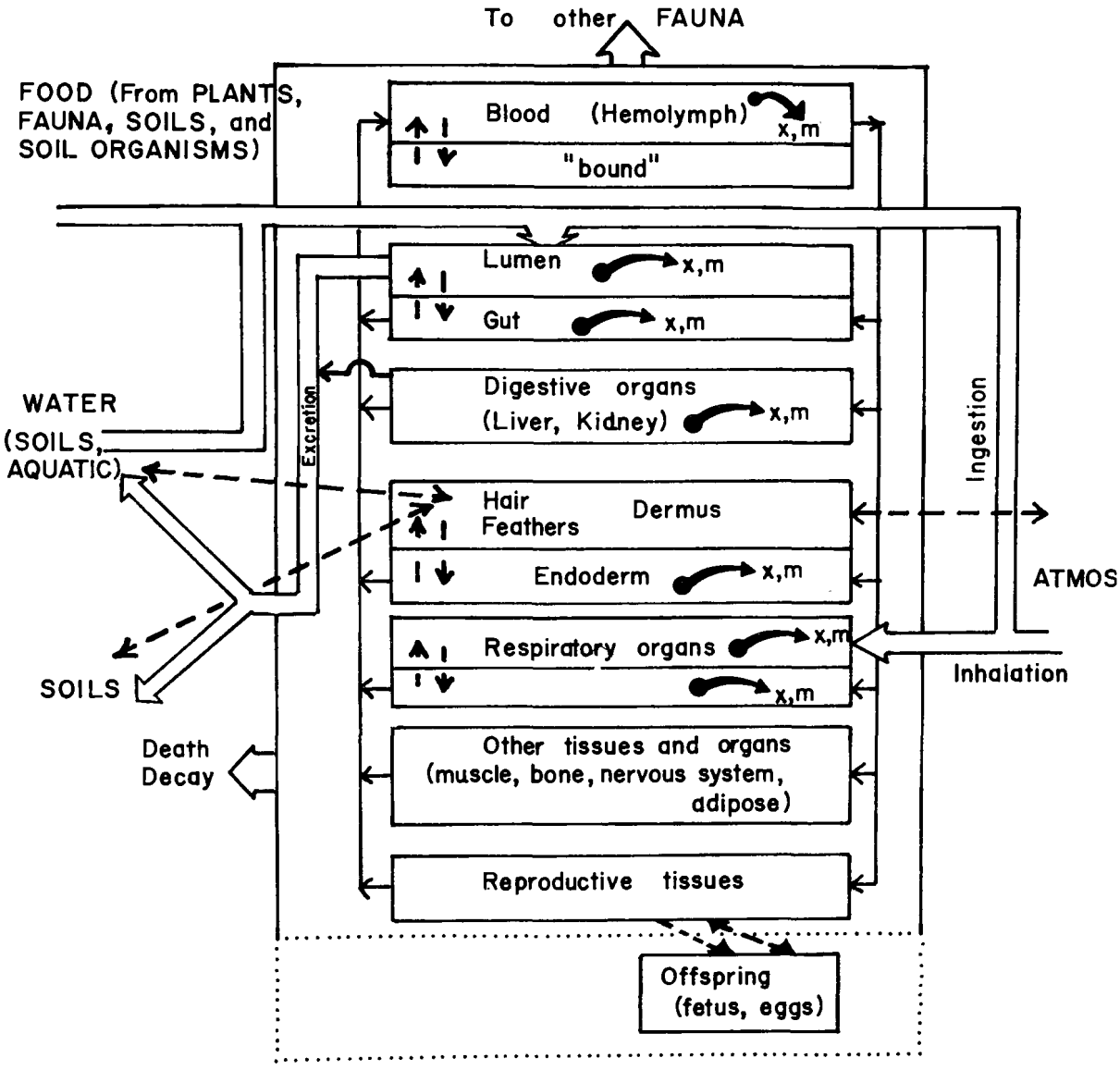


Figure 5: Diagram of Faunal Subsystem Model. (Based on BISCHOFF and BROWN, 1966). Arrows indicate flows or transfers of agent and/or compartment mass.

fecal matter, which then passes on to the SOIL or as food for other FAUNA. The portion absorbed may be distributed throughout the organisms to other tissues, which may alter the agent's structure to more easily excreted products, store the agent (for later release or for ingestion by a predator species), or provide for the agent's excretion.

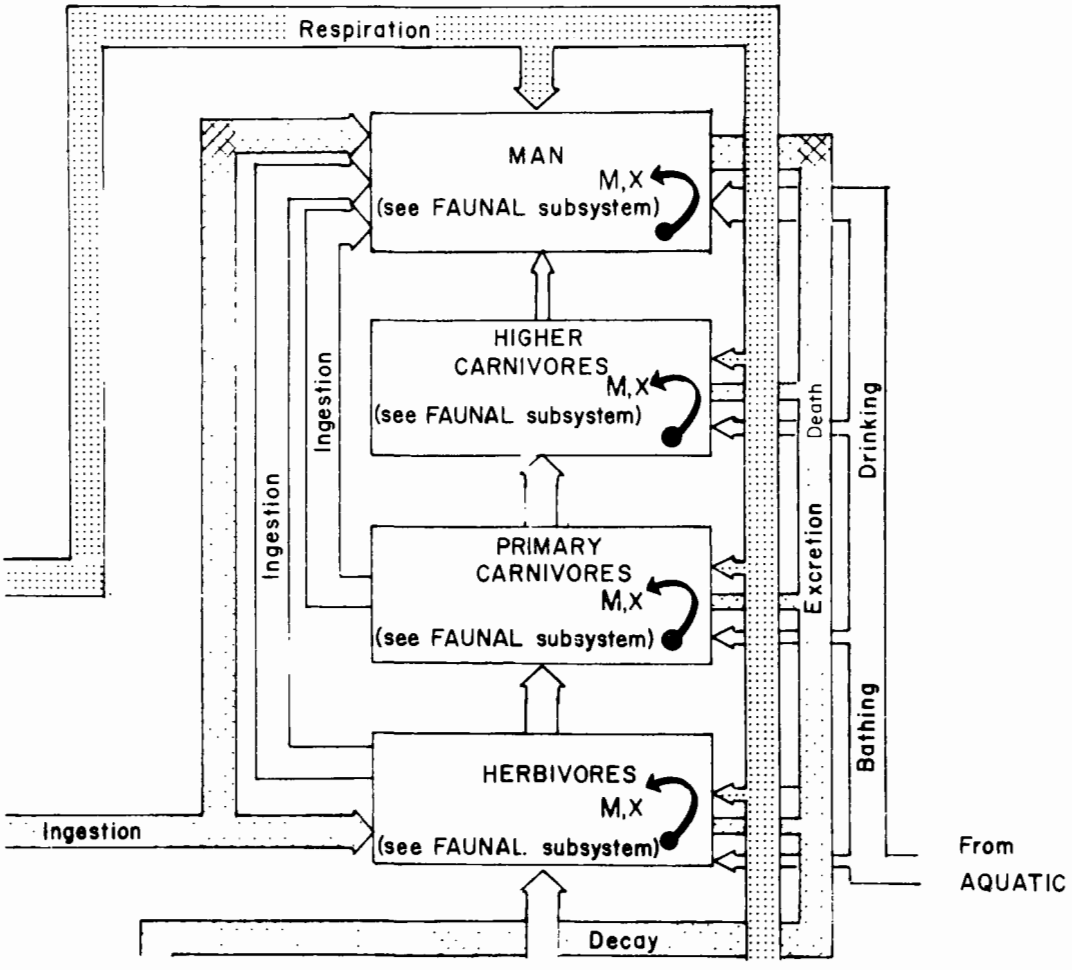


Figure 4B: FAUNA

In the higher vertebrates this process is complicated by functions such as the enterohepatic cycle (gut → liver → bile → intestinal lumen). In higher animals material may be lost through the skin, hair, or feathers. As noted earlier, these tissues also receive exposure from the ATMOSPHERE (and some instances SOIL). Some agents may be altered externally and some may not penetrate the dermal barrier. Unabsorbed material could volatilize or be adsorbed by atmospheric particles. Except for exhalation of unadsorbed material, pulmonary losses of chemicals taken into animals by other routes appear negligible.

Agents are also distributed to reproductive tissues, which can constitute a major outlet of chemical for the exposed animal. In female mammals this release can continue on through parturition into lactation. The route to offspring may be of great significance, since the young of many species serve as food for higher trophic levels.

Another major loss route, in addition to excretion, is the death and decay of tissues and organisms, leading to the entry of the material into the SOIL and WATER compartments (Figure 4D). Initially on the Soil Surface subcompartment, these materials become part of the organic particulates and later free water of that compartment, but are transferred by mechanical, geophysical, and biological action into the Subsurface compartment. Soil Organisms then ingest these particles, and one could propose an elementary version of the scheme in Figure 5 for disposition of the chemical in those organisms. Additionally some Soil Organisms may be purged of some chemicals by reversible sorption of materials in the gut lumen onto the out-going soil particles.

The other major biological compartment is that of FLORA (Figure 4C), represented as a generalized model with both Subsurface and Aerial

portions surrounded by a waxy cuticle. Materials can be deposited on this latter surface by fallout or precipitation, by condensation, or by reversible sorption. Some agents can pass on through into plant tissue or may be broken down chemically or photolytically on the surface. A portion may be washed off the leaves and added to the SOILS compartment. Material bound to the foliage will subsequently enter litter as decay occurs. An agent on the foliage may be volatilized off or sorb onto air particulates.

In the Subsurface zone, material may be brought into the plant by uptake of water or by sorbtion onto the root surface and subsequent penetration of the cuticle. Some may "leak" out or be released to the SOIL from the cuticle. Both the Aerial and Subsurface portions of FLORA are subject to herbivorous feeding, moving material into the Soil Organisms and other FAUNA, and an agent in either compartment is subject to chemical or biochemical alteration. Once an agent is in FLORA, it may be translocated to other tissues, including fruiting bodies associated with either portion. Similar to animals, a given species of herbivore may select only a limited tissue on which to feed; all portions of a plant are seldom ingested by a single creature at one time. Distribution within the plant of a given agent would therefore have a very marked effect on the subsequent nature and extent of movement of a chemical from FLORA to other major compartments. All of these movements would be less complex in photosynthetic microorganisms.

"Bound" agents, including material strongly sorbed (seemingly irreversibly) and material covalently reacted (but bearing the active groups intact) are difficult to define and determine. Some of the "bound" residues may be released by extraction, when sorption is reversed, or by chemical or enzymatic treatment, where the conjugating bonds are cleaved. In both FLORA and FAUNA (including Soil Organisms) materials considered metabolized or altered so as to leave the scheme may re-enter a compartment as a result of such action.

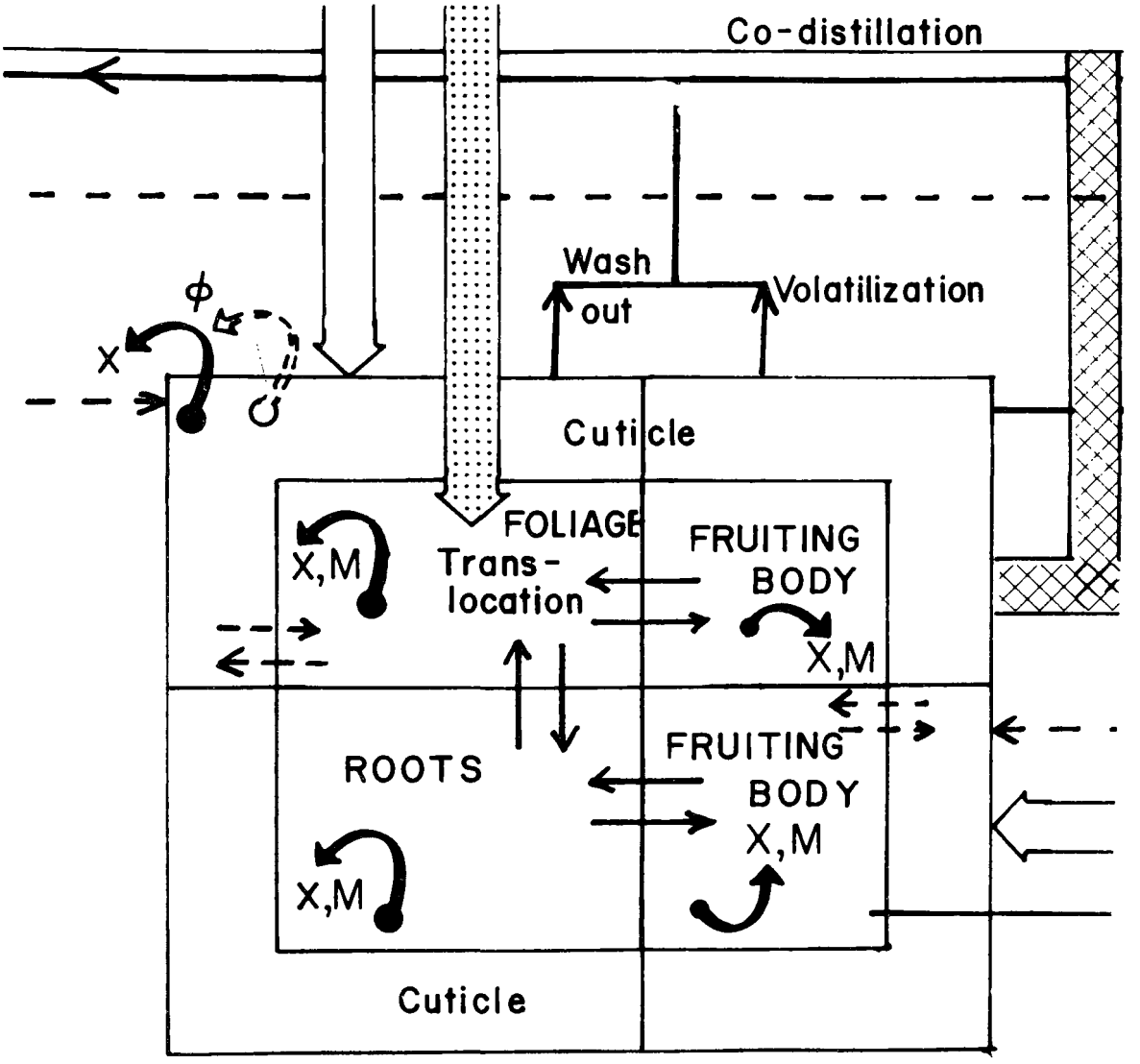


Figure 4C: FLORA

The most complex and probably most significant compartment in the disposition of an agent entering the terrestrial environment is that of SOILS and WATER (Figure 4D). Material can enter this compartment directly at the Surface by sorption from the ATMOSPHERE, condensation,

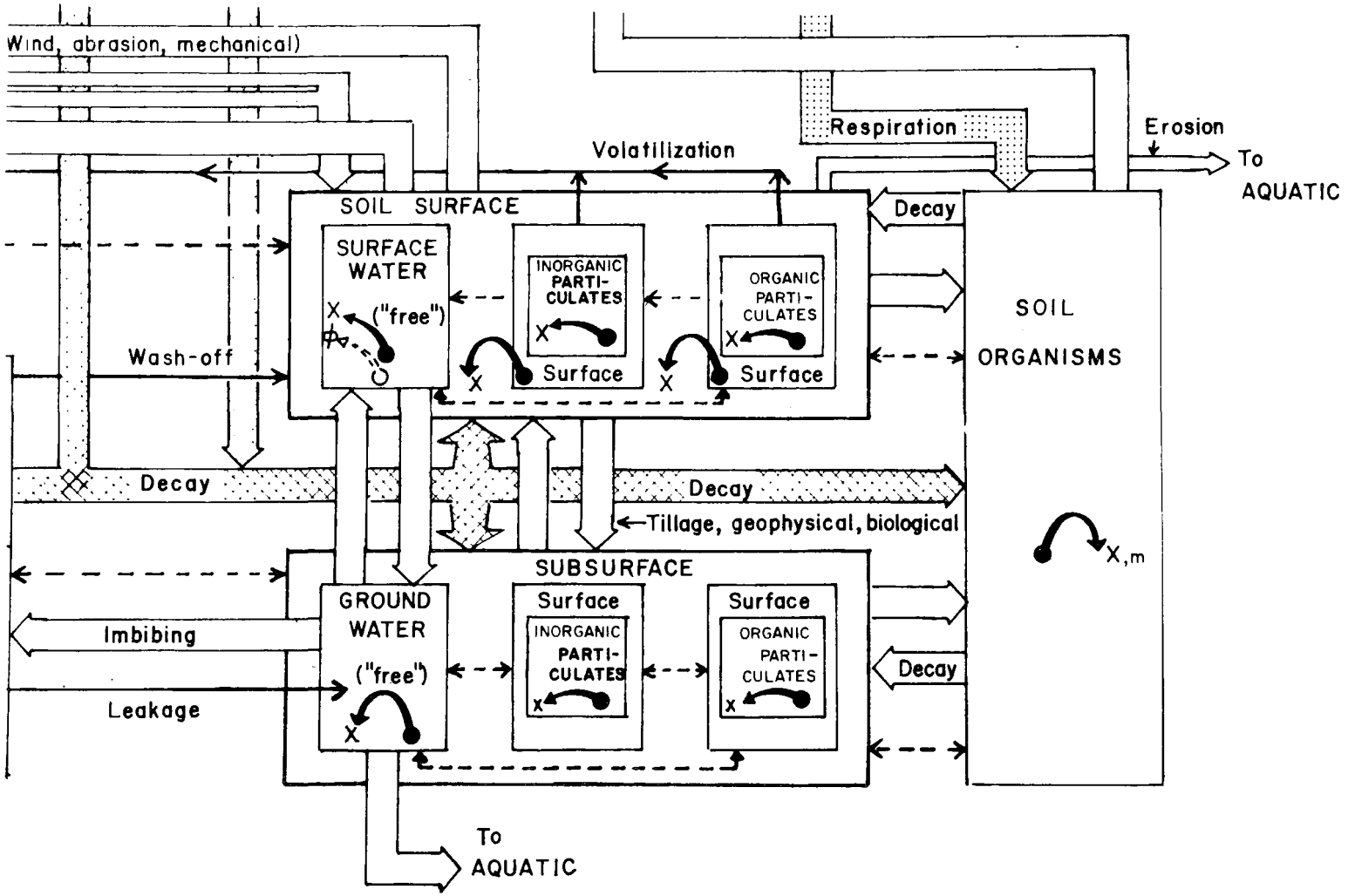


Figure 4D. SOIL and WATER

settling and fallout, and precipitation (including material washed off of plant surfaces). Excretion, exfoliation, and decay of animal tissues and defoliation, withering, litterfall, and subsequent decay of plant materials add to the routes of entry. Material can leave the Soil Surface by erosion (wind, water, or mechanical), by volatilization, by photochemical and chemical alteration, and by ingestion by Soil Organisms and other FAUNA. Some material is lost from the Surface by tillage, mechanical mixing (geophysical or biological), and "leaching." The movement of Surface water into the Ground water takes with it solubilized and reversibly sorbed materials. Within the Surface compartment, much as in the case of the Troposphere, materials can be bound to the surfaces of particulates. Surface waters can become contaminated by a Ground water-source containing an agent, which would then be distributed in the Soil Surface.

In the Subsurface zone, material can enter from the Soil Surface, be brought into the zone by Soil Organisms or FLORA (through translocation, leakage, and root decay), and can leave by routes noted earlier--sorption into FLORA and Soil Organisms (and to a lesser extent, other FAUNA), ingestion by Soil Organisms and FAUNA, and through the Ground water into FLORA and out into other waters (streams, lakes, estuaries--labeled AQUATIC). In actual cases, it would be necessary to characterize each soil horizon by iteration of interconnected Subsurface models.

SECTION VI
THE FRESHWATER AQUATIC MODEL

This section develops a systematic approach to an optimal representation of the behavior of pesticides in aquatic environments. A quantitative discussion of processes and parameters important to the fate and transport of pesticides-in-general is futile because of the diverse chemical and physical properties of pesticides. This is further complicated by the need to specify chemical, biological, and physical characteristics of the aquatic ecosystem. Therefore, a qualitative approach to studying and modeling the fate and transport of pesticides in aquatic ecosystems is discussed.

There has been a shift in many areas of science toward studies of wider scope. This has been brought about partly by increased emphasis on "the environment" and partly by wider knowledge of the techniques of system studies. According to MOORE (1967) the emphasis in pesticide studies has shifted from

Pesticide → Pest

to

Pesticide → Ecosystem

Past pesticide research resulted in few system studies and fewer mathematical analyses of such studies.

One area of system studies is that of microcosm or partial system studies (METCALF et al., 1971). These studies emphasize a particular short food chain largely as an index for comparison of various studies. Their quantitative applicability to real-world ecosystem is therefore limited. Nevertheless, they provide the basis of a large portion of our comprehension of the behavior of pesticides in the environment.

Global model studies are important in setting an overall framework within which smaller system studies may be placed. RANDERS and MEADOWS (1971) studied the movement of DDT in the environment, and WOODWELL et al., (1971) made a similar study. An important conclusion of both these papers was that the DDT concentration in food chain organisms would continue to increase long after the rate of application was decreased or terminated. This conclusion was based on computer simulation studies and comparative analyses.

Smaller system studies of greater detail bring us closer to interactions at the ecological level. Analyses of pesticide transformations and transports at the ecological level may make use of both ecological theory and various applications of systems theory. For example, EBERHARDT et al., (1971) applied system simulation to a field study as an aid in interpreting the data.

The above examples deal with specific pesticides in relatively defined ecosystems and are not generally applicable to a description of fate and transport. The presentation that follows is applicable to pesticides and aquatic ecosystems in general but can also be used as a starting point for any specific pesticide and system.

SKELETAL DIAGRAMS FOR A PESTICIDE IN THE AQUATIC ENVIRONMENT*

The most effective aggregation of storage components and rate processes varies as attention is turned from one aquatic regime to another. Even within the range of lentic systems, the diagrammatic representation for a deep dimictic lake would be inappropriate when used for a freshwater marsh. For this reason, several basic frameworks or skeletal models without detailed process embellishment are presented for different aquatic environments.

---

*See Appendix for detailed background.

The first skeletal diagram in Figure 6 is intended for a dimictic lake in which process dynamics are affected by the presence of a strong thermocline. The division between epilimnion and hypolimnion may allow for long-term storage and release from the sediments of the reduced forms of some chemical species (HUTCHISON, 1957; O'MELIA, 1972). The surface layer is isolated as a storage component in this vertical model because of the possibility of enrichment in heavy metals and pesticides (DUCE et al., 1972) and the neuston food web. The sediments are treated as a separate storage unit because of possible long-term storage (AHR, 1973), sorption-desorption process rates (HUANG, 1971) and the benthic food web.

A similar vertical skeletal structure without the hypolimnion may be used for a holomictic lake or a freshwater marsh. However, a shallow lake or a marsh may be better represented by a horizontal structure (SCHINDLER, 1974) as presented in Figure 7. Here the storage is divided among aquatic communities, which have varying response times and process rates.

The independent variable implied in both of these lake models is time. However, either one may be used as a two-dimensional stream or river model by choosing longitudinal distance (i.e., downstream) as the independent variable and including hydraulic and morphologic effects on settling and mixing.

Finally, the lotic system may be represented by a horizontal array of vertical column structures (similar to that of Figure 6) with longitudinal distance as the independent variable. The transfers between columns represent the transverse mixing in the system (HOLLEY and ABRAHAM, 1973). This concept is presented in the diagram of Figure 8.

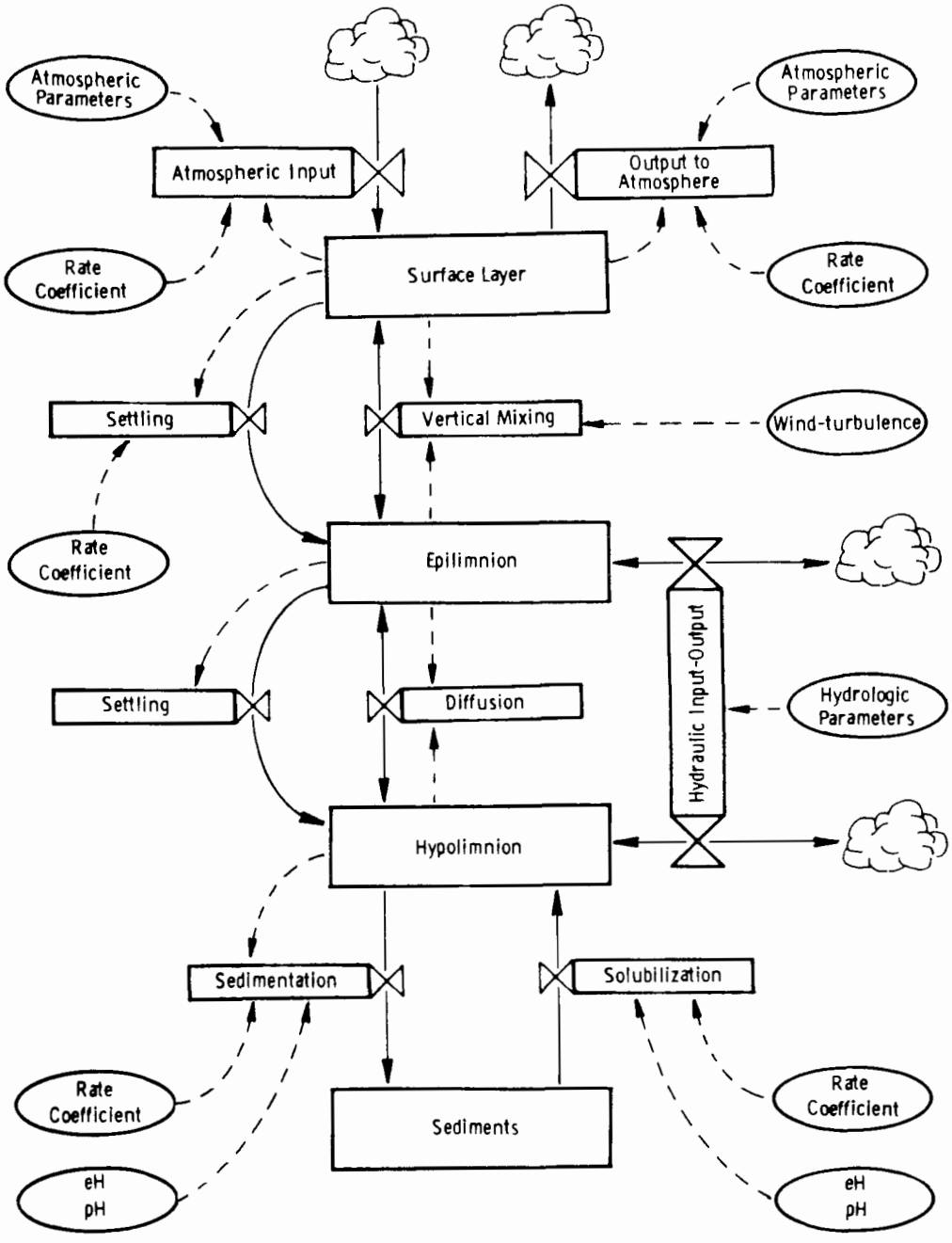


Figure 6: Vertical Representation of a Stratified Lake. Solid line - pathway of transfer of matter or energy; dashed line - control pathway of information transfer; cloud symbol - sink or source external to system boundaries; rectangle - storage of matter or energy; valve symbol - rate-controlling parameter or force; and circle - coefficients and parameters affecting flow rates. (Similar for Figures 6-13.)

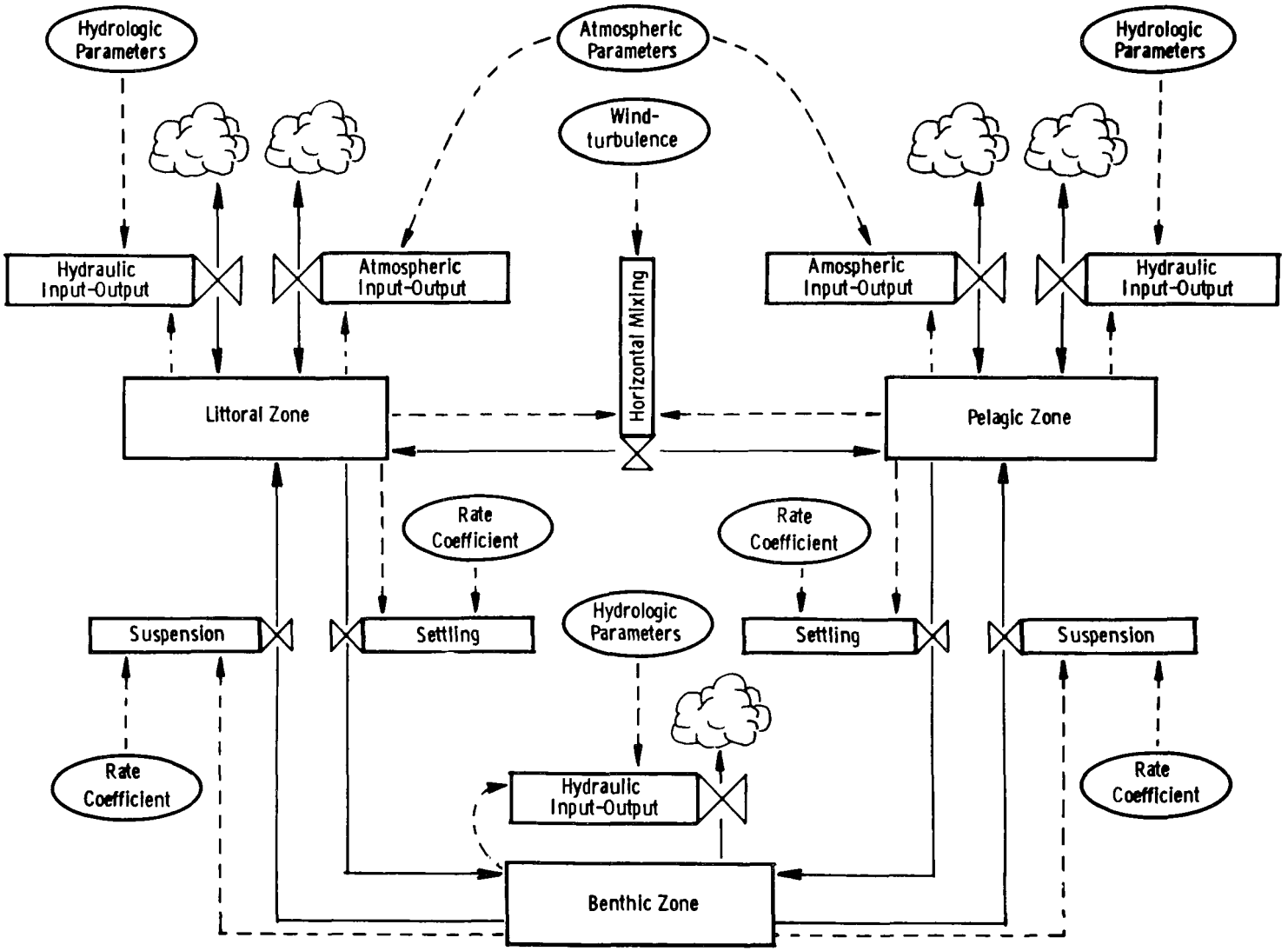


Figure 7: Horizontal Representation of a Lake.

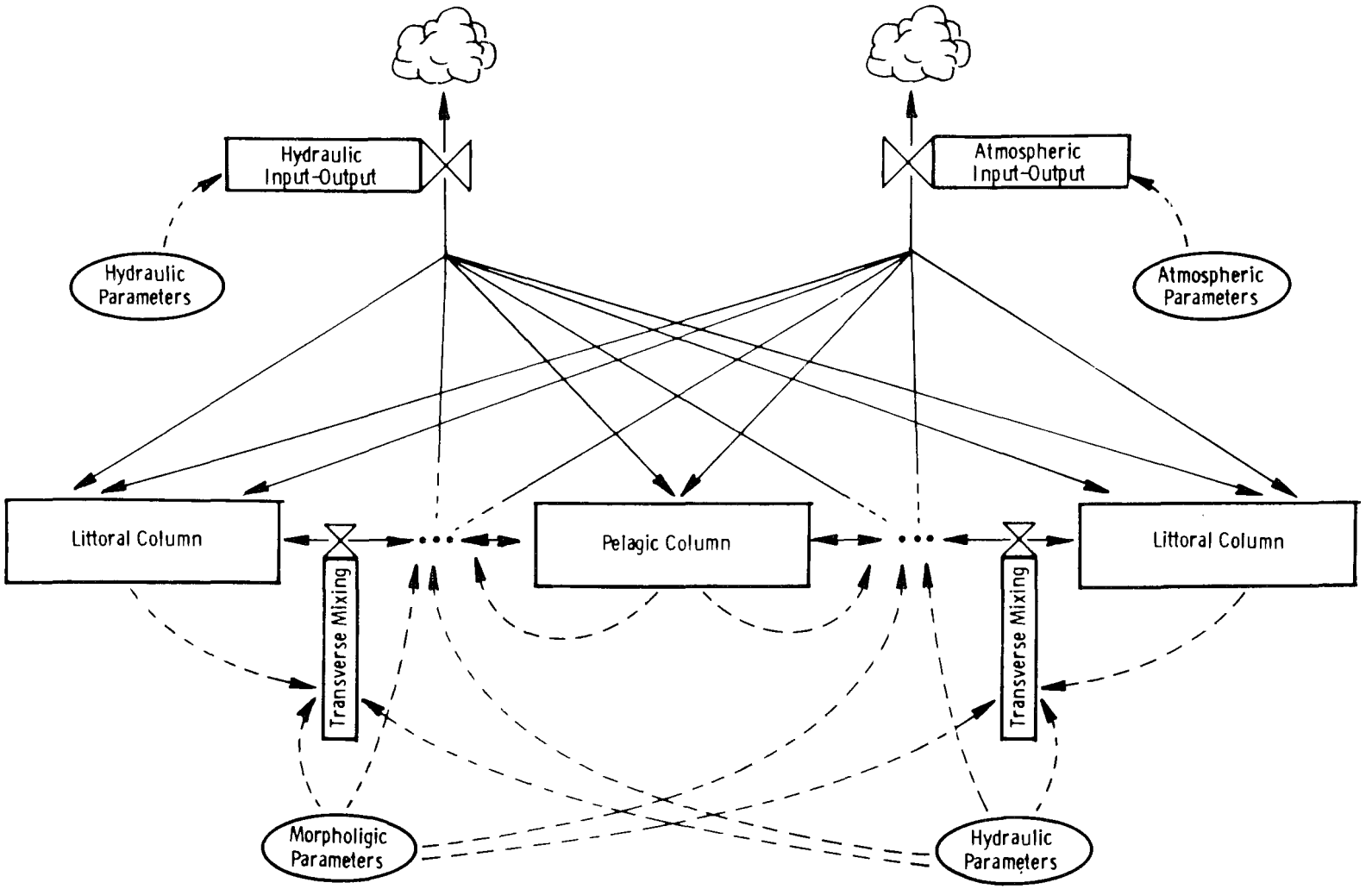


Figure 8: Horizontal Array of Vertical Columns for Representation of Lotic Systems.

In all of the preceding skeletal representations each storage component may be divided into discrete physical, chemical, and biological storage components with their associated transfers, rate coefficients, and coupled subsystems (e.g., food web biological uptake and storage with its associated growth, respiration, and trophic dynamics). A typical expansion of the surface layer storage component from the vertical representation of a lake in Figure 6 is shown in Figure 9.

Inputs and outputs may be expanded in a manner similar to the storage based upon their physical and chemical characteristics (dissolved, particulate, or absorbed). Expansion of the storage compartments may be necessitated by expansion of the hydraulic input-output to the epilimnion (Figure 10) into three separate inputs and outputs.

The storage labeled "Food Web" in Figure 9 is at the heart of most problems concerning pesticides in the environment (MOORE, 1967). A skeletal abstraction of a food web structure is shown in Figure 11 (compare to Figure 3). One method of allowing for the influence of food web dynamics upon the pesticide storages and fluxes in the model is to assume that the biomass levels in the aggregates representing the food web are at a steady state. Under this assumption the influence of the food web may be included in rate coefficients that affect the flux of pesticide between storage components representing pesticide concentration in the food web compartments. This is exemplified in the compartment diagram for the linear, donor-controlled model of DDT in the freshwater marsh of Figure 12.

Another method of allowing for the effects of the food web on pesticide behavior is to couple the explicit representation of the food web to the related pesticide storages. Thus, there will be two storage components coupled together in a manner similar to that of

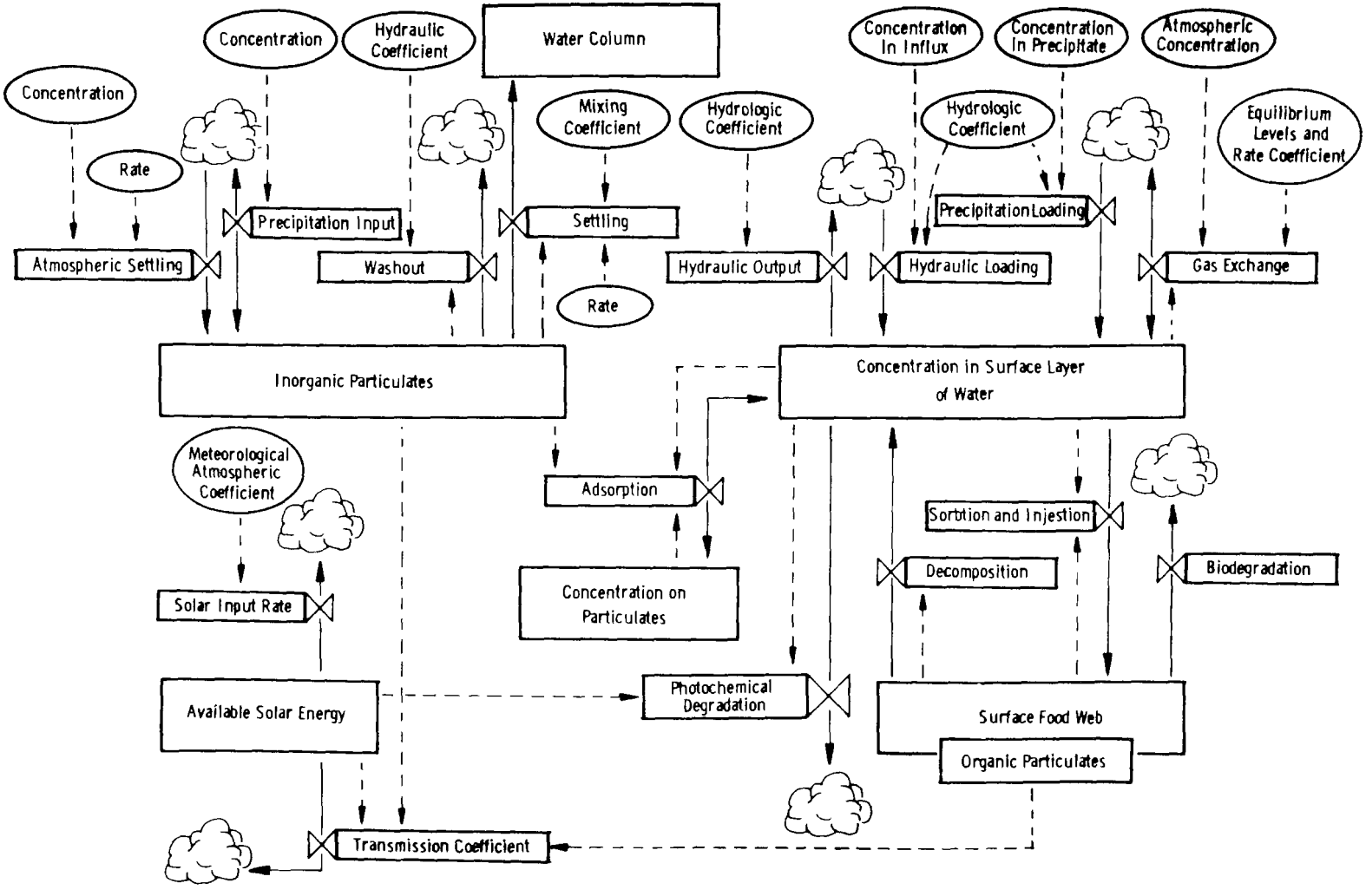


Figure 9: Some of the Storages, Processes, and Subsystems Associated with the Surface Layer Storage Compartment.

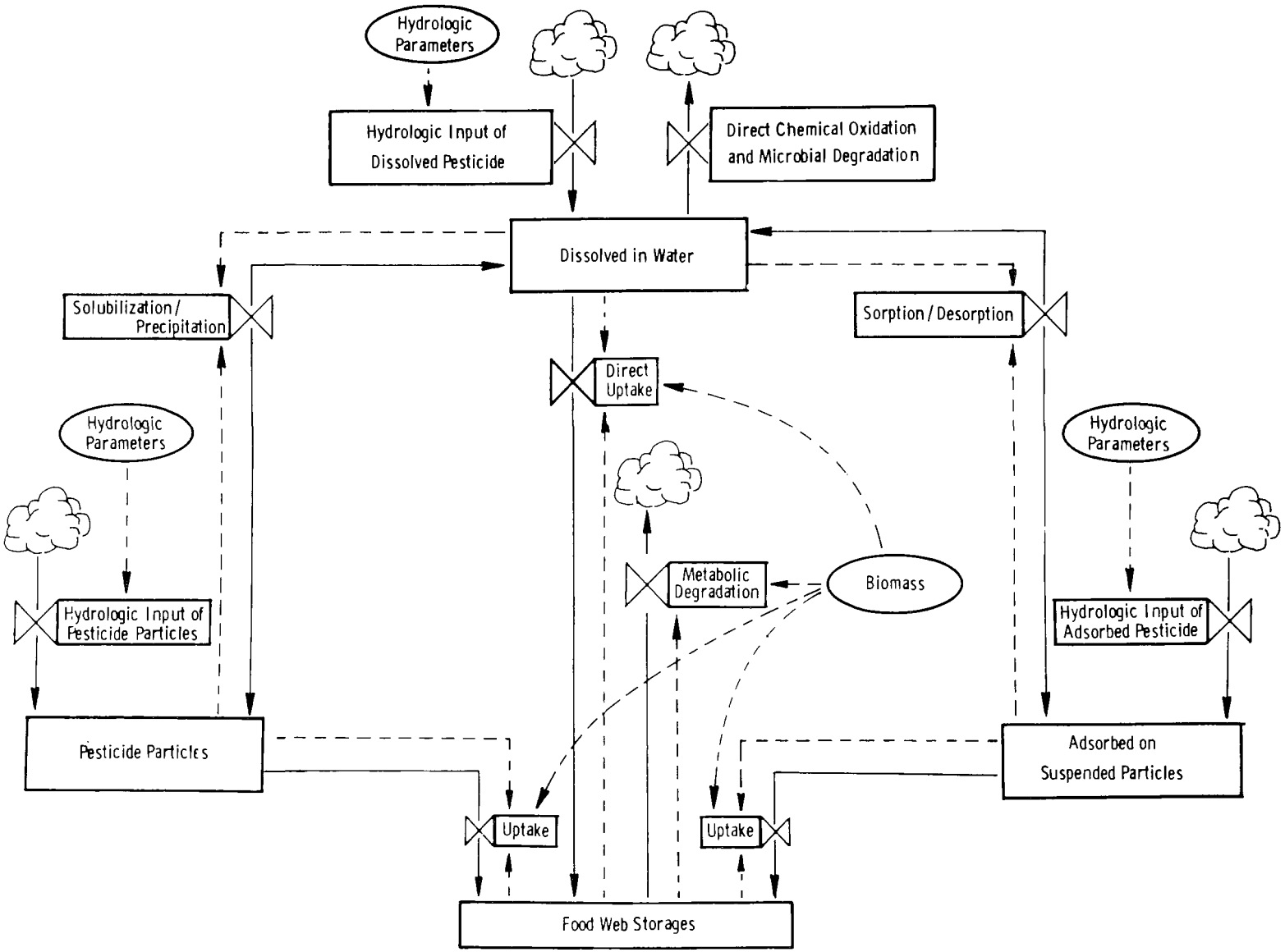


Figure 10: An Expansion of the Hydrologic Input.

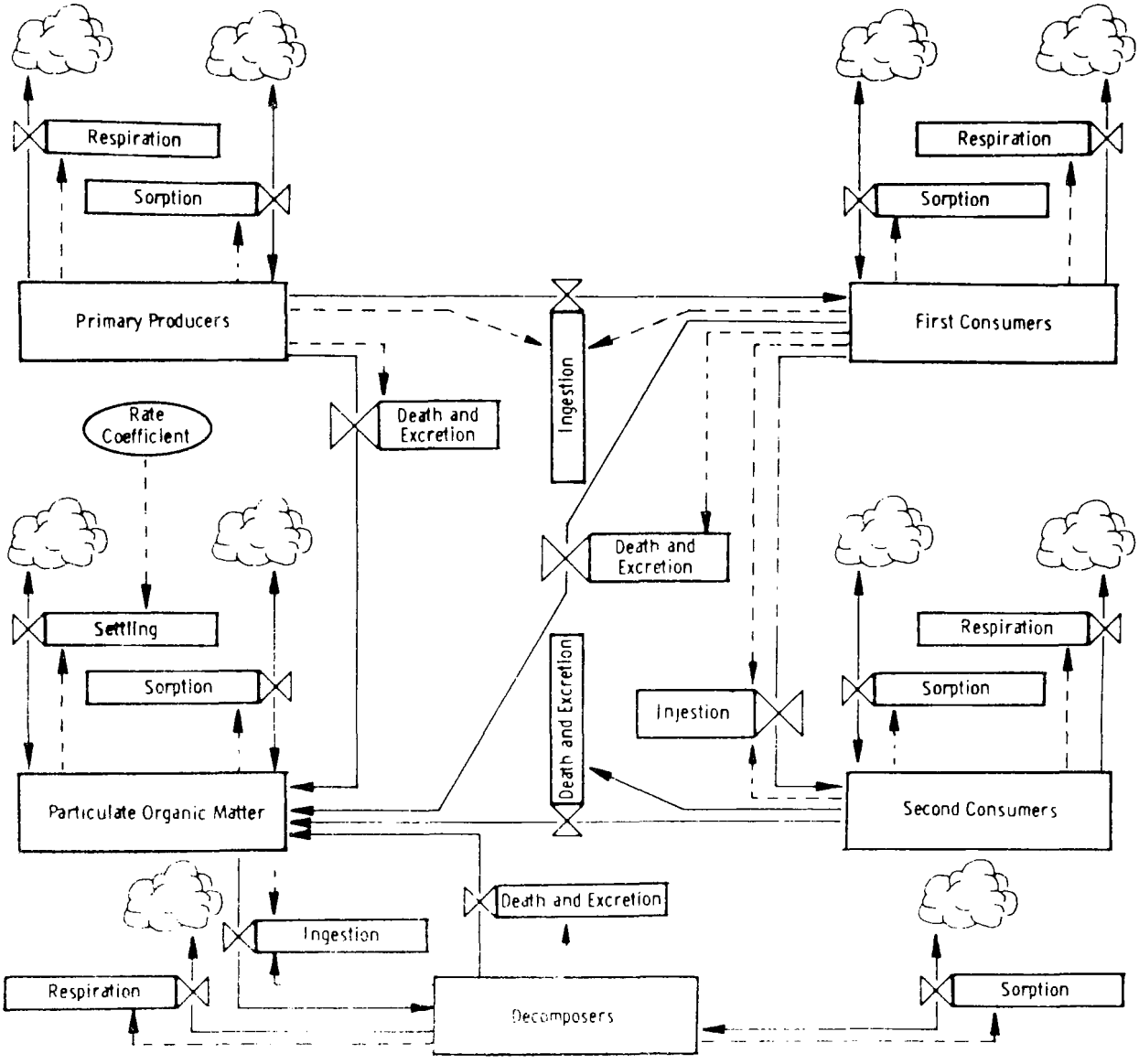


Figure 11: A Skeletal Abstraction of a Food Web.

Figure A-5 (see Appendix) for each storage of pesticide in a biotic component. The mathematical representation of this type of interaction is presented by HARRISON et al., (1971).

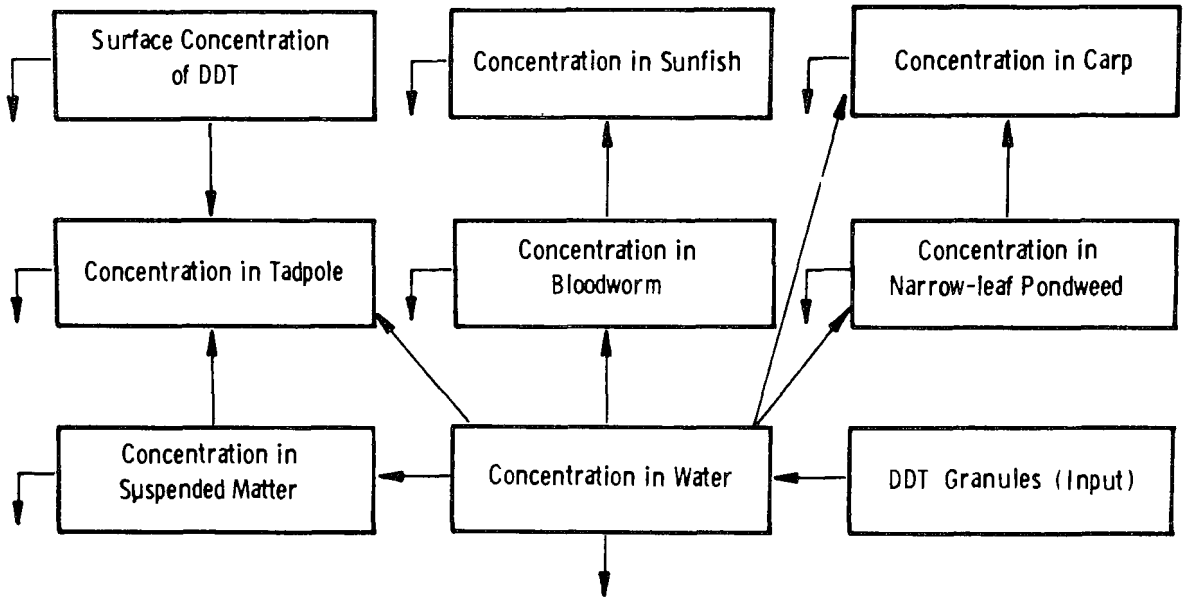


Figure 12: Food Chain Model of DDT in a Freshwater Marsh (from EBERHARDT et al., 1971).

Remembering that there is no best or correct representation, a plausible general model for a pesticide in the aquatic environment based upon the vertical skeletal structure is presented in Figure 13, an example of the result of the process of expanding the diagrams to include the system storages or processes that may be considered important. The system processes included in Figure 13 are intended to constitute a minimal set of parameters to be considered when investigating the movement and impact of a pesticide in the aquatic environment.

In a typical mathematical representation, which may be derived from the system diagram, each of the storage blocks accounts for one of a set of simultaneous differential equations. Also, each of the valve symbols accounts for a rate term in the set of equations. In addition, the coefficients or parameters in circles (many of which are omitted from Figure 13 for the sake of visual simplicity, e.g., pH, temperature, and Eh) appear as rate modifying coefficients in the equations. Each of these coefficients must be estimated from the literature or determined by a set of measurements on the system. Thus even the minimal set of variables of Figure 13 results in a complicated set of mathematical equations and requires a large data base for evaluation.

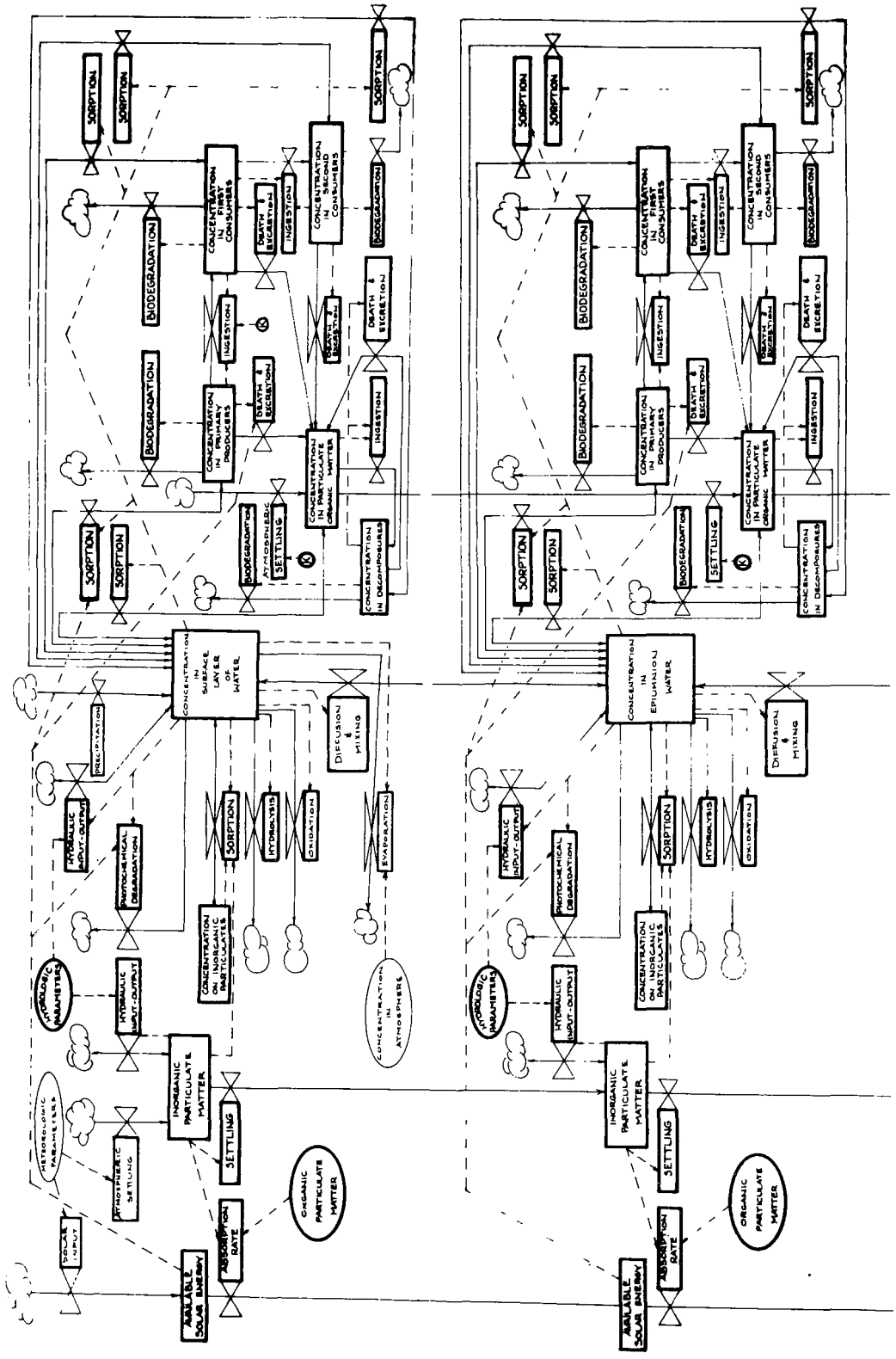
This complex representation can be reduced by aggregation of the least important variables for a particular pesticide and ecosystem. The inclination to eliminate the least important variables is usually intuitively focused either on very rapid processes, "which cannot be rate limiting," or conversely on very slow processes, "which cannot transport or transform much matter or energy," depending upon the investigator's objectives. The possible dangers in using these bases for eliminating variables lies in the synergistic behavior of causally closed environmental systems.*

The effect of an individual process on system behavior is dependent upon four levels of system interactions. These are

a. the rate coefficients and parameters for the process itself;
b. the hypothesized topology for the system interactions as presented in the system diagram;

---

*An ecosystem is a completely connected system (COMMONER, 1971) that is closed in the control sense (HUTCHISON, 1948; PATTEN, 1973). A closed control system or feedback system may exhibit "emergent properties" (CANNON, 1967) or "synergistic effects" (ODUM, 1971) that are dependent upon system structure or total system properties (BERTALANFFY, 1968). These properties can affect the influence of a specific process upon system behavior.


SORPTION
BIODEGRADATION
CONCENTRATION IN FIRST CONSUMERS
DEATH & EXCRETION
INGESTION
CONCENTRATION IN SECOND CONSUMERS
CONCENTRATION IN PRIMARY PRODUCERS
CONCENTRATION IN PARTICULATE ORGANIC MATTER
ATMOSPHERIC SETTLING
CONCENTRATION IN DECOMPOSERS
CONCENTRATION IN SURFACE LAYER WATER
CONCENTRATION IN EPILIMNION WATER
DIFFUSION & MIXING
PRECIPITATION
HYDRAULIC INPUT-OUTPUT
PHOTOCHEMICAL DEGRADATION
HYDROLYSIS
OXIDATION
EVAPORATION
CONCENTRATION IN INORGANIC PARTICULATES
CONCENTRATION IN ATMOSPHERE
METEOROLOGIC PARAMETERS
HYDROLOGIC PARAMETERS
INORGANIC PARTICULATE MATTER
ATMOSPHERIC SETTLING
SETTLING
ORGANIC PARTICULATE MATTER
SOLAR INPUT
AVAILABLE SOLAR ENERGY
ABSORPTION RATE

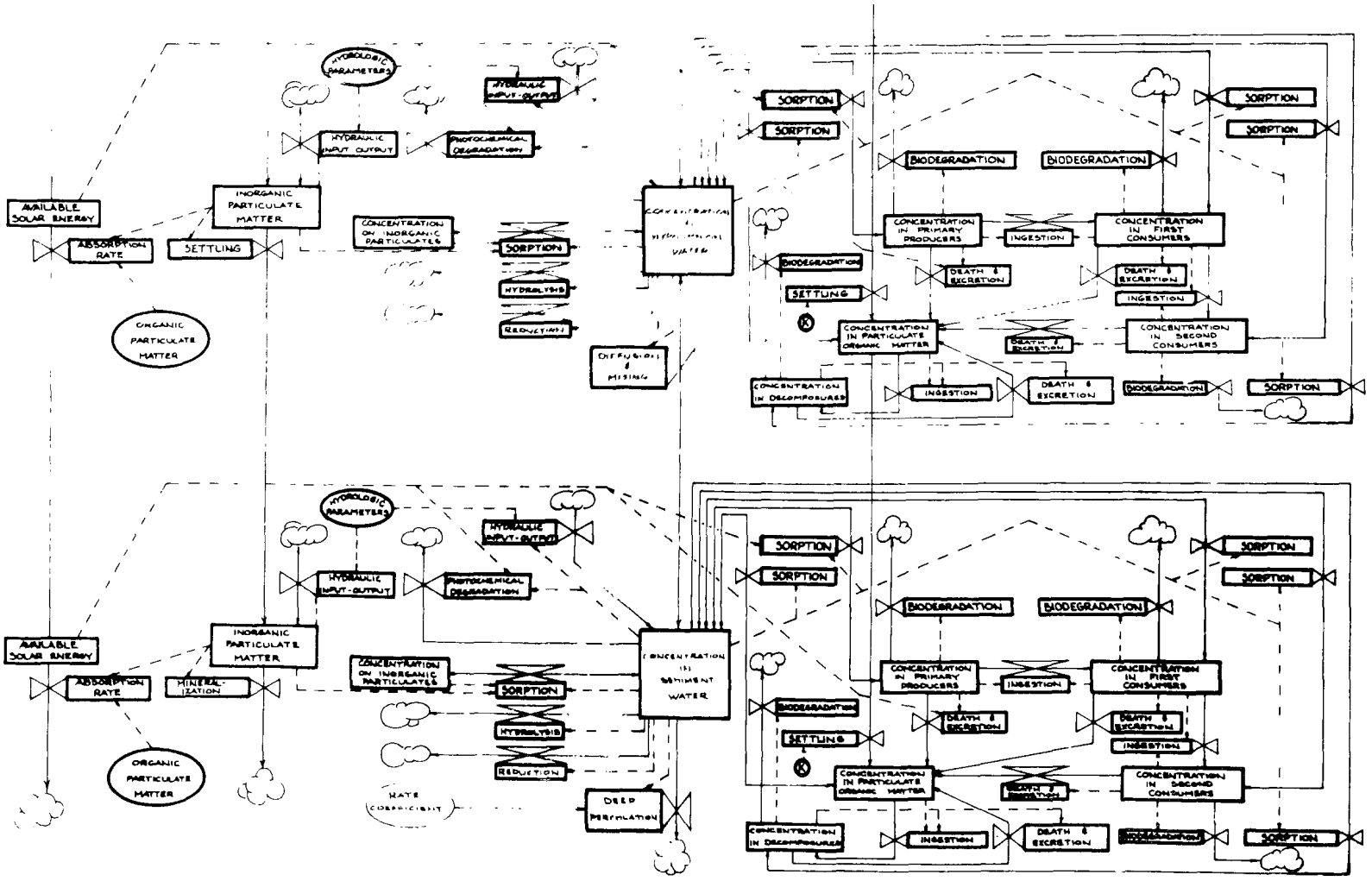


Figure 13: A Minimal Representation for a Pesticide in a Dimictic Lake.

c. the hypothesized system structure, which includes the influence of other process rates acting through the causal topology; and

d. the time series of inputs to the system.

Analytical techniques for estimating the effects of individual processes on the system behavior are summarized in Section I. The results provide an analytical basis for reduction of complex system diagrams.

SECTION VII
THE ESTUARINE/MARINE MODEL

The considerations incorporated in the freshwater aquatic model continue in relevance and validity into the estuarine/marine system, which can be viewed as specialized iterations of the general model (Figures 1 and 3). The relationship to the terrestrial and freshwater systems have been alluded to earlier (Figure 2). Thus, it is sufficient here to outline the significant differences and inter-relationships applicable to these regions of the environment.

The physical state of a compound in a system depends on its relation with the other components of the system, a behavior which can ideally be described by distribution constants when at equilibrium. For instance, the pharmaco-dynamic action of many drugs depends on their relative ability to bind to different sites. In such a fashion, the bloodstream may act as a reservoir permitting slow release of a drug to assure its long-term action. This ability to bind substances can occur anywhere. The toxicity of a pollutant must thus be evaluated in terms of the physical state(s) in which it shows toxicity and not merely by its observed concentration. With regard to availability to a carnivore, a pollutant adsorbed to detritus may be as unavailable as that adsorbed to a grain of sand.

Figure 14 is a schematic diagram of flow of a chemical through an estuary. It should be pointed out strongly that the estuarine system is exceedingly complex and any simulation will require time and caution.

Figure 15 is a representation of functional interactions at the interfaces between the estuary and other indicated ecosystems. The arrows indicate possible flux of chemicals without regard to form and origin. Interactions in the estuary are treated in a more precise conceptual fashion in Figure 14.

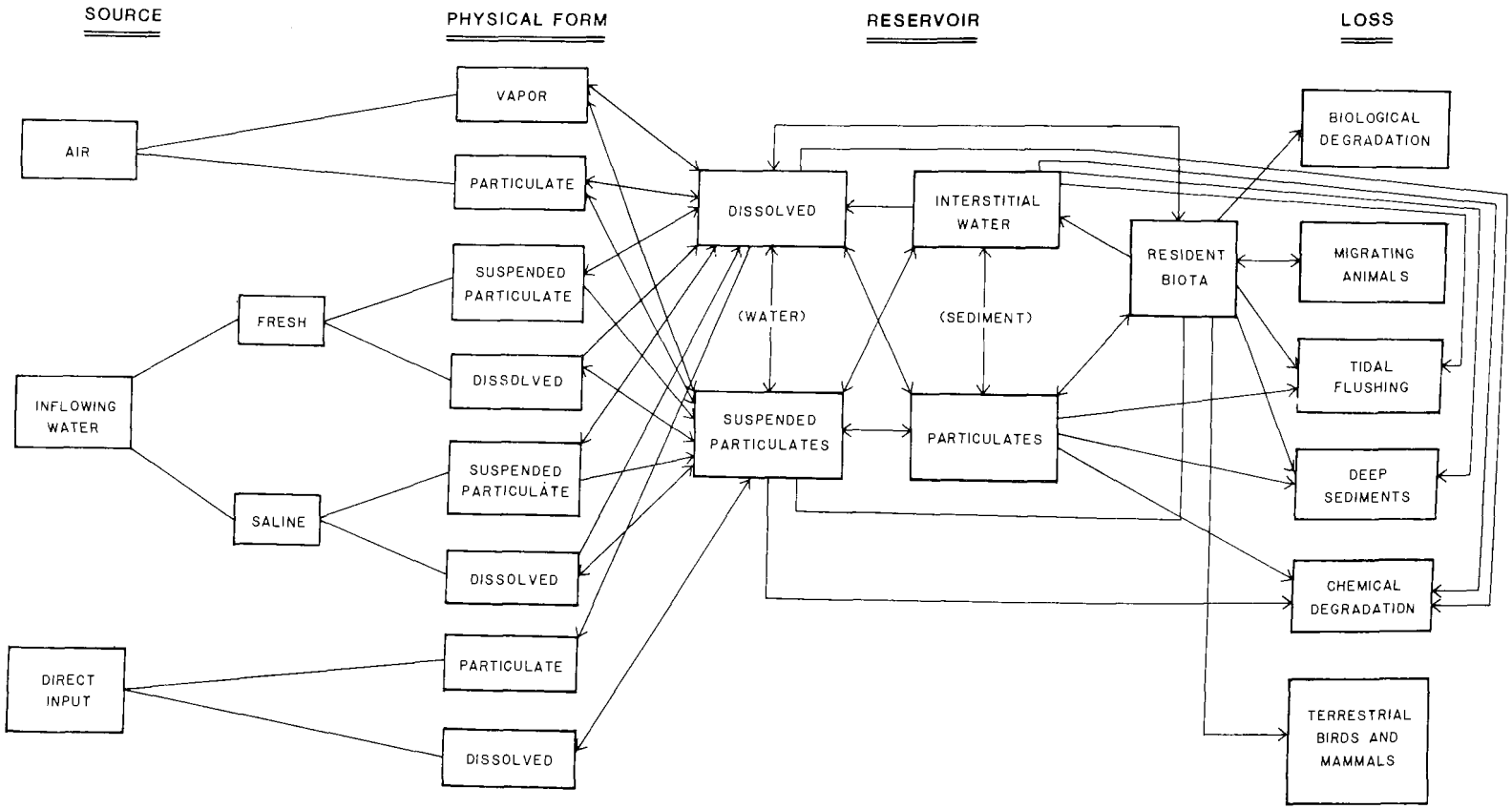


Figure 14: Simple Model of Transport in Estuaries.

The following definitions apply to Figure 15 only:

Run-off: Any transport from land adjacent to an estuary, including drainage not covered by river flow, such as non-specific drainage from swamps.

Tidal action: Any transport mediated by tidal flushing and tidal currents.

Biota-mediated flux: Any transport of organisms from one domain (sea coast, ocean, and fresh water) to another, such as in the case where a predator leaves its domain to feed in another domain, possibly itself becoming prey, or contamination through excretions (feces, urine, and regurgitated pellets). Emigration and immigration are also included.

River flow: Any transport mediated by a river or rivulet. This includes adsorbed as well as non-adsorbed materials.

Atmospheric disturbances: 1) Any transport caused by unusually high tides due to strong winds. 2) Any transport caused by agitation of the sediment or shore/bank by abnormally strong wave action or currents due to strong winds.

Turbulence: Any transport due to abnormal mixing caused by eddies (underwater storms).

Tides and Currents: Any transport due to normal tides and currents.

Fall-out: Any transport via the atmosphere.

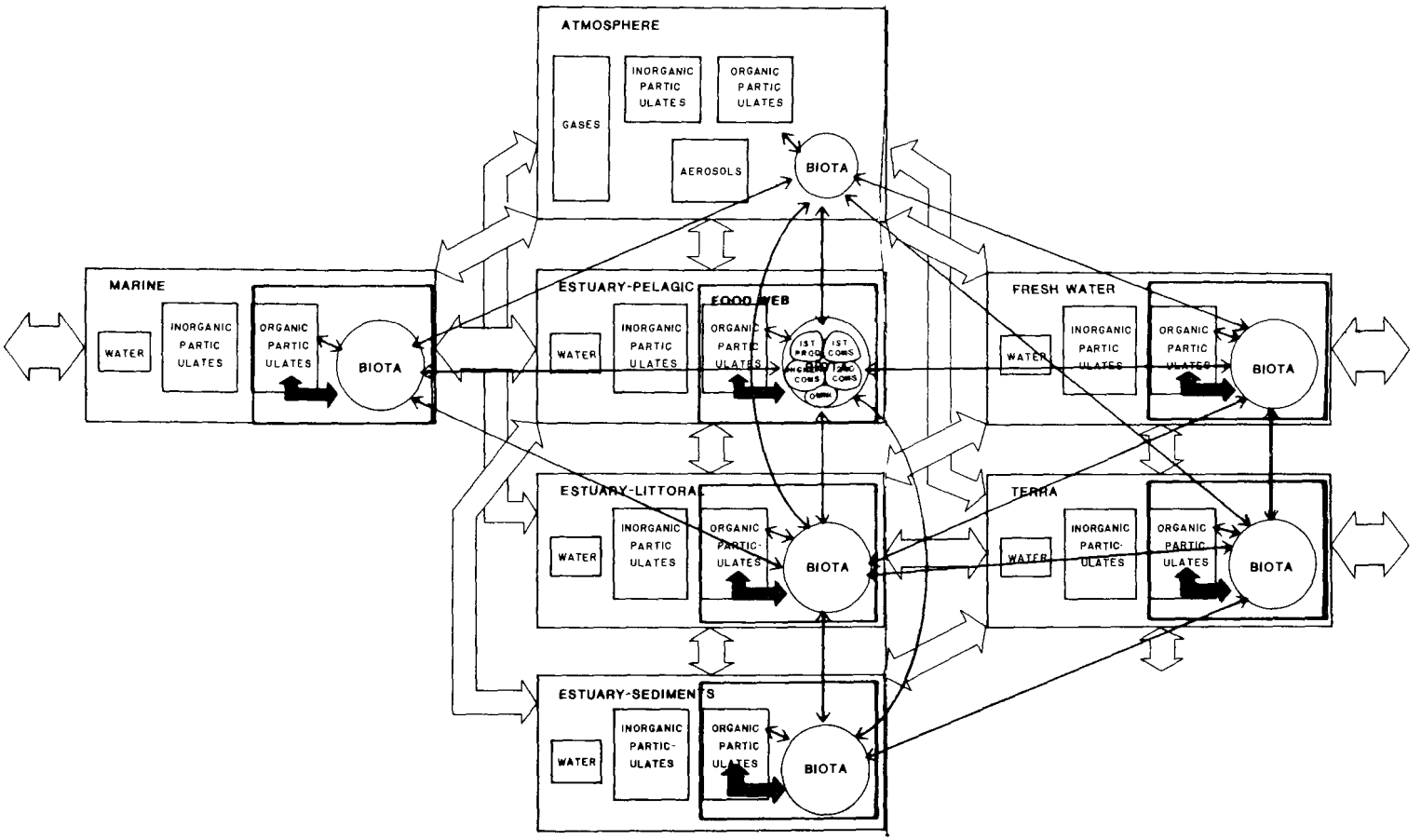


Figure 15: Expanded, Iterated Basic Chemical Module for Transport of Chemicals in Estuaries.

Open arrows indicate transport between modules via Run-off, River flow, Tidal action, Turbulence, Fallout, and Atmospheric disturbances.

Biota-mediated Flux (overlay).
Solid arrows indicate unspecified migration, predation, life cycle-related changes, and transport-dependent movement between food webs associated with chemical modules representing environmental regions.

SECTION VIII

REFERENCES

AHR, W. M. (1973). Long lived pollutants in sediments from the Laguna Atacosa National Wildlife Refuge, Texas. Geol. Soc. Amer. Bull. 84, 2511.

BERTALANFFY, L. VON. (1963). "General Systems Theory." George Braziller, Inc., New York. p. 289.

BISCHOFF, K. B. and R. G. BROWN. (1966). Drug distribution in mammals. Chem. Eng. Prog. Symp. Ser. A 66, 32.

CANNON, R. H. (1967). "Dynamics of Physical Streams." McGraw-Hill, New York. p. 1093.

CHILD, G. I. and H. H. SHUGART, JR. (1972). Frequency response analysis of magnesium cycling in a tropical forest ecosystem, In "Systems Analysis and Simulation in Ecology," Vol. II, B. C. Patten (ed). Academic Press, New York. p. 592.

CLOSE, C. M. (1963). "Notes on the Analysis of Linear Circuits." Rensselaer Polytechnic Institute, Troy, N. Y. p. 123.

COMMONER, B. (1971). "The Closing Circle." Alfred A. Knopf, Inc., New York. p. 326.

DeRUSSO, R. M., R. ROY and C. CLOSE. (1965). "State Variables for Engineers." John Wiley and Sons, Inc., New York.

DUCE, R. A., J. G. QUINN, C. E. OLNEY, S. R. PIOTROWICZ, B. J. RAY and T. L. WADE. (1972). Enrichment of heavy metals and organic compounds in the surface microlayer of Narragansett Bay, Rhode Island. Science 176, 161.

EBERHARDT, L. L., R. L. MEEKS and T. J. PETERLE. (1971). Food chain model for DDT kinetics in a freshwater marsh. Nature 230, 60.

ELZY, E., F. T. LINDSTROM, L. BOERSMA, R. SWEET and P. WICKS. (1974). Analysis of the movement of hazardous chemicals in and from a landfill site via a simple vertical-horizontal routing model. Oregon State Agricultural Experiment Station Special Report No. 414, Oregon State University, Corvallis, OR 97331. 110 pp.

FORRESTER, J. W. (1971). "World Dynamics." Wright-Allen Press, Cambridge, Mass. 142 pp.

HANNON, B. (1973). The structure of ecosystems. J. Theor. Biol. 41, 535.

HARRISON, H. L., O. L. LOUCKS, J. W. MITCHELL, D. F. PARKHURST, C. R. TRACY, D. G. WATTS and V. J. YANNACONE, JR. (1970). Systems studies of DDT transport. Science 170, 503.

HILL, J., IV (1973). Component Description and Analysis of Environmental Systems. Masters Thesis. Utah State Univ., Logan, Utah. p. 94.

HOLLEY, E. R. and G. ABRAHAM. (1973). Field tests on transverse mixing in rivers. J. Hydraulics Div. ASCE. HY12, 2313.

HUANG, J. (1971). Organic pesticides in the aquatic environment. Water and Sewage Works. May, 139.

HUTCHISON, G. E. (1948). Circular causal systems in ecology. Ann. N. Y. Acad. Sci. 50, 221.

HUTCHISON, G. E. (1957). "A Treatise on Limnology." John Wiley and Sons, Inc., New York. p. 1015.

KENAGA, E. E. (1972). Guidelines for environmental study of pesticides: determination of bioconcentration potential. Res. Rev. 44, 73.

KARNOPP, D. and R. C. ROSENBERG. (1968). "Analysis and Simulation of Multiport Systems." Massachusetts Institute of Technology. Cambridge, Mass. p. 221.

KUO, B. C. (1962). "Automatic Control Systems." Prentice-Hall. Englewood Cliffs. N.J. p. 504.

LEONTIEF, W. W. (1966). "Input-Output Economics." Oxford University Press, New York.

LINDSTROM, F. T., J. W. GILLETT and S. C. RODECAP. (1974). Distribution of HEOD (dieldrin) in mammals: I. Preliminary model. Arch. Environ. Contam. Toxicol. 2, 9.

MEADOWS, D. H., D. L. MEADOWS, J. RANDERS and W. H. BEHRENS, III. (1972). "The Limits to Growth" Universe Books, New York. p. 205.

METCALF, R. L., G. K. SANGHA and I. P. KAPOOR. (1971). Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. Environ. Sci. Technol. 5, 709.

MOORE, N. W. (1967). A synopsis of the pesticide problem, In "Advances in Ecological Research," Volume 4, J. B. Cragg, (ed). Academic Press, p. 75-128.

NISBET, I. C. T. and A. F. SAROFIM. (1972). Rates and routes of transport of PCBs in the environment. Environ. Health Perspect. 1, 21.

ODUM, E. P. (1971). "Fundamentals of Ecology." Saunders, Philadelphia. p. 574.

ODUM, H. T. (1972). An energy circuit language for ecological and social systems: Its physical basis, In "Systems Analysis and Simulation in Ecology," Vol. II, B. C. Patten, (ed). Academic Press. New York. p. 591.

O'MELIA, C. R. (1972). An approach to the modeling of lakes. Hydrologie 34, 1.

PATTEN, B. C. (1971). A primer for ecological modeling and simulation with analog and digital computers, In "Systems Analysis and Simulation in Ecology," Vol. I. B. C. Patten (ed). Academic Press, New York.

PATTEN, B. C. (1973). Need for an ecosystem perspective in eutrophication modeling, In "Modeling the Eutrophication Process," E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney (eds). Utah Water Research Laboratory, Logan Utah. p. 227.

PATTEN, B. C., W. G. CALE, J. FINN AND R. BOSSERMAN. (In Press). Propagation of cause in ecosystems, In "Systems Analysis and Simulation in Ecology," Vol. IV, B. C. Patten (ed.). Academic Press, New York.

QUINLAN, A. (1974). Personal Communication.

RANDERS, J. and D. L. MEADOWS. (1971). "System Simulation to Test Environmental Policy: A Sample Study of DDT Movement in the Environment." System Dynamics Group, Alfred P. Sloan School of Management, Massachusetts Institute of Technology. Cambridge, Mass. 52 pp.

SCHINDLER, J. (1974). Personal Communication.

TOMOVIC, R. and M. VAKOBRATOVIC. (1972). "General Sensitivity Theory." Elsevier, New York.

ULANOWICZ, R. E. (1972). Mass and energy flow in closed ecosystems. J. Theor. Biol. 34, 239.

WEBSTER, J. R., J. B. WAIDE and B. C. PATTEN. (In Press). Nutrient cycling and ecosystem stability, In, "Mineral Cycling in Southeast Ecosystems," F. Howell (ed). AEC Symposium Series.

WOODWELL, G. M., C. F. WURSTER, JR. and P. A. ISSACSON. (1967). DDT residues in an East Coast estuary: A case of biological concentration of persistent insecticide. Science 156, 821.

WOODWELL, G. M., P. P. CRAIG and H. A. JOHNSON. (1971). DDT in the biosphere: Where does it go? Science 174, 1101.

SECTION IX

KEY LITERATURE SOURCES FOR PESTICIDE EFFECTS RESEARCH

GENERAL TEXTS - CHEMISTRY, MODELING AND BIOLOGY

ASHTON, F. M. and A. S. CROFTS. (1973). "Mode of Action of Herbicides." John Wiley and Co., New York. 504 pp.

AUDUS, L. J. (1964). "The Physiology and Biochemistry of Herbicides." Academic Press, London. 555 pp.

DeRUSSO, R. M., R. ROY and C. CLOSE. (1965). "State Variables for Engineers." John Wiley & Sons, Inc., New York.

FEST, C. and K. J. SCHMIDT. (1973). "The Chemistry of Organophosphorus Insecticides; Reactivity, Synthesis, Mode of Action, Toxicology." Springer Verlag, Berlin; New York. 339 pp.

JACQUEZ, J. A. (1972). "Compartmental Analysis in Biology and Medicine." Elsevier, New York. 237 pp.

KARNOPP, D. and R. C. ROSENBERG. (1968). "Analysis and Simulation of Multiport Systems." Massachusetts Institute of Technology, Cambridge, Mass. 221 pp.

KEARNY, P. C. and D. D. KAUFMAN. (1969). "Degradation of Herbicides." M. Dekker, New York. 394 pp.

LUKENS, R. J. (1971). "Chemistry of Fungicidal Action." Springer Verlag, New York 130 pp.

MEYER, J. H. (1971). "Aquatic Herbicides and Algaecides." Noyes Data Corp., Park Ridge, N. J. 176 pp.

O'BRIEN, R. (1967). "Insecticides: Action and Metabolism." Academic Press, New York. 332 pp.

SONDHEIMER, E. and J. B. SIMEONE. (1970). "Chemical Ecology." Academic Press, New York. 336 pp.

REPORTS OF FEDERAL AGENCIES OF BROAD PROBLEMS

BALDWIN, I. L. (1962). Pest Control and Wildlife Relationships. National Acad. Sci., National Res. Council.

JENSEN, J. H. (1965). Report of the Pesticide Residues Committee. National Acad. Sci., National Res. Council.

_____. (1969). Report of the Committee on Persistent Pesticides. Div. of Biology and Agriculture, National Res. Council to USDA.

MacLEOD, C. M. (1963). Use of Pesticides. President's Science Advisory Comm.

MRAK, E. M. (1969). Report on the Secretary's Commission on Pesticides and Their Relation to Environmental Health. To U.S.D.H.E.W.

TECHNICAL DATA

ANON. (1972). Ecological Research Series. Office of Research and Monitoring. U.S. Environmental Protection Agency, Washington, D.C. Example: An Evaluation of DDT and Dieldrin in Lake Michigan. EPA-R3-72-003, August.

____. (1969). Effects of Pesticides in Water. A Report of the States. U.S. Environmental Protection Agency. Office of Research and Development.

____. (1969). "Fish and Chemicals." A Symposium on Registration and Clearance of Chemicals for Fish Culture and Fishery Management. 99th Annual Meeting of the American Fisheries Society, New Orleans, Louisiana. September 12, 1969.

____. (1958-59). Handbook of Toxicology. National Acad. Sci., National Res. Council, Saunders, Philadelphia.

Vol. I. Acute Toxicities of Solids, Liquids, and Gases to Laboratory Animals. W. S. Spector, ed.
Vol. III. Insecticides. W. O. Negherbon, ed.
Vol. V. Fungicides. D. S. Dittmer, ed.

____. (1972). Pesticide Study Series 2, 3, 5, 6, 7, 8, 9, and 10. Environmental Protection Agency, Office of Water Programs.

____. U.S. Department of the Interior. Office of Water Resources Research. Bibliography Series. Water Resources Scientific Information Center, Washington, D. C. Examples: DDT in Water - WRSIC 71-211; Dialdrin in Water - WRSIC 72-202; Aldrin and Endrin in Water - WRSIC 72-203.

____. (1972). Water Quality Criteria. A Report of the Committee on Water Quality Criteria. Environmental Studies Board. National Academy of Sciences. National Academy of Engineering, Washington, D.C.

____. (1971). Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Water Pollution Control Research Series 18050 GWV 05/71.

DYRSSEN, D. and D. JAGNER. (1972). "The Changing Chemistry of the Oceans." Proceedings of the 20th Nobel Symposium, August 16-20, 1971, Goteborg, Sweden. Wiley-Interscience, New York. 365 pp.

EISLER, R. (1970a). Factors affecting pesticide-induced toxicity in an estuarine fish. U.S. Bureau of Sport Fisheries and Wildlife Technical Paper No. 45.

_____. (1970b). Acute toxicities of organochlorine and organophosphorus insecticides to estuarine fishes. U.S. Bureau of Sport Fisheries and Wildlife Technical Paper No. 46.

EPSTEIN, S. S. and M. S. LEGATOR. (1971). "The Mutagencity of Pesticides." MIT Press, Cambridge, Mass.

GILLETT, J. W. (ed.). (1970). "The Biological Impact of Pesticides in the Environment." Environmental Health Sciences Series No. 1, Oregon State University, Corvallis, Oregon.

HEATH, R. G., J. W. SPANN, E. F. HILL and J. F. KREITZER. (1972). Comparative Dietary Toxicities of Pesticides to Birds. U.S.D.I., Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife. Special Sci. Report-Wildlife No. 152. Washington, D.C.

KRAYBILL, H. G. (ed). (1969). Biological Effects of Pesticides in Mammalian Systems. Ann. N. Y. Acad. Sci. 160.

PIMENTAL D. (1971). "Ecological Effects of Pesticides on Non-Target Species." Executive Office of the President, Office of Science and Technology. Washington, D.C.

ROSEN, A. A. and H. F. KRAYBILL (eds). (1966). "Organic Pesticides in the Environment." Adv. in Chem. Series 60. American Chemical Society, Washington, D.C.

STICKEL, L. F. (1968). "Organochlorine Pesticides in the Environment." U.S.D.I., Fisheries and Wildlife Service, Bureau of Sport Fisheries and Wildlife. Special Sci. Report-Wildlife No. 119.

TUCKER, R. K. and D. C. CRABTREE. (1970). Handbook of Toxicity of Pesticides to Wildlife. U.S.D.I., Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife. Research Publication No. 84, Washington, D.C.

WILKINSON, B. K., L. S. CORRILL and E. D. COPENHAVER. (1974). "Environmental Transport of Chemicals Bibliography." Oakridge National Laboratory (ORNL-EIS-74-68). 185 pp.

TECHNICAL JOURNALS AND PERIODICALS

Archives of Environmental Contamination and Toxicology. Springer Verlag (Quarterly).

Bulletin of Environmental Contamination and Toxicology. Springer Verlag (Monthly).

Comparative Biochemistry and Physiology. Pergammon (Monthly).

Environmental Science and Technology. American Chem. Soc. (Monthly).

Journal of Agricultural and Food Chemistry. American Chem. Soc. (Bimonthly).

Journal of Fisn Biology (Quarterly).

Journal of the Fisheries Board of Canada (Monthly).

Journal of the Water Pollution Control Federation (Quarterly).

Journal of Wildlife Management (Quarterly).

Limnology and Oceanography (Bimonthly).

Marine Pollution Bulletin (Monthly).

Nature (Weekly).

Pesticide Abstracts. EPA - Office of Pesticide Programs, Washington D.C. (Monthly).

Pesticide Biochemistry and Physiology. Academic Press. New York (Monthly).

Pesticide Monitoring Journal. EPA, Washington, D.C. (Quarterly).

Residue Reviews. Springer Verlag (Irregular - several volumes per year).

Science. Amer. Assoc. Adv. Sci. (Weekly).

Soil Science. Amer. Soc. Soil Science (Monthly).

Toxicology and Applied Pharmacology. Society of Toxicologists (Monthly).

Transactions of the American Fisheries Society. Allen Press, Inc. (Quarterly).

Water Pollution Control Federation Journal (Monthly).

Water Research (Monthly).

Weed Science. Amer. Weed Soc. (Bimonthly).

ABSTRACT SOURCES

Biological Abstracts (Semi-monthly).

Chemical Abstracts (Weekly).

Pesticide Abstracts (Monthly).

Sport Fishery Abstracts (Quarterly).

Water Pollution Abstracts (Monthly).

COMPUTER LITERATURE SEARCH DATA BASES

Medline (Biomedical) National Library of Medicine.

Toxline (Toxicology) National Library of Medicine.

WRSIC Water Resources Scientific Information Center

SIE Science Information Exchange. Smithsonian Institution, Washington, D.C.

NTI Search U.S. Department of Commerce.

ISI Institute for Scientific Information.

FEDERAL AND STATE RESEARCH LABORATORIES WHERE BOTH DATA AND INTERPRETATION OF DATA IS AVAILABLE

National Water Quality Laboratory. Pesticide Research Team. Mr. John G. Eaton, Coordinator. Duluth, Minnesota 55804.

Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, P.O. Box 862, LaCrosse, Wisconsin 54601.

Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route 1, Columbia, Missouri 65201.

Radiation and Metabolism Laboratory, U.S. Department of Agriculture, Fargo, North Dakota 58102.

Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561.

Newtown Fish Toxicology Station, U.S. Environmental Protection Agency, 3411 Church Street, Cincinnati, Ohio 45244.

Southeast Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601.

Perrine Primate Laboratory, Wenatchee Research Section, U.S. Environmental Protection Agency, P.O. Box 73, Wenatchee, Washington 98801.

U.S. Environmental Protection Agency Laboratory, Region 10, 15345 N.E. 36th Street, Redmond, Washington 98052.

Office of Pesticide Programs, Criteria and Evaluation Division, U.S. Environmental Protection Agency, Washington, D.C. 20250.

Gulf Coast Water Supply Laboratory, U.S. Environmental Protection Agency, P.O. Box 158, Dauphin Island, Alabama 36528.

Idaho Fish and Game Department, P.O. Box 25, Boise, Idaho 83707.

Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, Route 1, Box 9, Warm Springs, Georgia 31830.

Southeastern Fish Cultural Research Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, Marion, Alabama 36756.

U.S. Environmental Protection Agency, Pesticide Monitoring Laboratory, Bay St. Louis, Mississippi 29520.

Great Lakes Fishery Laboratory, Bureau of Commercial Fisheries, Fish and Wildlife Service, U.S. Department of the Interior, Ann Arbor, Michigan 48107.

Wisconsin Department of Natural Resources, P.O. Box 450, Madison, Wisconsin 53701.

Agricultural Research Service Laboratories (U.S. Department of Agriculture) Regional.
(Studies on nuisance aquatic insecticides, herbicides, etc.)

Department of Defense, Naval Ship Research and Development, Center, Annapolis, Maryland 21402.
(Anti-Fouling Agents)

National Agricultural Library, U.S. Department of Agriculture, Beltsville, Maryland 20705.

Alaska Department of Environmental Conservation, Pouch O, Juneau, Alaska 99801.

Conservation Library Center, Denver Public Library, 1357 Broadway, Denver, Colorado 80283.

Division of Pesticide Community Studies, Office of Pesticide Programs, Environmental Protection Agency, 4770 Buford Highway, Chamblee, Georgia 30341.

Gulf South Research Institute, P.O. Box 1177, New Lberia, Louisiana 70560.

Fish Control Laboratory, U.S. Bureau of Sport Fisheries and Wildlife, Route 1, Box 9, Warm Springs, Georgia 31830.

Fish Farming Experimental Station, U.S. Bureau of Sport Fisheries and Wildlife, Box 860, Stuttgart, Arkansas 72160.

National Agricultural Chemicals Association, 1155 15th St. NW, Washington, D.C. 20005.

Division of Biology and Agriculture, National Research Council, 2101 Constitution Ave. NW, Washington, D.C. 20418.

New Hampshire Pesticides Control Board, State House Annex, Room 201, Concord, New Hampshire 03301.

New York State Department of Environmental Conservation, 50 Wolf Rd., Albany, New York 12201.

Patuxent Wildlife Research Center. Laurel, Maryland 20810.

Toxicological Research Laboratory. Veterinary Sciences Research Division. Agricultural Research Service, USDA, P.O. Box 311, Kerrville, Texas 78028.

Community Study Pesticide Project. Idaho Department of Health, Statehouse, Boise, Idaho 83707.

Division of Wildlife Services. Bureau of Sport Fisheries and Wildlife. U.S. Department of the Interior, 1717 H Street NW, Washington, D.C. 20240.

Denver Wildlife Research Center. U.S. Bureau of Sport Fisheries and Wildlife, Building 16, Federal Center, Denver, Colorado 80225. from FLOR

COLLEGES AND UNIVERSITIES ASSOCIATED WITH
PESTICIDE RESEARCH OR PESTICIDE INFORMATION

Water Resources Research Institute, 314 Nuclear Science Center, Auburn University, Auburn, Alabama 36830.

Lake Ontario Environmental Laboratory, College at Oswego, State University of New York, Oswego, New York 13126.

Colorado State University, Fort Collins, Colorado 80521.

Department of Zoology, Mississippi State University, Mississippi State, Mississippi 39762.

Department of Fisheries and Wildlife, Michigan State University, East Lansing, Michigan 48823.

Department of Entomology, School of Life Sciences, University of Illinois, Urbana-Champaign, Illinois 61801.

Oregon State University, Corvallis, Oregon 97331.

Department of Entomology, Fisheries, and Wildlife, University of Minnesota, St. Paul, Minnesota 55101.

Cornell Pesticide Residue Laboratory, Cornell University, Ithaca, New York 14850.

Trace Level Research Institute, Purdue University, Lafayette, Indiana 47907.

Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, Ohio 45219.

Biological Sciences Library, University of New Hampshire, Kendall Hall, Durham, New Hampshire 03824.

College of Agriculture and Environmental Science, Rutgers--the State University, New Brunswick, New Jersey 08903.

Institute of Biological Sciences, School of Agriculture and Life Sciences, North Carolina State University, Box 5306, Raleigh, North Carolina 27607.

Rhode Island Agricultural Experiment Station, University of Rhode Island, 113 Woodward Hall, Kingston, Rhode Island 02881.

University of California, Berkeley, Department of Entomology and Parasitology, Berkeley, California 94720.

University of California, Davis, Department of Environmental Toxicology, Davis, California 95616.

University of California, Riverside, Department of Entomology, Riverside, California 92502.

Louisiana Cooperative Wildlife Research Unit. Louisiana State University, Baton Rouge, Louisiana 70803.

Massachusetts Cooperative Wildlife Research Unit. University of Massachusetts, Amherst, Massachusetts 01003.

South Carolina Community Pesticide Study. Medical University of South Carolina, 80 Barre Street, Charleston, South Carolina 29401.

College of Forest Resources. University of Washington, Seattle, Washington 98105.

SOME PRIVATE CORPORATIONS HAVE PERFORMED

PESTICIDE RESEARCH AS RELATED TO AQUATIC LIFE

Bionomics, Inc., P.O. Box 135, Main Street, Wareham, Massachusetts 02571.

Industrial Bio-Test Laboratories, Inc., 1810 Frontage Road, Northbrook, Illinois 60062.

Envirogenics Company, Division of Aerojet-General Corporation, El Monte, California 91734.

Union Carbide Corporation, Tarrytown Technical Center, Tarrytown, New York 10591.

Lakeside Laboratories, 1707 East North Ave., Milwaukee, Wisconsin 53201.

Syracuse University Research Corporation, Merrill Lane, University Heights, Syracuse, New York 13210.

APPENDIX

GRAPHIC REPRESENTATION OF PESTICIDES IN AQUATIC SYSTEMS

In approaching any problem, an investigator must first form a mental image or conceptual model of the system. This conceptual model usually is not well defined and varies considerably from one investigator to another. With the complex problems associated with environmental systems, solving and/or communicating the conceptual model requires translation into the nonempirical language of mathematics or symbolic logic.

Since direct translation of the conceptual model into mathematical representation is awkward and difficult, the initial description is best formulated into a graphic symbolism. The nature of the graphic description is dependent upon the investigator's conceptualization of the processes, the degree of resolution required, and data that are available or that can be measured from experiments with the system. The graphical representation is the heart of systematic experimental design because the applicability of the ensuing analysis is limited by the ability of the investigator to represent his conceptual model of the system processes in graphic form. There is no best or correct graphical representation of a system. They differ only in the degree of realism and utility.

Graphical representations can be improved by iteration. After application of analytical techniques, any unusual or unexpected storage levels or flow rates may require modification of the components or connectivity of the original graphical representation. The nature of the iterative interactions among the graphical representation, the mathematical model, and data acquisition is presented in Figure A-1.

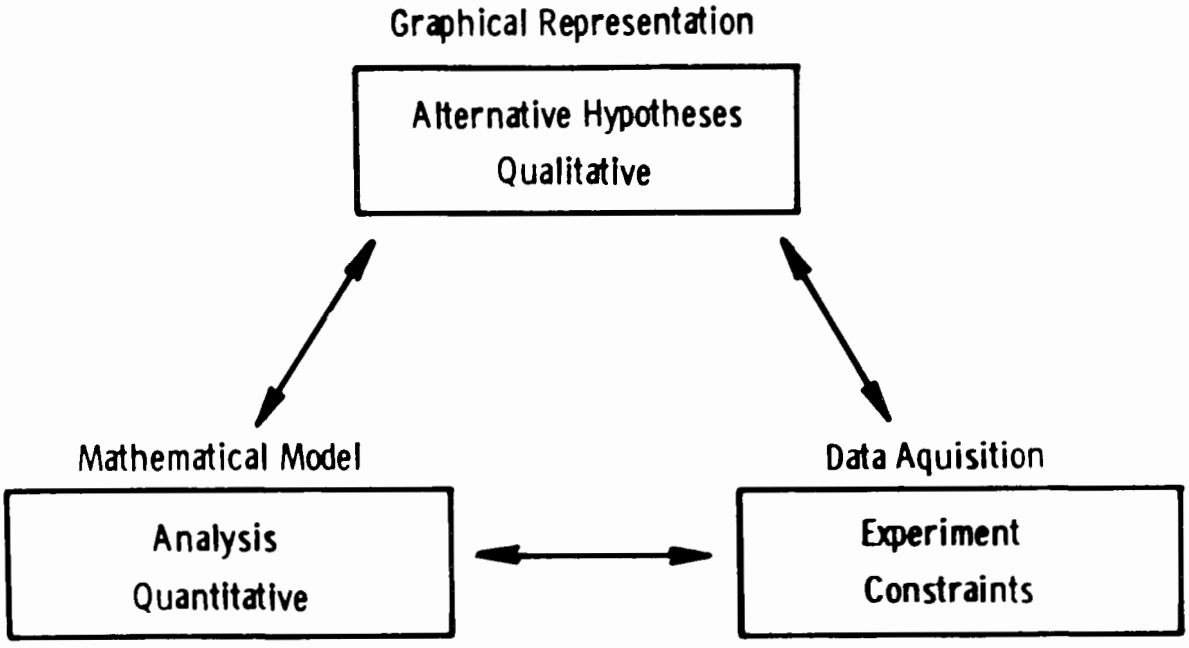


Figure A-1: Relationship Among Graphical Representations, The Mathematical Model and Data Acquisition (from QUINLAN, 1974).

Circuit diagrams (CLOSE, 1963) compartment diagrams (ODUM, 1971), block diagrams (KUO, 1962) signal flow graphs (KUO, 1962), bond graphs (KARNOPP and ROSENBERG, 1968), energy circuit language (ODUM, 1962), and Forrester diagrams (FORRESTER, 1971; MEADOWS et al., 1972) are all examples of graphical representations of systems. Each has advantages and disadvantages depending upon the nature of the system to be described.

Bond graphs are excellent symbolic representations for environmental systems in which energy flow is of primary concern and in which complementary variables (a potential and a related flux) may be defined. Compartment diagrams are useful representations of

environmental systems when mass or energy storage and their rates of exchange are of interest but complementary variables are not explicitly defined (ULANOWITZ, 1972). Signal flow graphs and associated control system analysis techniques are valuable when feedback control properties of the system are of primary concern. Forrester diagrams may be used in the general case to represent interactions, transformations, and transports of mass or energy without recourse to specific component equations or other constraints upon the system variables.

A Forrester diagram can be used to present a conceptual model of the transport and transformation of pesticides in the aquatic environment. From this presentation a reduced or working model in compartment form may be derived for a specific pesticide and specific ecosystem. The compartment diagram should include the mathematical form of the interactions and can provide a basis for preliminary system analysis as an aid to experimental design.

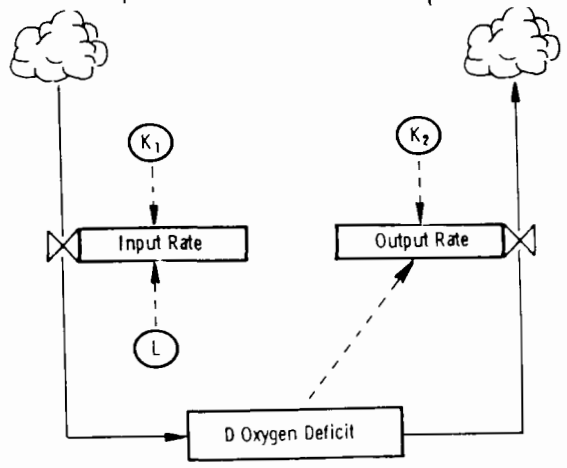
Forrester Diagrams

In Forrester diagrams of dynamic systems, six symbols are commonly used.

A solid line represents a directed pathway for transfer of matter or energy.
A dashed line represents a directed pathway for control or information transfer.
The cloud symbol represents a source or sink (input or output) outside the defined system boundaries.
A rectangle indicates storage of matter or energy.
The valve symbol indicates rates along the associated pathway.
Finally, the circle represents coefficients and parameters that affect flow rates.

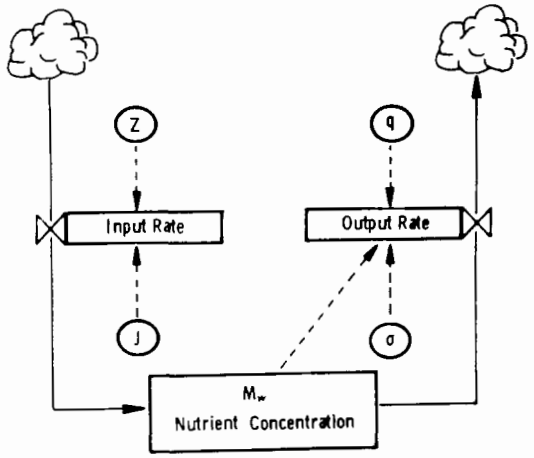
The degree of resolution or complexity of the Forrester diagram of a

system may vary considerably depending upon application and resources available for evaluating the hypothesis. While there appears to be no upper limit to the resolution of a model, the lower limit (a single storage component) is demonstrated for a stream and a lake in the following examples (Figures A-2 and A-3) from O'MELIA (1972). The low level of resolution in these examples does not necessarily imply that there is a better representation for a particular application.



$\frac{dD}{dt} = K_1L - K_2D$ where D = oxygen deficit, L = BOD remaining,
K_1 = deoxygenation coefficient, and
K_2 = reaeration coefficient.

Figure A-2: Streeter-Phelps Oxygen Deficit Model for a Stream.



$\frac{dM_w}{dt} = \frac{J}{Z} - (\sigma + q)M_w$ where M_w = concentration of nutrient,
J = flux of M to lake,
σ = sedimentation coefficient,
q = flow coefficient, and Z = mean lake depth.

Figure A-3: Vollenweider Lake Eutrophication Model.

One manner of increasing the resolution of a model is to divide a single storage component into sub-units, which may have differing rates of input or output for the stored variable. For example, the nutrient concentration in the lake (Mw) from Figure A-3 may be divided between abiotic storage (Ma) and biotic storage (Mb) with the result shown in Figure A-4. If the output rate (from sedimentation and flow) of the nutrient stored in the biotic component differs from that of the nutrient stored in the abiotic component, then the mean residence time is changed and the dynamic behavior of the nutrient output may be changed considerably from the single storage representation.

Another means of increasing the resolution of the representation is to include a time-varying parameter instead of the mean value of an exogenous variable that controls a rate of flow for an endogenous variable. Thus, instead of a mean lake depth (Z), a time varying lake depth [Z(t)] could be incorporated in the model.

Finally, the rate controlling flow and storage may be explicitly included in the representation and the two resulting subsystems can be realistically coupled (HARRISON et al., 1970). This is demonstrated in Figure A-5 for the biotic component of the lake model in Figure A-4. These expansions of the diagrams may continue until the point of diminishing returns is reached with respect to either application or resources.

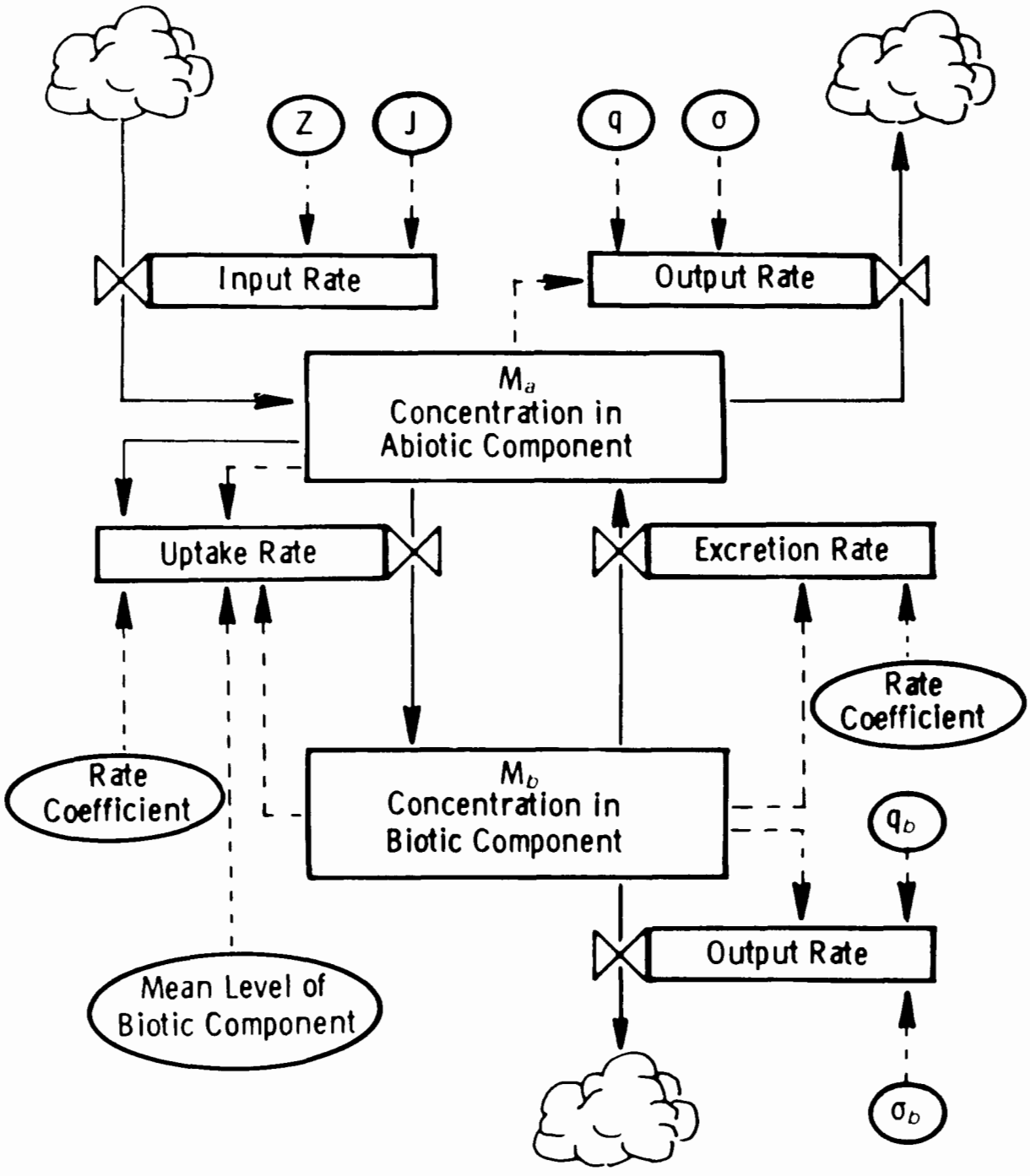


Figure A-4: Nutrient Model for Lake with Biotic and Abiotic Storage.

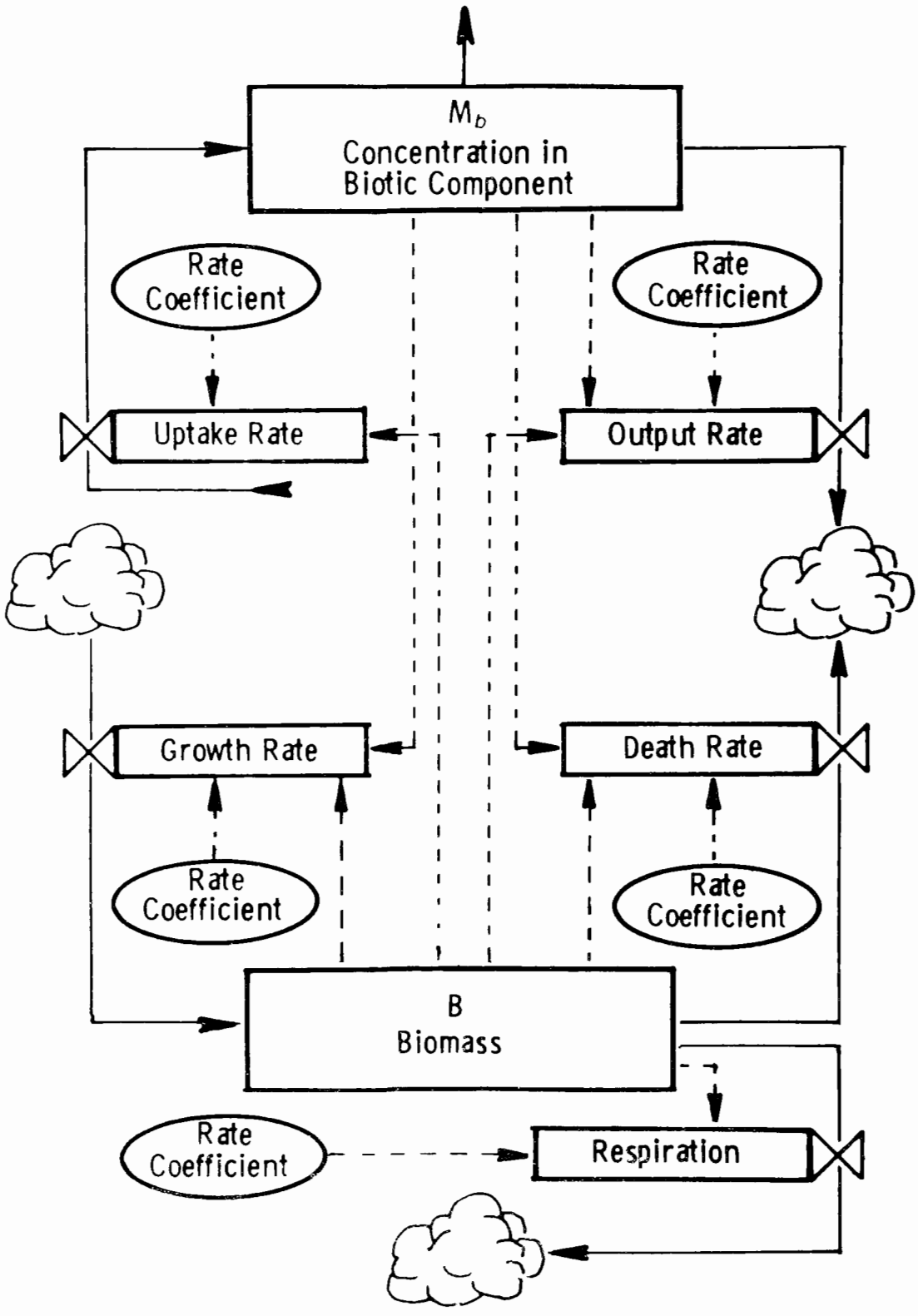


Figure A-5: Possible Coupling of Biomass (B) Subsystems with Nutrient Concentration (Mb) Subsystems.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.

4. TITLE AND SUBTITLE
A Conceptual Model for the Movement of Pesticides through the Environment: A Contribution of the EPA Alternative Chemicals Program

5. REPORT DATE
November 1, 1974

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
James W. Gillett, James Hill IV, Alfred W. Jarvinen and W. Peter Schoor

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
National Ecological Research Laboratory
Environmental Protection Agency
Corvallis, Oregon 97330

10. PROGRAM ELEMENT NO.
1EA487

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Same

13. TYPE OF REPORT AND PERIOD COVERED
Final

14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT This report presents a conceptual model of the movement and disposition of pesticides in the environment. A multi-media model is built up from simple modules representing basic processes and components of air, soil, and water. More specific models are exposited for the atmospheric/terrestrial, freshwater aquatic, and estuaring/marine environments. Through iterative operations of expansion and systematic reduction of the components and processes these models of segments of the environment can be joined to provide a holistic view of the disposition of a chemical and its attendant effects. Ultimately systems analysis and mathematical simulation techniques can be employed to evaluate the fate of a specific chemical in a particular environment. The conceptual model is thus a first step in organizing facts, assumptions, and hypotheses into a graphic and logical arm capable of exploitation in further experimentation of pesticide disposition and effects. While rejecting formulation of a model with global validity, the authors emphasize the commonalities of the basic processes and components in the various environments. Thus, a multi-media approach to disposition studies is made explicit even in the absence of a single all-media global model.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Air Pollution Conceptual Model Ecology Environmental Biology Hazardous Materials Mathematical Model Pesticides Systems Analysis Water Pollution	Alternative Chemicals Program Laboratory Microcosms Simulated Ecosystems	1201 0611 0606

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
	UNCLASSIFIED	
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (9-73)

☆ U. S. GOVERNMENT PRINTING OFFICE: 1974—697-650/65 REGION 10

CONTRIBUTION NO. 250

Gulf Breeze Contribution 250

In: Marine Bioassays
Proceedings of workshop sponsored by
The Marine Technology Society, 1975.

(Entire proceedings may be obtained from
The Marine Technology Society, 1730 M Street, N.W.,
Washington, D. C. $18.00).

HISTOLOGICAL AND PHYSIOLOGICAL EVALUATIONS IN SOME MARINE FAUNA

The development of pathology, as applied to aquatic toxicology, depends heavily on the knowledge of normal histology and physiology if anomalies, due to pollutants or disease, are to be accurately defined. However, at present, knowledge of normal morphology or metabolic activities is either incomplete or lacking for most marine or coastal organisms.

Obviously, well-coordinated efforts will be required to characterize normal ranges and interpret the morphological or physiological responses of aquatic organisms to various factors including pollutants. Most laboratories cannot justify well-defined pathological units, although the need often arises through governmental enforcement organizations and other activities. The techniques and examples thus presented are intended to offer some means of obtaining evaluations of aquatic organisms' well-being or disorders.

I - Histological Techniques

A.- Fixation

Freezing is useful for preserving tissue for chemical analyses, isolation of viruses or for sectioning on a cryostat if very small pieces of fresh tissue are frozen very rapidly as by immersion in liquid nitrogen. Specimens should never be frozen prior to routine histology because it destroys cells and produces artifacts which make diagnoses difficult or impossible and prohibits the taking of publishable photomicrographs. A common freezing artifact is rows of vacuoles resembling fat cells. If a chemical fixative is not readily available, refrigeration or wet ice, for up to 24 hours, and then fixing, is preferable to freezing.

Chemical fixation, which is one of the most important steps in the preparation of specimens for histology, should be at or very close to the time of death to insure that tissues are maintained as near to their natural state as possible. The chemical fixatives most commonly utilized for vertebrate and invertebrate marine organisms are Bouin's, Buffered Formalin, Davidson's, Dietrich's, Zenker's and Helly's.

1) Davidson's (Shaw & Battle, 1957) has proven to be an excellent fixative for marine organisms. This formula is close to that of Dietrich's, differing principally by using filtered seawater instead of distilled water, along with the addition of glycerol. Results are particularly gratifying when the seawater used is close to the salinity of water where organisms were obtained. For Davidson's, prepare a stock solution consisting of 1 part glycerin; 2 parts formalin; 3 parts 95% ethyl alcohol; and 1 part seawater. Before use, add one part glacial acetic acid to 9 parts stock solution. Fix in cold from 24 hours to one week. Store in 60-70% ethyl alcohol.

2) Ten percent formalin (1 part 40% formalin; 9 parts H_2O) is a good general fixative with several practical advantages: a) it can be transported as a concentrate and diluted with local water at the collecting site, greatly reducing the weight and volume that must be carried. After a minimum of 18 hours' fixation, the excess formalin can be discarded to facilitate the return of the specimens to the laboratory where they should be reformalized; (b) specimens can be stored for extended periods in neutral buffered formalin. If initial fixation is unbuffered, it should be replaced as soon as practical with buffered formalin; and (c) embedded formalin fixed tissue can be reprocessed for electron microscope studies with fair results. Tissues fixed with the acid fixatives are unsuitable for subsequent electron microscope studies.

The major disadvantage of formalin is that it penetrates slowly, allowing deep tissues to autolyze before being fixed. This disadvantage can be avoided if all body cavities are completely opened up and large tumors and organs are sliced part way through, every centimeter.

3) Bouin's (75 ml picric acid; 25 ml 40% formalin; and 5 ml glacial acetic acid) is excellent for fish tissue; it penetrates well and decals scales and small bones. Bouin's should be replaced with 70-80% ethanol between 18 and 48 hours and the alcohol changed regularly until the picric acid is mostly

removed (routine histological processing removes some picric acid, but if the initial burden is heavy, residual amounts will be carried through to the finished microscope slides and can be seen as crystals in the tissue).

4) Glutaraldehyde is recommended for tissues to be studied by electron microscopy. Porter's formula contains 3% glutaraldehyde; 0.1 M (2.14 gm/100 ml) sodium cacodylate buffer; 0.002 M (0.022 gm/100 ml) $CaCl_2$; and 10% glucose. Adjust pH to 7.4 with HCL or NaOH. Cut pieces of tissue with one dimension less than 1.0 mm with new alcohol-cleaned razor blades and immerse for a minimum of two hours. Keep refrigerated. Tissues can be safely stored in the fixative indefinitely.

5) Dietrich's FAA (10 ml 40% formalin; 2 ml glacial acetic acid; 30 ml 95% alcohol; 60 ml H_2O) is recommended for terrestrial and aquatic arthropods and molluscs. The arthropods should be fixed initially in a vacuum chamber to facilitate replacing the air in the trachea with the fixative. In the field, Dietrich's fixative has proven to be a very satisfactory fixative of marine organisms, based on the following criteria: (a) rapid tissue penetration, (b) tissue preparation does not require transfers to other liquids, (c) tissues, when fixed, are firm but not brittle, (d) fixed tissues may remain in this preparation for extended periods (not to exceed 6 months, preferably), (e) decalcification occurs simultaneously with fixation (in small teleosts and shrimps, decalcification is completed within approximately 10 days), and most important, (f) superior cellular detail is obtained with routine histological processing. Tissue-fixative proportion should not be less than 100 grams of tissue per liter of fixative, or 10 times the volume of fixative.

In the laboratory, excellent fixation of tissues for light microscopy is accomplished with Helly's or Zenker's fixative, but time and management of liquid changes can often be a deterrent to the use of these preparations. Therefore, Davidson's or Dietrich's fixative is used routinely in many laboratories.

Teleost tissue fixation and preparation for light microscopy should be done according to the following general guideline: teleosts having a total length of 4 cm or less are routinely immersed directly into the fixative, and require no further preparation prior to trimming for parafin embedding. Teleosts 4 to 12 cm in total length are prepared as follows: (1) immerse the whole animal in fixative for 5 to 10 minutes, (2) remove the whole animal and transect the caudal peduncle immediately posterior to the anal opening, (3) open the body cavity with a longitudinal, ventral incision, (4) remove the opercula, and (5) complete the sagital section of the organism after approximately 30 additional minutes of fixation. All fishes greater than 4 cm in length and all crustaceans should be decalcified prior to final tissues trimming. Commercially available products (Decal*) are available for tissue decalcification. Flat pieces from biopsies and autopsies, such as skin, should be backed by a piece of thin cardboard before fixing to prevent shriveling.

One of every five specimens, at least, are prepared for examination by transverse sectioning. Transections, at intervals of approximately 1 cm, are made beginning with ventral incisions that penetrate the soft tissues to the vertebral column. Following a preliminary fixation (approximately 30 minutes), the transection is completed with the scalpel by a "sawing action". However, some instances may require "crushing", or forced penetration of ossified and cartilagenous tissues.

The following additional steps are normally required to prepare teleosts having a total length greater than 12 cm: (1) excess dorsal musculature is trimmed away, (2) a dorsal-median incision is made to open the cranium, (3) lateral musculature of at least one flank is removed, and finally (4) the vertebral column is severed at intervals of approximately 2 cm with scissors. These tissues are returned to the fixative prior to trimming and embedding.

Fixation is accomplished by immersion of select tissues or whole animals into the fixative. Prior to immersion, soft-bodied species (i.e., those

(*) Omega Chemical, Cold Spring in Hudson, New York, N.Y. Mention of this product does not constitute endorsement.

without firm skeletons such as roundworms, flatworms, and octopods) are relaxed in $MgCl_2$, chlorobutanol, refrigeration, etc.), arthropods have their exoskeletons opened and trapped air evacuated from their trachea by vacuum chamber, and bivalve and gastropod molluscs are shucked from their shells. After an initial fixation of 10-15 minutes in Dietrich's and somewhat longer in Zenker's for large whole arthropods such as lobsters and certain crabs, one should: (1) remove appendages, (2) make a sagittal section through the carapace, and (3) remove visceral tissues and return them to the fixative. As a further aid to penetration of the fixative, whole large organisms such as the lobster may be step-sectioned according to convenience, but the slices should not exceed 2 cm in thickness. The larger molluscs should be sectioned at intervals of approximately 1 cm after initial fixation and returned to new fixative. When selected tissues are fixed rather than the whole animal, they should be suspended in the container by placing cheesecloth or absorbent paper towels on the bottom. Buoyant tissues, on the other hand, should be held immersed in the fixative by placing paper towels at the surface.

For final trimming, the tissue blocks are oriented so that the microtome sections will come off in the plane desired for examination. In preliminary studies of an organism (control or exposed), the tissues taken for histological examination should be as extensive and complete as possible.

Data accompanying organisms in shipment should be recorded on hard paper tags or keyed to numbered plastic tabs placed in the fixative with the respective tissue. Data accompanying a specimen's container is preferably recorded with lead pencil since inks (ballpoint) usually dissolve when placed in fixatives, or on package labels during freezing and thawing. Fixed tissues can be shipped in compact containers such as small, plastic "whirl bags" with excellent results. The volume of fixative in each tissue container need only be sufficient to immerse the previously fixed tissue or organism. Cotton is added to the container to insure that all the tissue remains moist in the event of leakage during shipment. All tissue containers should be placed in a shipment carton lined with a large, sealed, plastic bag to contain possible leakage during transit.

The rate at which postmortem changes or tissue autolysis occurs have been determined for the mummichog by light microscopy (Gardner, unpublished). Fifty-four adult mummichogs (Fundulus heteroclitus) were sacrificed in natural seawater (20°C and 20°/oo salinity) containing 2 mg/l or MS-222. Following death, the specimens were allowed to remain for preselected intervals of time in the aquaria prior to removal and fixation. Two specimens were removed every 2 minutes until 30 minutes and two every 15 minutes thereafter (or up to 3.5 hours).

Histological examination of these Fundulus revealed obvious tissue autolysis, first appearing apically in villus folds of the intestinal mucosa 10 minutes after death. Peripheral areas of the liver were atrophied at approximately 20 minutes. The changes proceeded to encompass the complete organ within a period of 3.5 hours. Autolysis also occurred in tissues adjacent to the gall bladder after 28 minutes, while changes in the intestinal track included the submucosa after 1 hour-30 minutes. The first indication of autolysis in the respiratory and oral epithelium occurred after 1 hour-45 minutes. The pseudobranch, sensory, renal, and gonadal tissues were noticeably affected after 2 hours-45 minutes. It must be realized that these autolytic changes will conceivably be intensified by increased temperature, for instance. Atrophy, or distortion of epithelial surfaces will also occur if the organism is removed from the aquatic environment and exposed to prolonged air-drying. Thus, it is imperative that tissue fixation be completed as quickly as possible. Moribund specimens are valuable for revealing the nature of a toxic response, however, those collected dead usually have little value for histopatholigical evaluation.

The importance of proper tissue fixation can be further illustrated by the observations of intestinal lesions produced in the ileum and rectum of adult Fundulus by 5500 mg/l (LC-50, 168 hrs.) of NTA (Eisler et al., 1972). The induced lesions originated at the apice of the villus structure as indicated by subacute NTA exposure (1 mg/l). It is easy to realize that inadequate fixation would have produced similar changes. Cadmium-induced damage in the

intestine of Fundulus also originates at the apice of the villus structure as early as 2 hours after exposure to the product (Gardner & Yevich, 1970). Therefore, delayed or improper fixation which promotes autolysis would undoubtedly conceal early development of lesions, as indicated above.

II Processing of Tissues for Microscopic Examinations

Detailed technical procedures for fixation, tissue preparation, staining and microscopy are to be found in a large number of publications. Among the more valuable sources for methods in histopathology, histochemistry and electron microscopy are: Jones (1966); Luna (1968); Galigher & Kozloff (1971); Pease (1964); Lillie (1965); Hayat (1970); and Zugibe (1970).

These texts can also be complemented by more specialized studies (Gardner & Yevich, 1970; Gardner & La Roche, 1973; Couch, 1974b; Couch & Nimmo, 1974a).

III Morphological and Physiological Changes Resulting from Pollutant Exposures

Select organs of some common species are briefly discussed as normal structures or morphological alterations resulting from toxicant exposures. These limited examples serve to illustrate the use of histology as an essential complement to marine bioassays.

The following species are among those considered to be valuable marine species for use in bioassays of toxic materials:

A.- American Oyster (Crassostrea virginica) an estuarine mollusc

1) Normal histological parameters:

As already indicated, an organism that would serve as an indicator of biologically damaging pollutants in estuaries must possess a number of characteristics. Wide geographic range is desirable to allow comparative studies of control and exposed organisms under differing environmental conditions and different locations. A species whose morphological and physiological properties are understood. The ability to rear the organism under controlled populations and specimens exposed to various changes of the natural environments.

The American oyster, Crassostrea virginica (Gmelin), fills these criteria. Its geographic range extends from Prince Edward Island, Canada, along the Atlantic coast to the Gulf coast of Texas. It has been claimed that the oyster is the best known marine organism. It is now feasible to spawn adult oysters, rear the larvae and maintain the spat and juvenile oysters under controlled laboratory conditions. For these reasons, the American oyster should make a valuable bioassay organism to evaluate estuarine conditions.

The voluminous literature on oyster biology is scattered through numerous scientific publications. However, for individuals initiating studies on the American oyster, biology and disease, several references may serve as entries into the field (Galtsoff, 1964; Cheng, 1967; Johnson, 1968; Sinderman, 1970; Sparks, 1972).

Histological study of oysters can be carried out using standard techniques of tissue fixation and staining. As in all histological work, investigators have developed special procedures found to be especially useful when particular cellular features are to be studied. Because of this, when a specific problem arises, one must consult the original literature for variant techniques. By utilizing the references in the literature, appropriate techniques can be found and adopted readily. One should be aware that most histological techniques developed for vertebrate tissues require variations to be useful for invertebrate tissues.

There is no single publication containing a detailed description of oyster histology, but Galtsoff's monograph (1964) contains much useful information and numerous references. Histological descriptions of various oyster tissue are scattered through the literature so that each tissue or organ may

be described with particular emphasis. For example, Loosanoff (1942) described the histology of gamete development in C. virginica when the laboratory was involved in development of techniques for artificial spawning. In 1957, Shaw and Battle published the definitive work on digestive tract histology while studying digestion in the oyster. Considerable information and speculation about "normal" oyster tissues has been published by persons studying oyster diseases; for example, Pauley & Sparks (1966) described acute inflammatory reactions and necessarily described the comparable "normal" tissue of control oysters. Thus, the best way to find a detailed histological description of oyster tissues is to seek appropriate references to original research papers.

Assuming one knows basic oyster histology, it is still necessary to identify common pathological conditions unrelated to the specific insult under consideration. Two examples of this problem are: (1) effects of non-specific stress and (2) infection. Oysters in unfavorable environments have a tendency to seal their valves and "ride out the storm". Thus, heavy rains, decreasing salinity below a tolerable minimum, or silting, or blooms of non-food organism, may cause oysters to close their valves tightly. With time, this cessation of feeding may lead to tissue and cell destruction, disruption of metabolism and reduction of reproductive potential. In some areas, infection of oysters by certain parasites is nearly universal (i.e. Nematopsis ostrearum in Delaware Bay), but little tissue damage is noted in most infected individuals. These natural occurences have to be evaluated individually and with some experience of local conditions, reasonable interpretations of tissue damage are possible. With this baseline information mastered, one may then turn to the effects of additional factors.

The oyster chiefly consists of parenchymal cells (leydig cells, vesicular connective tissue cells), abductor muscle, heart, kidney, gills, mantle, gut, gonad, and hepatopancreas, all bounded by various epithelial layers. The normal histology of the oyster is described in numerous publications, the most extensive of which is the book by Galtsoff (1964).

2) Toxicant-induced histopathology:

The following conditions may indicate altered well-being in oysters chronically exposed to toxicants when compared to oysters from control groups:

a) gross emaciation; watery, pale digestive gland (effects of post-spawning stresses also produce this condition);
b) abcesses or pustules on mantle or inner shell;
c) production of large amounts of pseudofeces;
d) loss of vesicular pattern of parenchymal cells with breakdown in supportive stromata; this may be associated with gross emaciation (see a);
e) massive or heavy infiltrates of leukocytes into regions of parenchymal cells and basement membrenes;
f) metaplasia in digestive diverticula, particularly involving the non-ciliated epithelium of the distal tubules, and/or the epithelium of the proximal tubular ciliated epithelium; atrophic changes in the distal epithelium of the digestive tubules may indicate a critical irritant response (Couch, in Lowe et al, 1972), and be associated with gross emaciation (see a) resulting from chronic interference with normal absorption of nutrients;
g) edema histological separation of parenchymal tissues from basement membranes of digestive tubules, stomach, or intestine due to fluid pressure;
h) hyperplasia or generally, metaplasia, of gill and mantle epithelia may produce abnormal epithelial tissues several cell layers deep, instead of normal cuboidal or columnar epithelia. Hyperplasia may indicate an irritant response of exposed epithelial surfaces;
i) higher prevalences of the following oyster pathogens in exposed versus control bioassay oysters:
   - Minchinia nelsoni (MSX) - Couch, 1966, 1967;
   - Minchinia costalis (SSO) Couch, 1967;
   - Labyrinthomyxa marina (Dermocystiduim marinum)- Ray, 1954;

Hexamita (flagellate);
Nematopsis (gregarine);

j) Finally, inhibition of gonadal development or gametogenesis as compared to that in control or feral oysters of the same size or age, during spring, summer and early fall seasons.

3) Physiological Indices:

a) Shell deposition

Under optimal conditions, yearling oysters usually grow rapidly and uniformly. A technique for determining suboptimal environmental conditions is to measure the lack of shell deposition in young oysters (Butler et al., 1960). Young oysters selected from a population for size, have the new shell filed off. Half of these oysters are placed in uncontaminated flowing water with an adequate food supply and the other half are placed in suspect water. Every other day, the total length of each oyster is measured.

After 3 weeks, each oyster is refiled to the original length and the experiment repeated. The presence of pollutants affecting the oyster will generally affect adversely shell growth rates when compared to oysters maintained in uncontaminated water (Andrews, 1961). To determine the permanency of the damage, oysters whose shell growth has been inhibited can be placed in uncontaminated water and their growth rate compared to controls. In most cases, the absence of toxic materials will allow a resumption of normal shell growth rate. Growth inhibition is not a specific reaction and independent chemical analysis of test waters are essential to identify toxic chemicals (Butler, 1966).

b) Water pumping

Lamellibranch molluscs are dependent on the flow of water over certain tissues to supply oxygen and food and to remove wastes. Water transport is accomplished by synchronized continuous beating of thousands of lateral cilia. The adductor muscle, the mantle edge, the gill muscles and ostea all play a part in regulation of the flow of water. Numerous techniques have been devised for determining rate of water flow and several are discussed in detail by Galtsoff (Chap. IX). Several groups of substances or changes in seawater will influence rate of pumping and, for this reason, care should be taken to establish reproducible control conditions before toxic response studies are undertaken. Considerable variations between individual oysters are also known to occur normally and must be taken into account. Toxic materials, excessive particulate matter, drastically modified salinities will, for instance, be sensed by oysters and will often result in decreased water pumping. This technique would thus appear to be relatively sensitive, but nonspecific in the establishment of water quality events which would affect this species. Kymograph recordings of shell movements of a group of test oysters, compared to comparable data from control animals, can yield valuable information about water quality (Butler, et al., 1960).

In oysters, water pumping (or transport) and respiration are intimately interdependent. Rate of oxygen uptake can be measured directly by microdetermination of oxygen content of water taken from the inhalent and exhalent currents of individual oysters. These procedures are discussed in Galtsoff (Ch. IX). Reproducible values are obtained with practice and toxic concentrations of pollutants in water would generally cause significant changes in the ability of oysters to extract sufficient oxygen from the water, even though pumping rates may remain unaffected for a period of time. Prolonged lowering of oxygen fixation will inevitably reduce physiological activities, including pumping rates.

c) Blood proteins

A traditional method for gauging the overall health of an organism is to monitor its blood protein composition or concentration. In vertebrates, this valuable information can assist in the diagnosis of certain anomalies; in oysters and other invertebrates, the information obtained is less definitive but may be useful in the establishment of disorders related to nutritional or metabolic activities.

Total blood cell (amoebocyte) counts can be made using simple enumerative techniques (Feng, 1965). Total hemolymph protein concentrations can be determined by standard methods (Lowry, et al., 1951). Electrophoresis of hemolymph protein can also be done with minor modifications of standard procedures (Feng and Canzonier, 1970). Lysozyme is found in oyster hemolymph and can be quantified (Feng and Canzonier, 1970). This sort of analysis of oyster hemolymph, to relate concentrations and distributions of natural products with overall health, can be a sensitive and valuable tool; however, this approach has not been fully exploited thus far.

d) Other tissue biochemistry

If oysters recognize and react to changes in their environments, it can be assumed that measurable biochemical changes may accompany their adaptive or toxic responses. For instance, gross observation of oysters subjected to prolonged exposure to low salinity demonstrates that they become "thin" and "watery" (i.e. the tissues have become translucent). This is often associated with loss of tissue glycogen; the product concentrated for eventual energy production under anaerobic conditions. Sophisticated studies show other effects of stress that are reflected in biochemical changes (Hammen, 1969). The basic patterns of tissue metabolism are similar to those found in other species. However, the development of selective analysis,identifying key enzymatic reactions and quantitative measures of products formed, need to receive further attention. A comparative approach, evaluating changes between control and exposed organisms, would be a powerful tool in the identification of environmental changes affecting the survival of oysters in a particular area.

e) Gonadal development

For the continued presence of the species to be assured, in a particular location, gamete formation and spawning must occur in a significant number of oysters each year. It is known that molluscan spawning follows irregular annual cycles, but it is evident that prolonged low yield reproduction will ultimately accompany the disappearance of a population. Colonization by planktonic oyster larvae from distant points via tidal movements is possible, but not nearly as efficient or effective as the successful spawning of the local population, particularly if local conditions limit the survival of setting organisms. Thus, any factor that decreases the ability of adult oysters to form gametes and insure adequate fertilization is a threat to the population.

In adult oysters, gamete formation is dependent upon obtaining adequate nutrients, active feeding and successful incorporation of elements essential for gamete synthesis. Anything interfering with feeding, digestion and metabolic activities for prolonged periods of time would adversely affect reproduction. Gamete production can be monitored by gross observation of individual oysters during the normal spawning season (mid-July through early-September, in the mid-Atlantic region: significant variability is notable in other areas). Semi-quantitative measurements of gonad development can be made (Tripp, 1974) and related to environmental parameters. This is done by cutting sections through the middle of the oyster so as to include the maximal cross-sectional gonadal area. The tissues are fixed, stained and examined microscopically. A semi-quantitative scale for measurement of gonad maturity is established. A rough measure of the condition of the population can be obtained by calculating the "gonad index" (the average degree of gonad development for several individuals sampled). This, in turn, can be correlated with environmental parameters and certain inferences can be drawn (Tripp, 1974). As indicated earlier for several measurable parameters, this type of biological reaction is not specific to any particular product or environmental changes. Probing environmental analyses are the only means of identifying the single or multiple causative factors inducing anomalies.

f) Larval development

Perhaps the most developed of all bioassay techniques involving oysters is the use of larvae as early indicators of water quality (Davis, 1961; Woelke, 1962, 1967 and 1972). This procedure requires collection of adult oysters from natural populations and maintenance under optimal developmental conditions until gonads are fully mature. At this time, spawning of individuals

may be stimulated by raising the temperature of running seawater to 25-30°C for 2-3 hrs. Embryos may then be collected and held in large cultures (20,000-30,000 fertilized eggs per liter) until 48 hrs. old. During this time, they are exposed to test water and, at the end of the 48 hr. test period, they are examined and compared to normal control larvae. The effect of any variable tested is described in terms of the per cent oyster larvae which develop abnormally (Woelke, 1967). Mortality can also be determined and LC_{50} calculated (Davis, 1961).

B.- Pink Shrimp (Penaeus duorarum) - an estuarine, marine decapod

The pink shrimp is found from Brazil to Virginia, in estuaries, as juveniles, and in the ocean, as adults and larvae. It is one of three commercially valuable penaeid shrimp species in the South Atlantic, and Gulf of Mexico (Williams, 1965). The species has been used successfully in many bioassays. These bioassays have included LC_{50} and EC_{50} determinations for several toxicants as well as estimates of effects on several physiological parameters (Nimmo et al., 1974). Couch (1974a,b) and Couch and Nimmo (1974a) have studied the histology, ultrastructure and pathology of this species in relation to toxicant exposures. The normal histology of select organs of the pink shrimp has also received considerable attention.

The hepatopancreas of the pink shrimp appears to be a sensitive organ in the histopathological detection of toxicant effects in crustacea. Both light microscopical and electron microscopical methods are proving useful in determining morphological changes (Couch, 1974b, Couch and Nimmo, 1974a).

1) Hepatopancreas normal appearance

This organ consists of acini or digestive tubules which extend from two hepatopancreatic ducts that have their origins in the lateral walls of the pyloric stomach. The functional and structural unit of the organ, the acinus, consists of a tube lined with epithelial elements of varied structurally and functionally defined cell-types. These cells are:(a) the embryonic cells, found at the blind-pouch end of the acinus, distal to the hepatopancreatic ducts; these cells are active mitotically and serve as generative or blastoid cells which give rise, through differentiation, to the cell types more proximal (in tubule epithelium) to the hepatopancreatic ducts; (b) absorptive cells, found distal-to-medial along the acinus, possess fine structural features similar to absorptive cells in the vertebrates, (i.e. microvilli, apical region of phagocytic vesicles, mitochondria and lysosomes, and a base nucleus); these cells have been studied intensively by light and electron microscopy following exposure of shrimp to toxicants (Couch and Nimmo, 1974a); (c) secretory cells, found medial and proximal to hepatopancreatic ducts along the acinus epithelium; these cells are characterized by the presence of large granules and vacuoles and may be both holocrine or apocrine in secretion of contents (presumably digestive and lubricative materials) into the lumen of the acinus.

The epithelium making up the walls of the ducts and tubules of the center of the hepatopancreas consists of cells usually in an atrophied condition. Renewal and growth of the whole organ occurs at the distal ends of the acini in the peripheral region of the organ where embryonic cells abound.

2) Hepatopancreas pathological alterations

Certain of the following histological and cytological alterations have been found in hepatopancreas of pink shrimp exposed to polychlorinated biphenyls (PCB's) in flowing seawater bioassays; most of these alterations are probably non-specific and, thus, may also be indicators of toxicity caused by other pollutants:

a) histological changes (light microscopy): hepatopancreatic epithelial cell lysis, nuclear pyknosis, vacuolization (larger vacuoles than found in normal secretory cells); large pyramidal, tetrahedral inclusion bodies of Baculovirus penaei in nuclei (Couch, 1974a,b,d).

b) histochemical abberrations: excessive lipid accumulation, glycogen loss, reticulin stromatic changes from normal, and selected - enzyme loss of activity.

c) ultrastructural changes in the cytoplasm, and nucleus of absorptive cells; loss of normal microvillus boarder at the apical end of cells; swelling of mitochondria; and formation of myelin bodies in cytoplasm; loss of normal composition of cytoplasm, i.e. abnormal increase in free ribosomes or loss of all organelles; nuclear hypertrophy, chromatin abberrations, or diminution and formation of vesicles within nucleoplasm (Couch and Nimmo, 1974a).

d) higher prevalence and intensities of viral hepatopancreatic infections (Baculovirus penaei, Couch, 1974a,b,d,) in exposed compared to control shrimp, i.e. possible augmentation of natural pathogens in the presence of pollutants (Couch and Nimmo, 1974b). Both light microscopy and electron microscopy are needed to detail the diagnosis.

Most of the above lesions have been found in experimentally exposed shrimp. However, more research is needed to clarify their significance in relation to functional anomalies.

C.- Spot (Leiostomus xanthurus) - an estuarine, marine fish

The spot is found in abundance along the Gulf and Atlantic coasts, in estuaries in the spring, summer, fall and even in the winter in the South. This fish is found as far north as New York and throughout the Gulf of Mexico coastal regions. It also may be obtained in several life-cycle stages, according to season of the year. The spot may be easily obtained for bioassay experiments in its range. For a biology of the spot, the reader is referred to Dawson (1958).

1) Liver - normal histological parameters

The normal liver of spot is of the tubulosinusoidal type (Elias & Benglesdorf, 1952). The liver parenchymal cells are arranged in cords or muralia which are usually two hepatic cells thick. There are no well-defined lobules, although the hepatic artery, portal vein and bile duct are found close together. The portal vein and bile duct are usually paralleled by disseminated pancreatic exocrine tissue (Couch, 1974c).

In normal, well-fed fish, hepatic cells show moderate to heavy PAS positive reactions and diastase lability indicating the presence of glycogen. Starved or stressed fish show less PAS affinity. Normal spot usually reveal few, small lipid droplets or no lipid in hepatic cells (oil Red O method, with frozen sections).

2) Liver - pathological alterations

The liver of this fish has been histologically and histochemically examined following exposure to the following toxicants: PCB's (Aroclors 1254, 1016; Couch 1974c), Dieldrin (Parrish, 1974), Endrin (Lowe, 1965) and Sevin (Couch, 1974c). The above listed chemicals may be found in various estuaries and marine environments. The pathological studies reported herein were performed on fish exposed in laboratory flow-through bioassays to which controlled low levels of the toxicant were administered continuously. The following changes in livers of spot exposed to any given toxicant may be observed in the course of such exposures:

a) alterations in the orientation of liver cord relationships and hepatic cells;
b) lytic or pyknotic changes in hepatic cells and nuclei;
c) vacuolization, fibrosis, cirrhosis and necrosis of hepatic tissues;
d) morphological anomalies in intrahepatic pancreatic exocrine tissues such as excessive vacuolization, loss of basophilia and nuclear degeneration;
e) glycogen loss or excessive accumulation; lipid increase, particularly "fatty liver" syndrome, or fatty metamorphosis (histochemical methods); researchers should be aware that these changes may also

result from normal seasonal variations;
f) prehepatomatic signs, and hepatoma or other neoplastic lesions;
g) cholangiolar proliferation;
h) red blood cell occlusion or stasis in portal or central veins;
i) excessive pigment deposition in or between hepatic cells (i.e. ceroid or hemosiderin);
j) ultrastructural changes (as observed with electron microscopy), such as abnormal proliferation of endoplasmic reticulum, lipid accumulation in hepatocytes, myelin body formation, and nuclear change.

The preceding list of possible morphological alterations is not meant to be exhaustive, by any means, but it includes alterations noted in livers from fishes exposed to known levels of toxicants (Couch, 1974c). Alert observers should note combinations of the above or other lesions not listed.

Certain dysfunctional states in the organism may be related to specific lesions in the list above. Identification of functional disorders associated with specific lesion or histopathological syndrome would have to be resolved by appropriate physiological or biochemical investigations.

D.- Remarks

Guided by observations made under natural conditions, exposure experiments of organisms to toxicants must be conducted under controlled laboratory conditions. With acute exposure studies, the researcher is generally able to estimate the concentrations of toxicants to be used in chronic bioassays. From the histological standpoint, organisms are evaluated for presence and reproductability of lesions in all instances. Whenever possible, the exposure and histopathologic studies are followed by, or associated with observations of field conditions to determine whether the reaction(s) of non-captive organisms exposed under natural conditions compare with laboratory findings. These results, combined with those from other scientific approaches, can provide the scientific basis for establishment of Water Quality Criteria (W.Q.C.). Histology provides an essential dimension which often confirms or explains the damaging effects of certain products before they are introduced into the environment, such as would have been the case with NTA (a proposed substitute to phosphate in laundry detergents) (Eisler et al., 1972).

Heavy metals, pesticides and petroleum products represent major concerns in terms of environmental contamination. Minimata, or Itai-Itai disorders are disturbing examples of what too much mercury in water can do to the human body. Too much cadmium affects the kidney of some fishes in a fashion similar to that documented for humans (Gardner & Yevich, 1970). For instance, accumulation of cadmium in the kidney to certain levels will induce irreversible damage in the renal tubules of Fundulus.

Copper is also known to promote lesions in the kidney of some fish and of some invertebrates. In addition, copper has demonstrated neurotoxic properties. Preliminary observations have indicated that behavioral changes were associated with exposures to sublethal copper concentrations in fish. Histological examinations of Fundulus and Menidia menidia (Atlantic silverside) exposed to copper confirmed the presence of morphological alterations in vital sensory organs (Gardner & La Roche, 1973). These lesions are significant because they involve the lateral line (mechanoreception) and the olfactory organs (chemoreception). Obviously, these organs are vital to perception and normal behavior patterns such as those of feeding, schooling, reproduction and migration. From the observations reported (Gardner & La Roche, 1972), it is unlikely that affected fish could cope with either preys or predators in a natural environment. Mercury and silver also damages some perception organs of the sensory system, although the lesions differ in appearance. Damage to sensory organs have also been demonstrated in menhaden (Brevoortia tyrannus) obtained from three different sites in the environment (Gardner & La Roche, 1973; Gardner, unpublished). In these instances, circumstantial evidence associates the lesions with high soluble copper concentrations in the water column at a power plant discharge (70 ppb), in Narragansett Bay, Warwick, R.I. (30-100 ppb) and in the Acushnet River, New Bedford, Mass. (230 ppb).

The prospect of combined metal wastes can be disturbing, especially when additive or synergistic responses are expected. The combination of cadmium and copper represent an example of two metals that can act synergistically (Eisler & Gardner, 1973). In Fundulus, low acute toxicity for a concentration of copper alone, added to a concentration of cadmium of undetectable toxicity by itself, becomes markedly toxic when both are added in short-term laboratory bioassays. For these low metal concentrations, copper would only induce sensory lesions, while cadmium alone would fail to elicit any renal lesion. However, in combination at these concentrations, the metals induced both neurosensory and kidney lesions and increased acute toxicity by several orders of magnitude.

Some physical factors are known to influence toxicity of some compounds, such as cadmium. Nevertheless, changes in salinity, temperature, pH, or dissolved oxygen, do not alter the eventual nature of lesions (Gardner & Yevich, 1969).

Various chemical pesticides, including chlorinated hydrocarbons and organophosphates, have been found to cause histological alterations in livers of fishes similar to those of mammals exposed to toxicants (Couch, 1974c). Preliminary histological evaluations of methoxychlor,exposed Fundulus heteroclitus have indicated that the compound causes lesions in the lateral line system (Gardner & La Roche, 1974).

Chemical carcinogens occur among the polycyclic hydrocarbons, N-nitrosocompounds, radioactive nuclides, mycotoxins, heavy metals, and other groups. Field collected cold-blooded vertebrates and invertebrates from numerous freshwater to marine environments are regularly discovered with neoplastic diseases suggesting that carcinogenic chemicals are present. The most likely sources of such chemicals include domestic and industrial discharges; leaching of pesticides, fertilizers, natural materials and residues from atmospheric fallout; oil spills, and natural synthesis when the precursors are available.

Examples of some lower animal neoplasms discovered in such possibly contaminated aquatic habitats are: (1) Russell & Kotin (1957) reported 10 out of 353 cases of oral non-invasive squamous-cell papillomas in white croakers collected in the Pacific Ocean, within 2 miles of the Santa Monica, California sewage outfall, while no similar lesions were found on 1,116 white croakers collected 50 miles away in unpolluted water. Subsequently, white croakers with oral papillomas were reported from Los Angeles Harbor (Young, 1964) and numerous examples were also discovered in white croakers feeding near the Santa Ana, California sewage outfall (Harshbarger, 1972, 1974); (2) Lucké and Schlumberger (1941) discovered 166 brown bullheads (Ictalurus nebulosus)with transplantable oral epitheliomas, in the Delaware and Schuylkill Rivers in southeastern Pennsylvania, presumably heavily polluted from effluents from Philadelphia. Harshbarger (1972, 1974) discovered similar neoplasms in Ictalurus nebulosus in lakes in central Florida, where there is extensive use of chemicals to control citrus pests and where water hyacinths in the lakes are sprayed with herbicides; (3) Rose (in press) has discovered a population of neotenic tiger salamanders (Ambystoma tigrinum) living in a treated, domestic sewage settling pond in which at least 50% of the population has developed conspicuous neoplasms of the skin - primarily epidermal papillomas, papillary dermal fibromas, and melanomas; (4) There have been numerous reports of invasive neoplasms in the filter-feeding (bivalve) molluscs from widely distributed estuaries receiving effluent from many sources since the initial report in 1968; previously, there were only occasional reports of benign neoplasms in these animals, going back to 1887. Wolf (1969, 1971) reported 12 invasive neoplasms of the mantle epithelium from Crassostrea commercialis from 2 rural (agricultural) estuaries in southeastern Australia, although one estuary had a pulp mill upstream. Pauley and Sayce (1972) described a single invasive mantle epithelioma in C. gigas from the lightly industralized estuary, Willapa Bay, Washington, while a second C. gigas from Willapa Bay had a ganglioneuroma (Pauley et al., 1968). Fourteen cases of gonadal neoplasms have been described in both male and female quahogs, Mercenaria mercenaria, from the Narragansett Bay area of Rhode Island, which is near a large metropolitan area (Yevich & Barry, 1969; Barry & Yevich, 1972). In the same general area, 40% of the soft-shelled clams (Mya arenaria) examined showed atypical gill and/or kidney epithelial hyperplasia (Barry et al., 1971). In the Chesapeake Bay and its

tributaries, hematopoietic neoplasms have been discovered in a large number of C. virginica (Farley, 1969; Couch, 1969; Frierman & Harshbarger, 1974), a reticulosarcoma-like lesion in a C. virginica (Couch, 1970), and an epizootic highly proliferative, anaplastic neoplasm arising from the gill epithelium of Macoma balthica (Christensen et al., 1974). An epizootic of an undifferentiated mesenchymal neoplasm in Mytilus edulis and hematopoietic neoplasms in Ostrea lurida have been found in Yaquina Bay, Oregon, downstream from a pulp mill (Farley & Sparks, 1970); and, (5) Following a spill of a combination of jet fuel and number 2 fuel oil, Barry (in preparation) associated a 22% incidence of neoplastic-like lesions in soft-shelled clams. Other lesions of a possible precancerous nature have also been demonstrated in animals exposed to crude oil. Gardner et al (1974) found such lesions in the olfactory organs of Menidia, apparently from the salt water soluble fraction of crude oil. In addition, the pseudobranch of Menidia was especially vulnerable to crude and waste oil and vascular lesions occurred in scallops, oysters, Menidia and Fundulus.

The neoplasms and related diseases correlated with environmental pollutants in the five examples just cited, all occurred in bottom-feeding fish and filter-feeding molluscs, a trend also borne out by the specimens which have been sent to the Registry of Tumors in Lower Animals for diagnosis and registration (Harshbarger, 1974), and by the other published, but not cited, cases of lower animal neoplasms (Dawe & Harshbarger, 1969). This is a logical observation since bottom-feeders and filter-feeders would be apt to encounter larger amounts of suspended and sedimented chemicals than surface feeders and it provides further circumstantial evidence of the harmful effects of water pollution.

Neurosensory lesions have also been linked to pulp mill waste exposures, since olfactory lesions have been demonstrated in the Atlantic salmon (Gardner, 1972). These lesions were induced in an experimental system within the natural waters of the St. Croix River. Thus, neurosensory lesions, detectable by histology, represent an important aspect of incidious damage to marine fish exposed to relatively low doses of varied chemicals likely to be introduced in the marine environment.

As previously stated, normal histology, accounting for seasonal and all other normal variations, forms the basis from which histological evaluations of anomalies must be made. Since the morphological and physiological states of marine organisms varies annually according to environmental conditions and including those of captivity, results may be greatly influenced by them. For this reason alone, it is imperative that researchers be convinced of the necessity to maintain and compare exposed organisms with appropriate controls.

The copepods (Acartia clausi and A. tonsa), routinely cultured for laboratory experimentation, serve to illustrate the importance of routine histological examination of experimental stocks maintained in captivity. Histological evaluations with these organisms have shown that captivity will affect the abundance of unicellular glands (Gonzalez et al., 1973). These glands are usually numerous in natural populations and in laboratory cultures that are well fed. These glands diminish in abundance from both laboratory and field populations when food densities are low. These glands can, furthermore, be used as an index of nutritional condition.

Investigations of the bay scallop, (Aquipectin irradians) have determined that these organisms undergo significant annual variations which can be followed by observing the content of acid-staining granules in its nervous system (Blake & Yevich, 1972). The annual cycles are considered normal and can be correlated with ambient water temperature. Therefore, it is essential that these histophysiological variations be recognized when appraising the effects of toxicants on aquatic species.

The above studies indicate that normal histology must be carried out on feral organisms taken from natural environment to appreciate both the morphological and physiological states of the species which may be affected by pollutant exposures. Although controls must be used in all experimental work, both control and exposed organisms may undergo changes in a laboratory system

which might not otherwise be recognized or evaluated in terms of significance. Normal histological evaluations of marine organisms are essential to better understand morphological anomalies which are due to pollutants. Weekly collections and examinations of stocks of the bay scallop (Aquipectin irradians), mussel (Mytilus edulis), soft-shelled clam (Mya arenaria), and Atlantic silversides (Menidia menidia), has been demonstrated to be reasonable means of establishing normal seasonal variations, especially in natural populations likely to be used experimentally (Yevich, 1974).

Because of a wide geographic distribution and commercial value, the oyster has been studied extensively for its responses to changes in water quality. In a few instances (i.e. to evaluate pulpmill effluents), it has been used routinely for acute larval responses. Some information on its morphological and physiological responses is also available. This is, generally speaking, quite rudimentary and it is hoped that systematic efforts will be made to improve techniques leading to an understanding of metabolic activities involved with specific environmental changes. With the increased attention being paid to pollution of estuarine waters, this important sedentary adult mollusc may serve as a biological indicator of the quality of these vulnerable waters.

An entirely new approach is now feasible as well. This is the rearing of oysters under controlled laboratory conditions and transferring them to field situations to determine their reaction to particularly suspicious environments. The advantage of this approach is to eliminate the possibility that biological damage or impairment observed in oysters from the field may limit the use of oysters as bio-monitoring elements.

Early histological evaluations must be as complete as possible to offer a broad survey of tissues which may be affected through specific exposures. In some instances, reported histological investigations are incomplete in that attention is focused too rapidly on one or two major tissues, such as to respiratory and hepatic tissues. In other cases, only the short-term study of one section of an organism may have been explored. Many lesions, reportedly due to toxicants or diseases, may appear questionable since they may only represent normal variations. The need for normal histology, established over extended periods of time, remains most important. In-depth studies on select tissues and organs is always indicated once they have been identified as targets of toxicants.

Respiratory tissues represent an area of prime histological interest in aquatic organisms. These tissues require a great deal of histological studies to properly assess normal and abnormal developments. In fish pathologist circles, it has been observed that "the degree of hyperplasia in respiratory epithelium depends largely on the angle of the cut". The statement is à propos.

Very often, histological diagnosis will acquire much greater significance by complementing routine histology with techniques available in histochemistry, autoradiography, electron microscopy and electron scanning. The light microscope and electron microscope are providing an increasing amount of knowledge about the morphological responses of marine organisms to acute concentrations of toxicants. The light microscope can, in many instances, indicate the sites of initial tissue reactions to chronic toxicity. In this manner, light microscope serves to guide the investigator in probes to define tissue damage with more elaborate diagnostic procedures including electron microscopy. In chronic toxicity evaluations, only a limited number of animals in any one population may become visibly affected in a specified time frame. However, these evaluations will generally supplement findings established at more acutely toxic levels. It is essential that diagnostics of other scientific endeavors such as behavior and physiology, for instance, be applied to the overall analysis of normal or abnormal performance by exposed species. Histopathology is essential as an element in these investigations, and by being applied broadly, it can offer realistic visible evidence of pollutant damage.

Histological evaluations, therefore, should be considered as essential elements in the scientific establishment of Water Quality Criteria. However, a successful approach to the problem of defining these criteria depends on well-developed interdisciplinary diagnostic strategies to identify the effects of pollutants.

REFERENCES

ANDREWS, J.D. 1961. Measurement of Shell Growth in Oysters by Weighing in Water. Proc. Ntl. Shellfish. Assoc. 52: 1-11.

BARRY, M.M. 1974. Incidence of Cancer in Soft-shelled Clam (Mya arenaria) from an Oil Spill. In: Neoplasms in Aquatic Animals. Prog. Exp. Tumor Res., F. Homburger (ed.), Vol. 21, S. Karger: Basel, München, Paris, New York, (in press).

BARRY, M.M. & YEVICH, P.P., 1972. Incidence of Gonadal Cancer in the Quahaug, Mercenaria mercenaria. Oncology 26: 87-96.

BARRY, M.M., YEVICH, P.P. and THAYER, N.H. 1971. Atypical Hyperplasia in the Soft-shell Clam, Mya arenaria. J. Invert. Pathol. 17: 17-27.

BLAKE, N.H. and YEVICH, P.P., 1972. The Neurosecretory Cycle of the Bay Scallop, Aquipectin irradians. Gen. Comp. Endocrinology, 18(3) abstr.

BUTLER, P.A. 1965. Reaction of Estuarine Mollusks to Some Environmental Factors. In: Biological Problems in Water Pollution. C.M. Tarzwell,(ed). U.S.D.H.E.W., P.H.S., Div. of Water Supply and Pollution Control, Cincinnati, Ohio. 424 pp.

BUTLER, P.A. 1966. The Problem of Pesticides in Estuaries. Amer Fish. Soc. Spec. Publication no. 3: 110-115.

BUTLER, P.A., WILSON, A.J. and RICK, A.J. 1960. Effect of Pesticides on Oyster. Proc. Natl. Shellfish Assoc. 51: 23-32.

CHENG, T. 1967. Marine Molluscs as Hosts for Symbioses with a Review of Known Parasites of Commercially Important Species. Advan. Marine Biol. 5: 1-424.

CHRISTENSEN, D.J. FARLEY, C.A.and KERN, F.G. 1974. Epizootic Neoplasms in the Clam Macoma balthica (L.) From Chesapeake Bay. J. Natl. Cancer Institute, 52(6): 1739-1949.

COUCH, J. 1967. Concurrent Haplosporidian Infections of the Oyster, Crassostrea virginica. Journ. Parasitology, 53: 248-253.

COUCH, J. 1969. An Unusual Lesion in the Mantle of the American Oyster (Crassostrea virginica. Nat. Cancer Inst. Monogr. 31: 557-562.

COUCH, J. 1970. Sarcoma-like Disease in a Single Specimen of the American Oyster. In Bibl. haemat. no. 36, ed. R.M. Dutcher, p. 647, Kager, Basel/München/Paris/New York.

COUCH, J. 1974a. Free and Occluded Virus, Similar to Baculovirus, in Hepatopancreas of Pink Shrimp. Nature, 247: 229-231.

COUCH, J. 1974b. An Enzootic Nuclear Polyhedrosis Virus of Pink Shrimp. I. Ultrastructure, prevalence and enhancement. J. of Invert. Path. (in press).

COUCH, J. 1974c. Histopathologic Effects of Pesticides and Related Chemicals on the Livers of Fishes. In: The Pathology of Fishes. University of Wisconsin Press (in press).

COUCH, J. 1974d. Possible Activation or Enhancement of Latent Baculovirus Infections in Penaeid Shrimp. In: Neoplasms in Aquatic Animals. Prog. Exp. Tumor Res., F. Homburger (ed.), vol. 21, S. Karger: Basel, München, Paris, New York, (in press).

COUCH, J., FARLEY, A. and ROSENFIELD, A. 1966. Sporulation in Minchinia nelsoni (Haplosporida, Haplosporidiidae) in Crassostrea virginica. Science, 153: 1529-1531.

COUCH, J. and NIMMO, D. 1974a. Ultrastructural Studies of Shrimp Exposed to the Pollutant Chemical, Polychlorinated Biphenyl (Aroclor 1254). Bull. of Pharmacol. and Environm. Pathologists, 11: 17-20.

COUCH, J. and NIMMO, D. 1974b. Detection of Interactions between Natural Pathogens and Pollutants in Aquatic Animals. In: Proc. Symposium on Diseases of Aquatic Animals (Regional) LSU-Sea Grant Sponsored (in press).

DAVIS, H.C. 1961. Effects of Some Pesticides on Eggs and Larvae of Oysters (Crassostrea virginica) and Coams (Venus mercenaria). Comm. Fish. Rev. 23: 8-23.

DAWE, C.J. and HARSHBARGER, J.C. (eds.) 1969. Neoplasms and Related Disorders of Invertebrate and Lower Vertebrate Animals. Natl. Cancer Inst. Monogr. 31, 772 pp.

DAWSON, 1958. A Study of the Biology and Life History of the Spot (Leiostomus xanthurus) with Special Reference to South Carolina. Bears Bluff Laboratory Contribution, no. 28, South Carolina, U.S.A.

EISLER, R. and GARDNER, G.R. 1973. Acute Toxicology to an Estuarine Teleost of Mixtures of Cadmium, Copper and Zinc Salts. J. Fish Biol. 5: 131-142.

EISLER, R., GARDNER, G.R., HENNEKEY, R.J., LA ROCHE, G., WALSH, D.F., YEVICH, P.P. 1972. Acute Toxicology of Sodium Nitrilotriacetic Acid (NTA) and NTA-Containing Detergents to Marine Organisms. Water Res. 6: 1009-1027.

ELIAS, H. and BENGELSDORF, H. 1952. The Structure of the Liver of Vertebrates. Acta Anatomica, 14: 24.

FARLEY, C.A. and SPARKS, A.K. 1970. Proliferative Disease of Hemocytes, Endothelial Cells and Connective Tissue Cells in Molluscs. In: Comparative Leukemia Research 1969, Bibl. Haemat., No. 36, R.M. Dutcher (ed.), pp. 610-617, S. Karger: Basel, München, Paris, New York.

FARLEY, C.A. 1969. Probable Neoplastic Disease of the Hematopoietic System in Oysters, Crassostrea virginica and Crassostrea gigas. Natl. Cancer Inst. Monogr. 31: 541-555.

FENG, S.Y. 1965. Heart Rate and Leucocyte Circulation in Crassostrea virginica (Gmelin). Biol. Bull. 128: 198-210.

FENG, S.Y. and CANZONIER, W.J. 1970. Humoral responses in the American Oyster (Crassostrea virginica) Infected with Bucephalus sp. and Minchinia nelsoni. In: Diseases of Fishes and Shellfishes, S.F. Snieszko, (ed.) Amer. Fish. Soc., Washington, D.C. 526 pp.

GALIGHER, A. and KOZLOFF, E. 1971. Essentials of Practical Microtechnique. Second edition. Lea & Febiger, Philadelphia. 531 pp.

GALTSOFF, P.S. 1959. Environmental Requirements of Oysters in Relation to Pollution. In: Biological Problems in Water Pollution. U.S.D.H.E.W., P.H.S., Div. of Water Supply and Pollution Control, Cincinnati, Ohio.

GALTSOFF, P. 1964. The American Oyster. Fishery Bull. 64: 1-480.

GARDNER, G.R. 1972. Appendix K, p. K1-K5. In: St. Croix River. A Study of Water Quality and Benthic Conditions in the St.Croix River, Grand Falls to Milltown, Maine and New Brunswick. U.S. Env. Protection Agency, Region I, Boston, Mass.

GARDNER, G.R.,LAROCHE, G. 1974. Chemically Induced Lesions in Estuarine or Marine Teleosts. In: W. Ribelin and G. Migaki (ed.) The Pathology of Fishes. Univ. of Wisconsin Press, (in press).

GARDNER, G.R., LA ROCHE, G. 1973. Copper Induced Lesions in Estuarine Teleosts. J. Fish. Res. Bd. Can. 30: 363-368.

GARDNER, G.R. and YEVICH, P.P. 1969. Toxicological Effects of Cadmium on Fundulus heteroclitus Under Various Oxygen, pH, Salinity and Temperature Regimes. Amer. Zool. 9(4): 1096.

GARDNER, G.R. and YEVICH, P.P. 1970. Histological and Hematological Responses of an Estuarine Teleost to Cadmium. J. Fish. Res. Bd. Can. 27: 2185-2196.

GARDNER, G.R., YEVICH, P.P. and ROGERSON, P.F. 1974. Morphological anomalies in Adult Oyster, Scallop and Atlantic Silversides Exposed to Emulsified Waste Motor Oil. (unpublished).

GONZALEZ, J., YEVICH, P.P., GENTILE, J. and LACKIE, N. 1973. Problems Associated with Cultured Marine Copepods (in press).

HAMMEN, C.S. 1969. Metabolism of the Oyster, Crassostrea virginica. Am. Zool. 9: 309-318.

HARSHBARGER, J.C. 1972. Work of the Registry of Tumors in Lower Animals with Emphasis on Fish Neoplasms. Symp. Zool. Soc. London, no. 30, pp. 285-303.

HARSHBARGER, J.C. 1974. Activities Report (of the) Registry of Tumors in Lower Animals 1965-1973. Smithsonian Institution, Washington, D.C. 141 pp.

HAYAT, M. 1970. Principles and Techniques of Electron Microscopy. Biological Applications. Vol. 1. Van Nostrand Reinhold Co., New York. 412 pp.

JOHNSON, P.T. 1968. An Annotated Bibliography of Pathology in Invertebrates Other Than Insects. Burgess Publ. Co., Minneapolis, Minn. 322 pp.

JONES, R. 1966. Basic Microscopic Technics. The University of Chicago Press, 334 pp.

LA ROCHE, G., GARDNER, G.R., EISLER, R., JACKIM, E., YEVICH, P.P. and ZAROOGIAN, G. 1973. Analysis of Toxic Responses in Marine Poikilotherms. In: Gary E. Glass (ed.) Bioassay Techniques and Environmental Chemistry. Ann Arbor Science Publishers, Ann Arbor, Michigan.

LILLIE, R. 1965. Histopathologic Technic and Practical Histochemistry. McGraw-Hill, New York, 715 pp.

LOOSANOFF, V.L. 1942. Seasonal Gonadal Changes in the Adult Oysters, Ostrea virginica, of Long Island Sound. Biol. Bull. 82: 195-206.

LOWE, J. 1965. Some Effects of Andrin on Estuarine Fishes. Proc. 19th Ann. Conf. Southeastern Assoc. Game and Fish. Comm. Tulsa, Okla. p. 271.

LOWE, J., PARRISH, P., PATRICK, J. and FORESTER, J. 1972. Effects of the pdychlorinated biphenyl Aroclor® 1254 on the American Oyster Crassostrea virginica. Mar. Biol. 17: 209-214.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. and RANDALL , R.J. 1951. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193: 265-275.

LUCKÉ, B. and SCHLUMBERGER, H. 1941. Transplantable Epitheliomas of the Lip Lower Animals 1965-1973. Smithsonian Institute, Washington, D.C. 141 pp.

LUNA, L.G. (ed.) 1968. Manual of Histological Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill, New York, 258 pp.

NIMMO, D., HANSEN, D., COUCH, J., COOLEY, N., PARRISH, P. and LOWE, J. 1974. Toxicity of Aroclor® 1254 and its Physiological Activity in Several Estuarine Organisms. Archives of Envir. Contam. and Toxicol. (in press).

PARRISH, R., COUCH, J., FORRESTER, J., PATRICK, J., and COOK, G. 1974. Dieldrin: Effects on Several Estuarine Organisms. Proc. of the 27th Ann. Conf. of the Southeastern Assoc. Game and Fish. Comm. (in press).

PAULEY, G.B. and SPARKS, A.K. 1966. The Acute Inflammatory Reaction in Two Different Tissues of the Pacific Oyster, Crassostrea gigas. J. Fish. Res. Bd. of Can. 23: 1913-1921.

PAULEY, G.B., SPARKS, A.K. and SAYCE, C.S. 1968. An Unusual Internal Growth Associated with Multiple Watery Cysts in a Pacific Oyster (Crassostrea gigas). J. Invert. Pathol. 11: 398-405.

PAULEY, G.B. and SAYCE, G.S. 1972. An Invasive Epithelial Neoplasm in a Pacific Oyster, Crassostrea gigas. J. Natl. Cancer Inst. 49(3): 897-902.

PAULEY, G.B. 1969. A Critical Review of Neoplasia and Tumor-Like Lesions in Molluscs. Natl. Cancer Inst. Monogr. 31: 509-540.

PEASE, D. 1964. Histological Technics for Electron Microscopy. Second Edition. Academic Press, New York, 381 pp.

RAY, S. 1954. Biological Studies of Dermo-cystidium marinum a Fungus Parasite of Oysters. Rice Inst. Pamphlet, Monogr. in Biology.

ROSE, F.L. 1974. Neoplasms and Related Lesions in a Population of Tiger Salamanders Living in Treated Sewage Effluent. In: Neoplasms in Aquatic Animals, Prog. Exp. Tumor Res., F. Homburger (ed.), vol. 21, S. Karger: Basel, München, Paris, New York (in press).

RUSSELL, F.E. and KOTIN, P. 1957. Squamous Papilloma in the White Croaker. J. Natl. Cancer Inst., 18(6): 857-861.

SHAW, B.L. and BATTLE, H.I. 1957. The Gross and Microscopic Anatomy of the Digestive Tract of the Oyster, Crassostrea virginica (Gmelin). Can. J. Zool. 35: 325-347.

SINDERMANN, C. 1970. Principal Diseases of Marine Fish and Shellfish. Academic Press, New York, 369 pp.

SPARKS, A.K. 1972. Invertebrate Pathology. Non-communicable Diseases. Academic Press, New York. 387 pp. General principles of invertebrate pathology with numerous references to oyster diseases throughout. A second volume on infectious diseases is in preparation, but probably will not be available before 1976.

TRIPP, M.R. 1974. Effects of Organophosphate Pesticides on Adult Oysters (Crassostrea virginica) In: Pollution and Physiology of Marine Organisms, F.J. Vernberg, (ed.) (in press).

WILLIAMS, A. 1965. Marine Decapod Crustaceans of the Carolinas. Fish. Bull. 65: 1-298.

WOELKE, C.E. 1962. Bioassays of Pulp Mill Wastes with Oysters. In: Biological Problems in Water Pollution, D.M. Tarzwell (ed.), U.S.D.H.E.W., P.H.S., Div. Water Supply and Pollution Control, Cincinnati, Ohio.

WOELKE, C.E. 1967. Measurement of Water Quality With the Pacific Oyster Embryo Bioassay. Water Quality Criteria, ASTM STP 416, Am. Soc. Testing Mats, 112-120.

WOELKE, C.E. 1972. Development of a Receiving Water Quality Bioassay Criterion Based on the 48-hour Pacific Oyster (Crassostrea gigas) Embryo. Washington, Dept. of Fisheries, Tech. Report no. 9, pp. 1-93.

WOLF, P.H. 1969. Neoplastic Growth in Two Sydney Rock Oysters, Crassostrea commercialis (Iredale & Roughley), Nat. Cancer Inst. Monogr. 31: 563-573.

WOLF, P.H. 1971. Unusually Large Tumor in a Sydney Rock Oyster. J. Natl. Cancer Inst., 46(5): 1079-1084.

YEVICH, P.P. and BARRY, M.M. 1969. Ovarian Tumors in the Quahog, Mercenaria mercenaria. J. Invert. Pathol. 14: 266-267.

YEVICH, P.P. 1974. Unpublished.

YOUNG, P.H. 1964. Some Effects of Sewer Effluent on Marine Life. Calif. Fish Game, 50(1): 33-41.

ZUGIBE, F. 1970. Diagnostic Histochemistry. C.V. Mosby Co., St. Louis, 366pp.

The presentation: "Histological and Physiological Evaluations in Some Marine Fauna" is a composite of information compiled by (in alphabetical order):

John Couch
Gulf Breeze Environmental Research Laboratory,
United States Environmental Protection Agency,
Gulf Breeze, Florida 32561
Tel. no. (904) 932-5326

George Gardner
National Marine Water Quality Laboratory,
United States Environmental Protection Agency,
South Ferry Road,
Narragansett, Rhode Island 02882
Tel. no. (401) 789-1427

John C. Harshbarger
Director,
Registry of Tumors in Lower Animals,
National Museum of Natural History,
Smithsonian Institution,
Washington, D.C. 20560
Tel. no. (202) 628-4422

M. R. Tripp
Department of Biological Sciences,
117 Wolf Hall,
University of Delaware,
Newark, Delaware 19711
Tel. no. (302) 738-2275 or 2281

Paul P. Yevich
National Marine Water Quality Laboratory,
United States Environmental Protection Agency,
South Ferry Road,
Narragansett, Rhode Island 02882
Tel. no. (401) 789-1427

RESPIROMETRY

Although respirometry, applied to aquatic organisms, cannot be claimed as a technique with any degree of specificity in the identification of toxic mechanisms, it is valuable in detecting signs of metabolic involvements. Well-standardized respirometry may also be relatively accurate in measuring the degree of metabolic changes associated with specific treatments (Voyer & Morrison, 1971, Cheng & Sullivan, 1973).

A number of aquatic poikilotherms are suited for these measurements and it would appear that respirometry is among the simplest and earliest means of establishing quantitative assessments of toxicant effects by comparing control and test organisms. Test organisms used were aquatic gastropod molluscs: Biomphalaria glabrata, Bulinus truncatus, and Nassarius obsoletus. Results on the mudflat snail, N. obsoletus, are presented herein.

I - Materials and methods

The specimens of the estuarine snail N. obsoletus used in this study were collected at Wickford Harbor, North Kingston, Rhode Island, during November 1973. They were brought back to the laboratory and maintained in 170-gallon aquaria at 22°C. Overcrowding was avoided by maintaining no more than 500 snails in each aquarium. The salinity of the artificial seawater (Instant Ocean)* was 30-31°/oo and the snails were fed frozen turbot and fresh clams, Mercenaria mercenaria, ad libitum.

All of the snails used measured between 18-22 mm in shell length and all had been ascertained to be free of helminth parasites.

In preparation for respirometric determinations, the exterior of the shell of each snail was cleaned and blotted dry prior to being placed in a reaction vessel of a Model GRP 14 Gilson Differential Respirometer (Middletown, Wisconsin). The vessel also contained 73 ml of the test solution, (i.e. seawater plus an experimental additive).

One ml of potassium hydroxide was placed in the center well of each flask, together with a filter paper fan (Whatman No. 2) for the absorption of the CO_2 evolved. One control snail was placed in a similar reaction vessel containing seawater with each determination and respirometric data obtained for these served as controls. By following this procedure, data obtained for the control snails can be compared with those of test snails with greater reliability than if the determination of oxygen consumption of controls and experimentals were made separately, at different times.

Once the molluscs are in place, reaction vessels are submerged in the water bath maintained at 20°C and allowed to equilibrate for 50 minutes with shaking at 80 oscillations per minute. After this period, respirometric readings are recorded at 20-minute intervals for a 2-hour period.

At the end of the 2-hour period, snails were dissected from their shells, rinsed in deionized water, dried overnight at 70°C, and weighed. Respirometric data were converted to microliters of oxygen consumed per gram dry weight.

II - Test solutions

Simulated pollutants added to the seawater were 1 ppm of Zn as $ZnSO_4$, 1 ppm Cu as $CuSO_4$, 1 ppm Cu as copper ethylenediamine tetraacetic acid (CuEDTA), 1 ppm Bayluscide, and 1 ppm Frescon. Copper sulfate was selected because it is known to be a molluscicide (Cheng, 1974 for review) and CuEDTA was selected because it is known not to be molluscicidal, at least to Biomphalaria glabrata (Cheng & Sullivan, 1973). Both Bayluscide and Frescon are commercial molluscicides, and $ZnSO_4$ was chosen because certain zinc-containing compounds, such as zinc dimethyldithiocarbamate, have molluscicidal properties (Malek & Cheng, 1974).

---

* Aquarium Systems, Inc., Eastlake, Ohio.

CONTRIBUTION NO. 251

Gulf Breeze Contribution No. 251

BEHAVIORAL BIOASSAYS

In: Marine Bioassays
Proceedings of workshop sponsored by
The Marine Technology Society, 1974.

(Entire Proceedings may be obtained from
The Marine Technology Society, 1730 M Street, N.W.,
Washington, D. C. $18.00).

BEHAVIORAL MEASURES OF ENVIRONMENTAL STRESS

Bori L. Olla, Chairman and Editor

Jelle Atema
Charles C. Coutant
Patricia De Coursey
David Hansen
James S. Kittredge
John J. Magnuson
Don Miller
Mark J. Schneider
Winona Vernberg

AFFILIATIONS

Jelle Atema
Woods Hole Oceanographic Institution
Woods Hole, Massachusetts 02543

Charles C. Coutant
Ecological Sciences Division
Building 2001
Oak Ridge National Laboratory
P.O. Box X
Oak Ridge, Tennessee 37830

Patricia DeCoursey
Belle W. Baruch Coastal Research Institute
University of South Carolina
Columbia, South Carolina 29208

David Hansen
Environmental Protection Agency
Gulf Breeze, Florida 32561

James S. Kittredge
Marine Biomedical Institute
University of Texas
200 University Blvd.
Galveston, Texas 77550

John J. Magnuson
Laboratory of Limnology
Department of Zoology
University of Wisconsin
Madison, Wisconsin 53706

Don Miller
U.S. Environmental Protection Agency
National Marine Water Quality Laboratory
South Ferry Road
Narragansett, Rhode Island 02882

Bori L. Olla
U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Middle Atlantic Coastal Fisheries Center
Sandy Hook Laboratory
Highlands, New Jersey 07732

Mark J. Schneider
Battelle Northwest
Ecosystems Department
Richland, Washington 99352

Winona B. Vernberg
Belle W. Baruch Coastal Research Institute
University of South Carolina
Columbia, South Carolina 29208

INTRODUCTION

Until fairly recently, water quality bioassay techniques have been limited to observations of the lethal concentrations of a pollutant. Such measures as LD_{50}, TL_{m50}, and LC_{50} were, and still are, commonly used to assess the acute effect of a pollutant, usually based on the mortality of adult organisms. While this approach was probably inevitable as a step in the evolution of both the philosophy and techniques of bioassaying, it has become abundantly clear that this concept has serious limitations as a measure of the effects of a pollutant on the environment.

The need for additional, more comprehensive measures of organismic response to contaminants has stimulated the search for new testing techniques in a variety of disciplines, including behavior. The most important advantage to using behavior as a tool to measure stress is that the results of behavioral tests often lend themselves to direct interpretation regarding environmental quality as related to possible consequences at the population and ecosystem levels. Also complex biochemical and physiological responses of an organism may be reflected in rather easily observable acts. Although behavioral measures may suffer in regard to quantification because of the high degree of inherent variation, they are highly sensitive to stress.

The general aim of the Workshop was to explore various aspects of applying behavioral measures to bioassay. Although in a few instances the use of behavioral bioassays has reached the standard test stage, the state of the art is still very young. Consequently, the scope of the discussion was intended as a beginning toward integrating a variety of basic research techniques into logical steps towards developing standard tests.

SPECIES TO STUDY (INDICATOR ORGANISMS)

Assuming that the objective of behavioral or any other bioassays is to protect the structure and function of marine ecosystems from degradation by pollutant sources, the organisms selected for study must be representative of the ecosystem that will receive the impact. The exact selection will, by necessity, differ from ecosystem to ecosystem. The kinds and number of species required to adequately represent various ecosystems will differ according to the complexity of the ecosystem.[1] Rarely will selected species transcend ecosystems in general. The examination of "standard" test organisms, nationally designated or from commercially-produced laboratory stocks will not be sufficient, except when there is an obvious commonality of species of similar ecosystems.[2] In essence, the subject of bioassays should be determined on an ecosystem-by-ecosystem basis and often on a site-by-site basis where discrete locations for waste disposal or discharge have been identified.

The following criteria are offered as guidelines for selection of species. There are two principal categories: social (wherein the importance is predominantly humanistic) and ecological.

---

[1] No hard and fast number can be recommended, but will in most cases exceed 10.

[2] Broad functional generalizations should be encouraged wherever possible. However, even when tests are on the same species from widely-spaced locations the applicability of numerical results may be questionable.

Social

Species of economic importance

Species which sustain economically important commercial or recreational harvests should be included. These may not represent the most important species ecologically, but economic conoerns usually will justify their preservation or enhancement.

Rare and endangered species

National law protects species judged to be rare or endangered (see lists published by the U.S. Dept. of the Interior). Wherever these species are found they must be evaluated if at all practical. Of course, it is obvious that many of the endangered species may be so limited in number as to preclude extensive assays.

Nuisance species

Some species may be judged as nuisances from both social and economic viewpoints, and events which promote expansion of these species are thus deemed undesirable. For example, thermal modifications may allow northward proliferation of undesirable southern fish species. These expansions may be possible through a variety of means including behavioral changes caused by elevated temperatures. It is also possible that a particular environmental stress may favor less desirable species causing imbalance in population structure.

Ecological

Interaction of the organism with the pollutant

Bioassays should be designed on an ecologically realistic basis wherein there is a significant potential for interaction of the organism with the pollutant. Some knowledge of the engineering design, hydraulics or chemistry of the waste discharge will be necessary to predict the components of the ecosystem that will be susceptible. Species selected should be spatially representative of the zone affected by the pollutant. For example, a shoreline discharge will probably first influence nearby littoral species and not benthic organisms a mile or more offshore. Temporal considerations may be as important as spatial in some instances, as for example, when seasonal discharges such as food processing wastes are discharged into waters with transient, migratory fishes.

Trophic level representation

Major trophic levels should be represented in the overall scope of bioassays. While behavioral assays of primary producers may be a rarity, the several levels of consumers (herbivores, carnivores, detritivores, etc.) should be represented. Of particular importance will be those species which contribute a major portion of the biomass or which functionally have a rapid material or energy transfer function. These may be temporarily short, but none the less important for the ecosystem (e.g., meroplankton, fish larvae).

Habitat structure

Some organisms should be considered for assays because they may be ecologically important by virtue of their role in directly serving as habitats for other organisms (e.g., corals and attaching bivalves) or secondarily affecting other species that serve as substrates. For example, behavior or larval corals (especially settling) will determine the locations of coral reef structures. Feeding behavior of sea urchins affects the kelp of southern California waters, which is a dominant "physical" habitat feature there.

Community structure

Certain species are particularly important because they strongly structure the remainder of the biotic community. For example, starfish of the Pacific coast maintain a characteristic community of the rocky intertidal zone through selective predation (Paine, 1969). In the Great Lakes invasion of the marine lamprey has greatly changed the indigenous community structure (Smith, 1973).

Consideration of life history phases

Particularly among invertebrates, there has been ecological diversification within the life cycle. Different life phases are behaviorally diverse and exhibit different levels of sensitivity to pollutants. Key phases should be examined independently.

Organisms with behavioral traits pertinent for pollutant effects

All ecologically significant species will not have behavior patterns that are sensitive and ecologically pertinent in terms of having high probability of interacting with the specific contaminant. Since the assays will be of behavior, the normal behavioral patterns of the indigenous fauna must be known and pertinent behaviors selected for study.

Coverage of sensory repertoire

Behavior patterns that depend upon diverse sensory mechanisms (e.g., olfaction, vision, acoustic, electrical) should be evaluated depending upon the nature of the pollutant. For example, a chemical discharge may interfere with chemoreceptors important for feeding behavior while at the same time cause turbidity which would affect visually-dependent behavior. The relative importance may not be readily apparent.

Species with behavior patterns that can be accommodated in the laboratory

Practicality dictates that behavioral traits of species to be studied must be amenable to laboratory (or controlled field) examination. This will involve (a) maintenance of organisms in the laboratory, (b) ability of the organism to perform under controlled conditions, and (c) capability of investigators to isolate relevant behaviors, especially those that may be influenced by the pollutant in question. It seems clear that much behavioral research must precede selection of suitable behavioral bioassays. (This subject is covered more fully below.)

SOURCE OF TEST ANIMALS

Preferably, local test animals should be used in assessing possible pollution effects. Few areas in the world are completely free today of man-induced pollutants; since each pollutant can significantly alter physiological and behavioral responses to environmental factors, effects of an added stress should be assessed on these organisms. If species are not available from a local area, then representative species from nearby localities offer an alternate source. As a last resort, commercially available species can be utilized for "first-cut" tests.

Variables to be Considered

One of the prime reasons local organisms should be used is that even within the same species there may be significant differences in the way an organism responds physiologically between separate populations. Some of these differences have been recognized for many years. For example, Mayer (1914) found that populations of the horseshoe crab, Limulus, from Woods Hole died at 38.5°C, but specimens from Florida survived up to 46.2°C. In 1936, Sparck, Fox, and Thorson demonstrated independently that the physiological responses of species from northern waters were not the same as those from some more southern latitudes when measured at the same

temperature. Since these earlier works, there have been many studies documenting the many physiological parameters that differ between populations of the same species (for review see Vernberg and Vernberg, 1972a).

Thermal history must also be considered. For example, phototactic response of larvae of the fiddler crab, Uca pugilator, reared at 20.0°C is not the same as larvae reared at 25.0°C (Vernberg, DeCoursey, and Padgett, 1973). Temperature may also interact with pollutants to modify behavioral responses. For example in goldfish, increase in water temperature from 21.1°C to 21.5°C in the presence of $CuCl_2$ can increase the attractiveness of the water (Kleerekoper et al., 1973), while exposure to DDT can modify the thermal preference of salmon fry (Ogilvie and Anderson, 1965).

Stage in the life history is another important variable. Larvae of U. pugilator are several magnitudes more sensitive to Hg poisoning than are adults or the eggs (DeCoursey and Vernberg, 1972; Vernberg, DeCoursey, and O'Hara, 1974) or the salinity preference of mosquitofish, Gambusia affinis (Hansen, 1972).

The use of animals cultivated in the laboratory is debatable. Preliminary evidence indicated that animals reared in the laboratory under optimum conditions often do not respond physiologically in the same manner as do field animals. There may, however, be some utility in testing limited numbers of cultivated animals for obtaining reference data (e.g., for comparing techniques among different laboratories).

Many abiotic and biotic factors must be considered before making valid assessments of the effects of a particular pollutant. In the estuarine environment, which is potentially the most likely to have the greatest pollution, salinity, dissolved oxygen, and photoperiod all fluctuate daily and seasonally. In the temperate zone, temperature is also an important consideration. Thus, multiple factors must be taken into account. The studies of Haefner (1970) on Crangon septemspinosa illustrate the interaction of some of these factors. These shrimp are well adapated to the normal temperature-salinity fluctuations of their estuarine environment, and calculations of surface response survival curves of adults indicate that they can tolerate a wide range thermal-salinity regime. However, when the dissolved oxygen levels are reduced to 2 ppm in contrast to 6 ppm, survival is markedly narrowed. Lowered dissolved oxygen levels often are indicative of polluted areas.

Often the effects of a pollutant cannot be properly assessed under optimum environmental conditions. When U. pugilator adults were exposed to 0.18 ppm mercury under optimum conditions, the crabs could survive indefinitely. However, under thermal-salinity stress (33.0°C, 5 °/oo) female crabs died after 26 days; male crabs after an 18-day exposure (Vernberg and Vernberg, 1972b). Phototactic responses are not modified in U. pugilator larvae reared under optimum conditions, but marked modification of response to mercury is noted when larvae are reared under suboptimal regimes (Vernberg, DeCoursey, and Padgett, 1973).

FIELD OBSERVATIONS: PREREQUISITE FOR THE DESIGN OF LABORATORY BEHAVIORAL BIOASSAY

Behavior is best suited for pollution bioassay when the particular behavioral modifications may be related to possible consequences at the population and ecosystem level. Thus, it is important that the choice of a behavioral bioassay arise from extensive knowledge of natural behaviors in the environment which, if altered, may intefere significantly with certain defined vital life processes. Key among these are ones closely related to reproductive success, survival to maturity and body growth. Experiments conducted under controlled laboratory situations have much greater significance when they can be compared and verified. Further observations in nature are essential to choices of realistic laboratory conditions, proper choice of experimental organism, and life history stage.

Direct and Indirect Field Observations

Many advances have been made in techniques of both direct and indirect observation of behavior of aquatic organisms. Table I provides a number of pertinent examples of how problems involved with in situ observations have been solved by a variety of approaches and techniques. Animals can be observed from above, on and below the sea surface. Limitations of direct observation center around the tolerance and fatigue of the observer, technological limits to access to particular habitats, and the responses of organisms to the observer and his facilities. The deeper or more distant the observation, the more difficult and costly it is.

Technological advances in the use of indirect methods such as closed-circuit television, acoustic and radio telemetry, and active sonar have greatly increased our abilities to observe in the aquatic environment. While these techniques do not have the resolution of direct visual observation, they nevertheless have extended the capabilities of observing aquatic organisms. Limitations of these techniques primarily relate to species identification and a variety of technological limitations.

Sampling as a Behavioral Tool

Insights into an organism's behavior in nature can often be gained by not observing behavior at all, but by sampling changes in the spatial distribution of the animals over time with conventional gear such as nets, traps and dredges. Knowledge of habits such as on and offshore movements, migration into estuaries and rivers and vertical migrations may be gained. Variations in number caught per unit of sampling effort help determine whether animals are distributed in uniform, random, or clumped spatial arrays. To some extent, even changes in the relative locomotory activity can be followed over time by changes in catch per unit effort with passive or stationary sampling gear.

Likewise feeding behavior can be inferred by stomach content. Locomotory behavior, feeding behavior, and sensory ability can also be inferred to some extent from anatomy with elusive marine species. These methods are often used and in some cases, the only available.

The Bridge Between the Laboratory and the Field

Relating what we observe in the field to what we observe under controlled laboratory conditions is the crucial link before the behavioral bioassay may be employed to assess and predict pollution effects. The following hypothetical example illustrates the interrelation between the field and laboratory.

To study the effects of an industrial waste we select an animal important in the trophic ecology from the proposed site. This animal is observed to feed on worms and to mate once a year. After its feeding behavior is studied in the laboratory using a similar substrate and food, it is established that feeding is not modified in the laboratory. Mating behavior is too infrequent to be useful for bioassay or, alternatively, may not withstand the transition to the laboratory negating the use of reproduction as a bioassay. After testing the effects of the effluent on feeding, we find that feeding behavior is altered. The animal takes a significantly longer time to locate a certain amount of food. Its efficiency has decreased. From this simple observation we conclude that the animal will grow more slowly, an effect which will be felt at the population and ecosystem level. Fewer individuals will reach maturity and populations of the next generation will be smaller (Atema and Stein, 1974).

In some cases, the use of a particular behavioral bioassay may be promising for development even though direct links of this behavior with survival of the species have not been demonstrated. An example is the effect of pollutants on learning ability of aquatic organisms. It would seem counter-productive to discard this bioassay at the present, as it seems evident that learning affects many aspects of an animal's daily life.

Table I. Techniques used in field observations of behavior.

Category	Technique	Reference
Direct Observation		
Surface	Boat	Newman (1956); Wisby and Nelson (1964); Jenkins (1969); Nelson et al. (1969); Keenleyside (1971)
Underwater	Snorkel (free diving)	Keenleyside (1962); Brown et al. (1973); Ogden and Buckman (1973); Reinboth (1973)
	SCUBA	Hobson (1965), (1968), (1971), (1972); Starck and Davis (1966); Myrberg et al. (1967); Olla et al. (1969); Green and Farwell (1971); Sale (1971); Reese (1973); Olla et al. (1974)
	Submersibles	Ballard and Emery (1970)
	Underwater habitats	Clarke et al. (1967); Collette and Earle (1972); Sartori and Bright (1973)
	Boat/rafts with viewing portals	Strasburg and Yuen (1960); Hobson (1963); Gooding and Magnuson (1967); Nakamura (1972)
	Periscope	Magnuson and Karlen (1970)
	Field enclosure	Jenkins (1969); Magnuson and Karlen (1970); Popper et al. (1973)
Remote Sensing	Photography	Yuen (1961), (1963), (1966); Nakamura (1972); Reinboth (1973)
	Photography-Sonar	Groot and Wiley (1965)
	Sonar	Cushing (1973)
	Underwater television-Video tape	Tyler (1971); Myrberg (1972); Stevenson (1972)
	Sound (acoustic methods)	Winn (1964); Tavolga (1964), (1967); Norris (1966); Myrberg et al. (1971); Bright (1972); Sartori and Bright (1973)

Table I. (cont'd)

Category	Technique	Reference
	Telemetry	
	Acoustic tags	Johnson (1960); Henderson et al. (1966); Poddubnyi et al. (1966); Haler et al. (1969); Yuen (1970); Stasko (1971); Dodson et al. (1972); McCleave and LaBar (1972); Young et al. (1972); Carey and Lawson (1973); Scholz et al. (1973); Stasko et al. (1973); Olla et al. (1974); Rochelle and Coutant (1974)
	Radio tag	Lonsdale and Baxter (1968)

BEHAVIORAL PATTERNS IN LABORATORY BIOASSAYS

The modification of a normal behavioral response or impairment of any physiological function by a toxicant may in many cases so diminish the changes of survival of an organism that it will be eliminated. These impairments may affect a spectrum of activities from the rate of photosynthesis by algae, to the feeding efficiency of herbivores, to the detection and avoidance of predators or success in prey capture (DeCoursey and Vernberg, 1972; Vernberg and Vernberg, 1972a; Sprague, 1971). The survival of a species may also be seriously threatened if the sublethal levels of a pollutant impair the location of sexual partners or capacity to mate, though not affecting the survival of the adult organism (Takahashi and Kittredge, 1973). Furthermore, the life cycles of many marine invertebrates involve a series of developmental stages occupying different niches, utilizing diverse food sources and being susceptible to different pollutants. An example might be the numerous larval forms (e.g., gastropod veligers) that feed in the natural surface film of neretic waters and thus are most susceptible to oil pollution at this stage. Larval forms may also be less tolerant to a given toxicant, for example, the adults of the fiddler crab, Uca pugilator, can tolerate 0.18 ppm Mercury for two months while this concentration kills the stage I larvae in one to three days and the stage V megalops in six hours (DeCoursey and Vernberg, 1972).

The question then becomes the detection of the sublethal effects of pollutants on the various life stages of crucial representative species of the marine ecosystem under study. The initial decision, as considered in an earlier section, is the optimum choice of species and developmental stage. The behavioral criteria utilized for detecting and measuring effects of contaminants in the laboratory is the primary subject of this section.

Experimental Design

Behavioral analysis

Most neretic marine organisms have evolved adaptive behavior to overcome the stresses imposed on them by the environment. Quantitative studies of these altered behaviors can provide the most sensitive bioassays of pollution stress. Behavioral studies are also necessary to define the limits at which the behavioral adaptations cannot compensate for the imposed stress and the organism's response is impaired.

The design of a behavioral bioassay should be based on both field and laboratory observations of the behavioral repertoire of the species. These observations will suggest both the scope and the limits of the behavioral characteristics of the organism that may be amenable to analysis. While a complete analysis, documented to form a behavioral ethogram, is desirable, that is seldom feasible. The analysis should, however, be sufficiently complete to allow the investigator to define the major behavioral sequences and to recognize those components that are relatively stable and those that are variable.

The field studies should initially establish the prime characteristics of the behavior of the species. Although covered in an earlier section it would be illuminating to reiterate certain points. These include:

a. The spatial distribution of the species in the water column, or, for benthic species, the type of substrate occupied or niche preference;

b. Spontaneous activities, e.g., ventilation or pumping rate, locomotor activities;

c. Feeding behavior, e.g., detection, capture, satiation;

d. Sexual behavior, e.g., attraction, courtship, copulation, spawning;

e. Parental care, e.g., nest preparation, guarding;

f. Social behavior, e.g., aggregation, aggression, territorial instincts, social structure;

g. Defensive behavior, e.g., warning displays, chemical secretions, withdrawal;

h. Evidence of phototactic or thigmotactic behavior or other taxes;

i. Behavioral response to changes in light intensity or sound;

j. The presence or absence of diel or tidal rhythms in their behavior.

Laboratory studies based on the information derived from adequate field studies will maximize the opportunities for true measures of the effects of the experimental stress on the "normal" behavior of the animal. This does not imply that the laboratory situation must mimic the field conditions in every detail. One must, however, understand the behavior in the field in order to recognize which features of the laboratory design must match the natural environment. Often one can succeed with a relatively simple experimental approach if it satisfies a few basic criteria. Most investigators recognize that proper substrate and water quality are essential. Light levels and disturbing vibrations are the two physical parameters often given insufficient attention. Both the appropriate light intensity and light/dark cycle must be provided for most organisms. One can often reverse the normal light/dark cycle in studying the behavior of nocturnal organisms. Random fluctuations in light intensity should be avoided. Due to the incompressibility of water, most fish and invertebrates will respond to sources of vibration that the observer may be unaware of; circulation pumps and refrigeration compressors are prime sources of these vibrations.

When the appropriate laboratory situation has been established, a more detailed study of the behavior observed in the field can follow with some confidence that the results observed resemble the natural behavior. The criteria for the selection of the behavioral traits best suited for the development of a bioassay should include:

a. Is the behavior obviously important in the survival of the species?

b. Can the behavior be analyzed as a sequence of components?

c. Which components of the behavioral sequence are stable and reproducible?

d. Does the behavior have a well-defined endpoint?

e. Is the stimulus that triggers the behavior easily presented?

f. Can the strength of the stimulus be quantified?

g. Is there a potential for quantifying the response or is it "all or none"?

It is also desirable that the behavioral characteristics selected for study yield data that can be evaluated in the field.

Statistics

At this stage in the design of the bioassay, it is imperative that the statistical methods to be utilized in the analysis be considered before the fact rather than after data has been collected. It has been our experience that parametric statistics are often inappropriate for the data generated by behavioral bioassays. One seldom has any assurance that the spread of the observed criteria in the population has a normal distribution. Nonparametric techniques must be applied in analyzing data when the distribution of the measurement in the population is unknown. It is, however, a common misconception that only nonparametric statistics are useful with small samples. A number of shortcut parametric tests have been developed that are applicable to small samples. The price one pays for the use of nonparametrics is usually a loss of power in testing hypotheses and some loss of accuracy in estimating confidence intervals. Thus, if it can be established that the data are drawn from a normal population, the power of the short cut parametric tests can be utilized. If the sample size is approximately fifty or greater, one may apply the chi-square test for the goodness of fit to

determine whether the frequencies in the classes of a sample distribution differ significantly from the theoretical normal frequencies. Alternately, one may utilize the Kolmogorov-Smirnov test to examine whether cumulative percentage distribution of the sample differs significantly from a normal distribution. This test is easier to apply than the chi-square test and it can be used for both grouped and ungrouped data. In addition, the data can be plotted on normal distribution paper together with the parallel straight lines delimiting the confidence bands. An excellent example of small sample parametric tests is the shortcut t-test (Lord's test). When it can be assumed that the two independent samples are randomly drawn from a normal population of equal dispersion, this test, utilizing only the two means and the two ranges, provides an estimate of confidence limits that is nearly as good as the standard t-test in small samples.

Data from behavioral tests may be continuous or discrete, but often the data may provide only ranking or only indicate the presence or absence of a criterion. We will suggest only a few of the nonparametric tests that are both powerful and quick in evaluating this data.

The rationale of both parametric and nonparametric inference rests on the randomness of the sampling. In behavioral bioassays, randomness in the distribution of the results from a long sequence of tests is a measure of lack of bias in the results that might have been imposed, for example, by gradual changes in the stock of experimental animals. When the results are in the form of a simple two-sided alternative, a Run Test will indicate the presence or absence of randomness in the sequence.

Many times an inference must be derived from a single set of samples. The Rank-Difference Correlation test can be applied to bivariate data available only in ranks or to ordered or continuous data to test the correlation between two variables in a set of data. It provides a ready measure of the independence (or conversely, the degree of relationship) or two parameters, as, for example, the size of an animal and its response time. Another test of independence, the Contingency Test, can also be applied when the variables are continuous, discrete, or qualitative. It is simple and extremely general. Its application in a 2 x 2 contingency table is one of the most common statistical applications; however, it should be limited to those cases in which the expected frequence in any cell is greater than four.

Often in behavioral bioassays one wishes to draw inferences from two samples. If the two samples are independent and capable of being combined into a single ordered series, the Rank Test, or Wilcoxon T test, is frequently used. This test is the nonparametric test most nearly analogous to the standard parametric t test and it is approximately ninety percent as powerful. It is ideally suited for calculating, for example, whether the response times of two different species of animals exposed to the same stress are significantly different. If the two samples are related, as occurs, for example, when each test organism is measured before and after exposure to a stress, or the individuals in the experimental group are matched with individuals in the control group, the tests of significance designed for independent samples may give over-conservative results (these procedures reduce sampling error). The most useful nonparametric tests for two related samples are the proportions, the sign and the signed-rank tests.

Variability and noise

The final stage of the development of a behavioral bioassay is a study of the "noise" in the system. Initially there is usually considerable scatter in the results which can be reduced by considering separately each of the abiotic and biological components of the assay. This refinement is largely a matter of trial and error, determination of the optimum temperature and the effects of a temperature fluctuation, unexpected variations in the water supply, proper aging of the experimental tanks, etc. A major part of the variability is usually due to variations in the physiological condition of the experimental organisms. It is usual to provide for an acclimation period for the experimental animals before they are used in an experimental situation. Paired experiments will reveal

the importance of acclimation and statistical analysis will provide a measure of the contribution of this factor to the variability of the measurements. It is perhaps unnecessary to point out that large numbers of replicate measurements are often not the optimum solution to overcoming the variability. Studies which statistically define the contribution of acclimation, or feeding cycle, or age of the organisms, etc., to the variability provide both for the selection of the optimum conditions and often allow one to reduce the work required in the bioassay by permitting one to discard some of the initial precautions without increasing the variability.

It is often desirable to raise the experimental organisms under controlled conditions. As an example, in refining a study of the locomotor activity of the fiddler crab, Uca pugilator, it was found necessary to select the test animals from hatches which exhibited 90% or greater viability in order to reduce the variability of the assay (DeCoursey and Vernberg, 1972).

It is sometimes suggested that there is a real dichotomy between those bioassays that are used as research tools and the bioassays suitable for water quality monitoring. Primarily this apparent dichotomy is an expression of the extent of the debugging that has been applied to the bioassay. There are some research bioassays that are inordinately time consuming or require, in their present stage of development, elaborate instrumentation making them unsuitable for routine use. Often a research worker will tolerate undesirable variability because the course of his study permits him to make inferences from a minimum number of replications and it would thus be uneconomical for him to refine the technique further. Many behavioral bioassays, however, can be developed into routine assays.

Presentation of pollutant

A wide variety of pollutants are currently being added to both the marine and freshwater ecosystems. The class of the pollutant(s), mode of action and where and how it may be potentially added to the environment will determine the choice of species and the specific behavioral assay. Conversely, chemical contaminants may be introduced into an ecosystem in high concentrations as in a "slug" dose, e.g., vast dumping from a barge or effluent from an outfall. These high concentrations will decrease with time depending on certain environmental parameters (e.g., turbulent diffusion, flocculation, biodegradation, etc.). It is also possible that the concentration of a chemical pollutant may be relatively constant over prolonged periods as in the leaching of a toxicant from dyked dredge sediments (DeCoursey and Vernberg, 1974: submitted for publication).

No matter how added, the effect of exposing a test organism to an environmental stress will generally be some form of an inverse relationship between the intensity of the stress and the length of exposure for a given biological effect. The design of the bioassay should include provisions for the exposure of the organisms to a range of concentrations of the pollutant and to several lengths of exposure. Generally, it is most economical to explore short-term exposures to a series of three or more concentrations differing by an order of magnitude. Realistic concentrations of a chemical pollutant for this exploratory phase may range from 10^{-4} to 10^{-8} wt./vol. The results of these short-term exposures can then guide the selection of concentrations for long-term exposures. It has been our experience that, for any long-term exposure experiment, one must provide for maintaining the concentration of the pollutant, either by infusion into an intermittent or continuously flowing system or by dialysis against an immiscible solvent. Both the concentrations and the time intervals selected should be realistic when compared to the field considerations under study.

Selection and application of the bioassay

In selecting and refining a behavioral bioassay for routine application in water quality studies, there are additional criteria beyond those considered initially in establishing the bioassay that will contribute to its potential application:

a. Is the species readily available throughout the year?

b. Is the species hardy?

c. Are there undesirable changes in its behavior during its spawning season?

d. Are larvae readily available?

e. Is there a potential for automating the measurement of the behavior?

Unfortunately, undue consideration of the above criteria is a major factor in often selecting inappropriate organisms in water quality bioassays. The species selected for study should be part of the normal biota and susceptible, at a behavioral level, to the range of pollutant concentrations that are dangerous to the other species in the ecosystem under study.

A major advancement in water quality bioassays has been the trend toward automation. Several simple behaviors such as locomotion, phototaxis and filtration rate, are readily automated. Others, such as the pumping rate of oysters, can be automated but require technical preparation of the oyster. The sensitivity of this assay and the minimum time required per assay, offset the set-up time. Many other behaviors of marine invertebrates, such as the defensive withdrawal time of many species of Polychaeta, Mollusca and Tunicata, the feeding response of Anoumura and Brachyura, the feeding movements of the Cirripedia, should be amenable to automation. The locomotor activities of certain marine fish species are also amenable to automation (for example, Schuyf and de Groot, 1971; Gibson, 1973). Automation, however, of a poorly designed experiment may obscure the results. We are reminded of feeding response studies which were automated and analyzed in a computer to produce histograms that were almost uninterpretable. In most instances, there is no substitute for simple observation.

Another factor to consider in selecting a behavior for water quality studies is the advantage of studying behaviors that are responses to sensory stimuli as compared to spontaneous behaviors. These responses necessarily reflect the integrity of sensory organs, the central nervous system and the musculature involved. One may measure quantitatively the threshold of the receptors for response or the time before a response is elicited. A sequence of many measurements can be completed in a short period of time. Although too few examples exist to permit a comparison, elicited behaviors probably provide the most sensitive types of bioassay available.

Behavioral bioassay literature

A large number of behavioral responses have been used at the research level to measure the effects of sublethal concentrations of pollutants on aquatic organisms. They are shown in tabular form, grouped for convenience into two major groups (Table II). The performance of individual organisms has been studied at the level of the sensory organs, an endogenous time sense, motor activities, motivation and learning phenomena, as well as physiological responses closely related to the measurement of particular behavioral patterns. A second group of responses involving the interactions of animals has been classified as inter-individual responses. The examples of each behavioral response are annotated with the species studied, the stress administered and the literature citation.

INTEGRATION OF BEHAVIOR WITH STANDARD BIOASSAY TECHNIQUES

Behavioral observations used in conjunction with standard acute and chronic bioassays can significantly increase the value of tests. Bioassays which use functional death as the measure of effect are ignoring the fact that alterations in behavior may be occurring well before death. In this section we will present procedures that can be used by an observer in the course of other testing which will serve to improve the sensitivity of bioassays.

Table II. Summary of Sublethal Behavioral Bioassays Used Primarily as Research Tools.

A. Performance of Individuals

Category	Specific behavior	Species	Pollutant or stress	Reference
Sensory capacity	Phototaxis	Uca pugilator larvae	Mercury	Vernberg et al. (1973)
		Calonodia americana	Xanthine dyes	Bjornberg and Wilbur (1968)
		Acartia lillejeborgi		
		Paracalanus crassirostris		
	Salinity preference	Gambusia affinis	DDT and malathion	Hansen (1972)
	Visual-optomotor (flicker frequency)	Lepomis macrochirus	Parathion	Scheier and Cairns (1968)
	Temperature preference	Salmo salar	DDT	Ogilvie and Anderson (1965)
		Poecilia reticulata	Sodium pentobarbitol	Ogilvie and Fryer (1971)
		Carassius auratus	Copper	Kleerekoper et al. (1973)
		Cyprinus carpio	Various	Neill et al. (1972)
		Lepomis macrochirus		
		Micropterus salmoides		
		Pomoxis nigromaculatus		
		Ambloplites repestris		
		Perca flavescens		
	Sensor inhibition	Ictalurus natalis	Detergents	Bardach et al. (1965)
	Chemical attraction (chemotaxis)	Nassarius obsoletus	Kerosene fraction	Jacobsen and Baylou (1973)

Table II. (cont'd)

Category	Specific behavior	Species	Pollutant or stress	Reference
Sensor capacity (cont'd)	Chemoreception	Homarus americanus	Crude oil	Atema and Stein (1974)
		Pachygrapsus crassipes	Crude oil fractions and aromatic hydrocarbons	Takahashi and Kittredge (1973)
	Rheotaxis	juvenile salmon	Temperature	Keenleyside and Hoar (1954)
	Lateral line sensitivity	Salvelinus fontinalis	DDT	Anderson (1968)
Time sense	Endogenous timing of daily activity	Uca pugilator adult	Mercuric chloride	Vernberg et al. (1974)
Motor activity	Avoidance of contaminants	Salmo gairdneri	Zinc sulphate	Sprague (1968)
		Cyprinodon variegatus	Pesticides	Hansen (1969)
		Gambusia affinis	Pesticides	Hansen et al. (1972)
	Attraction to contaminants	Homarus americanus	Kerosene fraction	Atema et al. (1973)
	Shelter seeking	Lepomis macrochirus	Zinc	Sparks et al. (1972)
	Equilibrium	Oncorhynchus tshawtscha	Temperature	Coutant and Dean (1972)
		Salmo gairdneri		
	Swimming performance and spontaneous locomotor activity	Oncorhynchus nerka	Temperature	Brett (1967)
		Pomatomus saltatrix	Temperature	Olla and Studholme (1971)
		Uca pugilator (larvae)	Mercury	DeCoursey and Vernberg (1972)
		Carassius auratus	DDT	Davy et al. (1972)

Table II. (cont'd)

Category	Specific behavior	Species	Pollutant or stress	Reference
Motor activity (cont'd)	Swimming performance and spontaneous locomotor activity (cont'd)	Lepomis macrochirus	Zinc	Cairns et al. (1973)
		Salvelinus fontinalis	Copper	Drummond et al. (1973)
		Uca pugilator (adult)	Mercury	Vernberg et al. (1974)
		Paleomonetes pugio	Cadmium and low dissolved oxygen	White (1974)
		Carassius auratus	Copper	Kleerekoper et al. (1972)
		Carassius auratus	Copper	Kleerekoper (1973)
Motivation and learning phenomena	Feeding motivation	Brachydanio rerio	Detergent	Cairns and Loos (1967)
		Pomatomus saltatrix	Temperature	Olla and Studholme (1971)
		Homarus americanus	Crude oil	Atema and Stein (1974)
		Uca (sp.)	Mercury	Klein and Lincer (1974)
	Learning	Salvelinus fontinalis	DDT	Anderson and Peterson (1969)
		Salvelinus fontinalis	DDT	Anderson and Prins (1970)
		Carassius auratus	Various metals	Weir and Hine (1970)
		Salmo salar	Four insecticides	Hatfield and Johansen (1972)
Physiological responses	Ventilation rate	Crassostrea virginica	Hydrocarbons	Galtsoff et al. (1947)
	Breathing rate	Lepomis macrochirus	Zinc	Cairns et al. (1973)

Table II. (cont'd)

B. Inter-individual Responses

Category	Species		Pollutant or stress	Reference
Social motivation	Ictalurus natalis		Thermal	Todd et al. (1972)
Intraspecific visual attraction	Mugil cephalus		Thermal	Olla (1974)
Aggregation and schooling	Pomatomus saltatrix		Thermal	Olla and Studholme (1971)
Aggression	Lepomis cyanellus		Turbidity	Heimstra et al. (1969)
	Lepomis macrochirus		Zinc	Sparks et al. (1972)
	Ictalurus natalis		Electric shock	Todd et al. (1967)
Predation vulnerability	Predator	Prey		
	Gulls	Fish	Thermal	Prentice (1969)
	Amia calva	Fish (varied species)	Poor condition	Herting and Witt (1967)
	Oncorhynchus kisutch	Oncorhynchus nerka	Thermal	Sylvester (1972)
	Micropterus salmoides	Gambusia affinis	Mercury	Kania and O'Hara (1974)
	Salmo gairdneri (adult)	Salmo gairdneri (juvenile)	Thermal	Coutant (1973)
		Oncorhynchus tshawatscha		
	Micropterus salmoides	Gambusia affinis	Radiation	Goodyear (1972)
	Micropterus salmoides	Micropterus salmoides	Thermal	Coutant et al. (1974)
		Ictalurus punctatus		

Acclimation

Standard Methods for the Examination of Water and Waste Water (1971) suggests that before routine bioassays are performed, test animals should be acclimated for a week or longer to laboratory conditions that are similar to test conditions. During a period of four days immediately preceding a test, incidence of death or disease must be less than ten percent. Also, specimens must show no abnormalities in appearance or behavior at the time of their transfer to the test containers. It is at this point that knowledge of the normal habits of the animal in both the field and laboratory can aid in determining if the experimental subjects are in fact behaviorally acclimated. The results of these observations may be considered a reflection of the relative health and state of the test organisms.

Knowledge of test organisms' normal habits and environmental requirements as learned from field observations are essential in determining such factors as the size and shape of holding and testing aquaria, water quality, feeding schedules, and the various physical parameters such as temperature, salinity, pH, etc. The aim is to provide facilities which will enable the organism to express certain critical behaviors which, if inhibited, may significantly alter their physiological condition. For example, elliptical shaped aquaria should be provided for fast swimming pelagic forms; suitable substrate for burying and burrowing forms; cover for reef residents; enough space as defined by normal observations for animals which tend to be territorial; light levels appropriate for the species.

Determining the fitness of a stock of animals for bioassays may utilize observable behavioral activity in addition to more conventional criteria. The use of behavioral characteristics in a diagnostic manner assumes that the behavior of the species is already well understood by the investigator. Diagnostic behavior characteristics that one might use in determining fitness might include feeding activity with the organisms expected to feed during acclimation, particularly if they are offered food that comprises their normal diet (as determined by field observations and stomach analysis). Alterations in photo-responsiveness, increases in excitability, changes in motor activity, schooling configuration, agonistic displays, and opercular movements are all observations which may indicate that organisms are not yet acclimated or suitable as test subjects.

Acute Bioassays

Standard Methods (1971) suggests that physiological death should be the prime measure of effect for routine bioassays. It also suggests that bioassayists keep records of the number of animals which are alive but show pronounced symptoms of intoxication and distress, such as loss of equilibrium and other markedly abnormal behavior. Because death can be predicted if certain behavioral activities are altered, the usefulness of bioassays can be significantly improved by accurate and consistent reporting of the number of animals with impaired behavior, a description of the symptoms of the impairment, and a discussion of its probable ecological significance. Bioassays will also be improved if the habitat and behavioral requirements of the animals are considered when designing the bioassay.

Most behavioral observations that were applicable in improving acclimation are also applicable for bioassays. Animals should be provided adequate physical, chemical and biological surroundings and the animals should orient normally to them. For example, phototropic animals, such as crab larvae, should react to light, burrowing animals should burrow, gregarious animals should interact and animals should exhibit normal rhythms of activity. An inventive researcher can improve observations by devising procedures, such as timing ventilation rates, to quantify changes in behavior without disturbing animals or interrupting the progress of the bioassay.

Long-term Bioassays

In chronic bioassays, i.e., tests that are conducted over extended time periods and/or over entire life cycles it is particularly important to provide a suitable environment for the test organisms to grow, mature, and reproduce. The more closely the environmental requirements of the species can be met in the laboratory the more representative will be the behavior of the animals. In bioassays that include the total life cycle of the animals this may be particularly important since such behaviorally involved phases as courtship and reproduction may be altered, and in turn the significance of the results. Since the end point in chronic bioassays is not necessarily death, the significance of behavior to the interpretation of the data is much more critical. Detailed and careful observations of behavior during the course of an experiment may also provide explanations for trends in the data.

PROCEDURES AND FACILITIES FOR CAPTURING AND HOLDING ORGANISMS

Great care and expense may be committed to the major aspects of the actual experimentation, but often little attention is given to the critical aspects of animal acquisition and care. Questions that should be considered by the experimenter working with marine organisms include:

a. How may organisms be captured and transported from the field to the laboratory with minimal mechanical damage and physiological stress?

b. What physical and biological conditions must be provided in laboratory holding facilities for keeping aquatic organisms in the best possible shape?

c. How is disease to be monitored and controlled?

d. How can the build-up of metabolites be controlled in closed systems?

e. Which animals might be raised in the laboratory?

To reiterate what was mentioned in an earlier section of this chapter, there is no substitute for defining the normal requirements of study organisms by a variety of techniques and methods of study in the natural environment.

What we present in this section may be considered an introduction to some of the more important aspects of acquisition, maintenance and culture of marine bioassay organisms, with reference to some of the most significant publications on the subject.

Field Collection and Transport

It is obvious that the capture and transport of organisms should be done in a manner which minimizes mechanical injury and physiological trauma. Fishing gear used in commercial endeavors or in normal scientific collecting, when taxonomy or biological surveys are the aim, are not often adaptable to collection of live animals for laboratory use.

The individual characteristics of the organism, including their hardiness, will determine how best to capture and transport them. For example, delicate types of fishes such as Atlantic silversides, Menidia menidia, or any of the herrings and anchovies are easily captured by shore seine. However, rather than beaching the seine, it is far less deleterious to the fish to scoop them with a bucket or soft net from the water and into a transport container while they are still swimming within the enclosed net in shallow water. Another excellent method of catching animals with little injury is with barbless hook and line. A variety of adult species including tuna (Nakamura, 1972) bluefish, Pomatomus saltatrix and Atlantic mackerel, Scomber scombrus, (Olla, unpublished) have been collected successfully in this way. Juvenile bluefish, spot, Leiostomus xanthurus, and winter flounder, Pseudopleuronectes americanus, have also been successfully collected in this way. Collection of slow-moving benthic animals by hand, with the aid of SCUBA, is less damaging to the animal than traps or

dredges. This method has been successfully employed in capturing tautog, Tautoga onitis, while the fish were in a quiescent sleep phase during the night (Olla et al., 1974). Collecting positively photo-responsive invertebrate larvae and adults and a variety of other juvenile organisms can be accomplished with a night light. Innovative modifications in plankton nets, which avoid mechanical injury, are also employed. When beam or otter trawls must be used, slow tows which are very short in duration are suggested.

Transport containers, especially for highly motile species, should be elliptical in shape to prevent animals from crowding in corners or damaging themselves by striking the walls (Nakamura, 1972). Verheijen (1956) suggested holding sardines and anchovies in plastic bags, a method which has proved satisfactory (Tardant, 1962). Plastic bags or a plastic curtain suspended in a way which forms a cushion from the container wall will greatly aid in preventing physical damage.

Maximum density in a container will vary with the requirements of the species, as well as with the water quality and the duration of holding. Aggressive animals should be isolated in separate containers.

When these temporary holding and transporting measures must be sustained for much more than 30 minutes, adequate safeguards must be taken to shield the animals from sunlight and from extreme temperature and dissolved oxygen changes. Styrofoam coolers and addition of ice around the outside of uninsulated containers provide temporary temperature maintenance. The use of anesthetics should be avoided, if at all possible, during transport and handling due to potential after effects on organism behavior (Goddard et al., 1974).

For many intertidal organisms, especially sessile types, large volumes of water are not needed. Items such as wet seaweed may be an ideal transport medium for these animals. If containers with appreciable volumes of water must be transported by motor vehicle, an elliptical tank with a "conning tower" will prevent sloshing of the water and organisms (Nakamura, 1966).

Laboratory Holding Requirements

Basic specifications for designs and materials for marine aquarium facilities have been detailed by a number of authors: Clark and Clark (1964), Hagen (1970), Congres International d'Aquariologie (1962-63), Olla et al. (1967), Spotte (1970, 1973). The chapter by Atz in Clark and Clark (1964) is particularly useful for such basic principles and practices as avoidance of toxic materials and maintenance of water condition. Still one of the most useful papers on marine closed system maintenance is by Saeki (1958) (also consult Davis, 1953 and Lewis, 1963). The suitability of various construction materials in culture systems is further discussed in papers by Dyer and Richardson (1962) and Bernhard et al. (1966). While the problem of disease is discussed by a number of workers, Sindermann (1970) presents a useful and definitive treatment of marine fish and shellfish diseases.

When organisms are to be used in behavioral bioassays, the basic requirements iterated above must be supplemented. Ideally, conditions in the laboratory should approximate the organism's normal requirements. For example, natural cycles of light, temperature and salinity must be maintained, as well as habitat needs and food which quantitatively and qualitatively approach natural requirements. The quality of the light should also be considered. Acute illumination changes are to be avoided with the most desirable method of light introduction as part of a daily cycle employing a system which slowly increases and decreases light levels approximating sunrise and sunset. Other physical conditions such as current, pressure, turbulence and natural diurnal and tidal ranges of temperature may also be required.

Suitable animal density depends partly on natural conditions (see Section II). Benthic and cover-dependent organisms should be provided with suitable substrate and/or habitat materials.

Type of food and mode and time of feeding should approximate natural conditions as closely as possible as determined from field observations. Although it is often not possible to duplicate the exact kinds of food the animal eats, it is often possible to approximate their nutritional level and quality. At the most basic level, carnivores should be supplied with a diet that suits their specific requirements, which are obviously different from those of herbivores. At present, unfortunately, knowledge of the nutritional requirements of marine organisms is at best deficient in many aspects.

Failure to eat is common in animals recently brought into the laboratory. This initial period of non-feeding may be shortened by social facilitation, i.e., adding animals which are already feeding to the holding tank. When laboratory animals do not begin to eat or stop eating, once having begun feeding in the laboratory, this may be indicative of an unhealthy condition in the holding facility, rendering the animals useless for experimentation.

Laboratory Culture of Organisms

Laboratory culture techniques are now sufficiently developed for certain species to consider culture as a feasible method for obtaining experimental larval material, when the much more desirable method of using animals from the particular locale that will receive the impact is not possible (see earlier section of this chapter). Cultivation of marine organisms was the topic of the International Helgoland Symposium in 1969 (Kinne and Bulnheim, 1970). Methods of bivalve rearing are reported by Loosanoff and Davis (1963). Facilities for bivalve larval bioassay are discussed by Woelke (1972). For crustacea culture, numerous references are cited by Costlow and Bookhout (1968) and others in a symposium volume on decapod larval development. A method for polychaete culture has been developed by Reish and Barnard (1960). For two very useful general works on invertebrate culture, see Galtsoff et al. (1937) and Costello et al. (1957). A bibliography of marine fish culture efforts has been compiled by May (1971).

The prime requirement for laboratory reared organisms is that they be physiologically compatible with natural populations. Physiological compatibility should be tested by comparing the results of bioassays on both laboratory animals and on natural populations. Other criteria must be developed for larval forms. Histological comparison, which will indicate tissue normality and nutritional condition, can also be used.

LITERATURE CITED

Anderson, J.M. 1968. Effect of sublethal DDT on the lateral line of brook trout, Salvelinus fontinalis. J. Fish. Res. Board Can. 25:2677-2682.

Anderson, J.M., and M.R. Peterson. 1969. DDT: Sublethal effects on brook trout nervous system. Science 164:440-441.

Anderson, J.M., and H.B. Prins. 1970. Effects on sublethal DDT on a simple reflex in brook trout. J. Fish. Res. Board Can. 27:331-334.

Atema, J., S. Jacobson, J. Todd, and D. Boylan. 1973. The importance of chemical signals in stimulating behavior of marine organisms: effects of altered environmental chemistry on animal communication, p. 177-197. In G.E. Glass (ed.) Bioassay Techniques and Environmental Chemistry. Ann Arbor Science Publishers Inc., Ann Arbor, Michigan.

Atema, J., and L. Stein. 1974. Effects of crude oil on the feeding behavior of the lobster Homarus americanus. Environ. Pollut. 6:77-86.

Atz, J.W. 1964. Some principles and practices of water management for marine aquariums. p. 3-16. In J.R. Clark and R.L. Clark (ed.) Sea-water systems for experimental aquariums. U.S. Fish Wildl. Serv. Res. Rep. 63.

Ballard, R.D., and K.O. Emery. 1970. Research Submersibles in Oceanography. Marine Technological Society, Washington, D.C., 70 p.

Bardach, J.E., M. Fujiya, and A. Holl. 1965. Detergents: Effects on chemical sense of the fish Ictalurus natalis (Le Sueur). Science 148:1605-1607.

Bernhard, M., A. Zattera, and P. Filesi. 1966. Suitability of various substances for use in the culture of marine organisms. Pubbl. staz. zool. Napoli 35:80-104.

Bjornberg, T.K.S., and K.M. Wilbur. 1968. Copepod phototaxis and vertical migration influenced by xanthine dyes. Biol. Bull. 134:398-410.

Brett, J.R. 1967. Swimming performance of sockeye salmon (Oncorhynchus nerka) in relation to fatigue time and temperature. J. Fish. Res. Board Can. 24:1731-1741.

Bright, T.J. 1972. Bio-acoustic studies on reef organisms. Nat. His. Museum, Los Angeles County Sci. Bull. 14:45-69.

Brown, J.H., M.A. Cantrell, and S.M. Evans. 1973. Observations on the behavior and coloration of some coral reef fish (Family: Pomacentridae). Mar. Behav. Physiol. 2:63-71.

Cairns, J., Jr., and J.J. Loos. 1967. Changed feeding rate of Brachydanio rerio (Hamilton-Buchanan) resulting from exposure to sublethal concentrations of zinc, potassium dichromate, alkyl benzene sulfonate detergent. Proc. Penn. Aca. Sci. 40:47-52.

Cairns, J., Jr., R.E. Sparks, and W.T. Waller. 1973. The relationship between continuous biological monitoring and water quality standards for chronic exposure, p. 383-402. In G.E. Glass (ed.) Bioassay Techniques and Environmental Chemistry. Ann Arbor Science Publishers Inc., Ann Arbor, Michigan.

Carey, F.G., and K.D. Lawson. 1973. Temperature regulation in free-swimming bluefin tuna. Comp. Biochem. Physiol. 44A375-392.

Clark, J.R., and R.L. Clark (ed.) 1964. Sea-water systems for experimental aquariums. U.S. Fish Wildl. Serv. Res. Rep. 63:1-92.

Clarke, T.A., A.O. Flechsig, and R.W. Grigg. 1967. Ecological studies during Project Sealab II. Science 157:1381-1389.

Collette, B.B., and S.A. Earle. 1972. Results of the Tektite program; ecology of coral reef fishes. Nat. Hist. Museum, Los Angeles County Sci. Bull. 14:1-179.

Congres international d'aquariologie, I^{er} Monaco 1960. 1962-1963. Bull. Inst. Oceanogr. Monaco numero Special IA-ID.

Costello, D.P., M.E. Davison, A. Eggers, M.H. Fox, and C. Henley. 1957. Methods for obtaining and handling marine eggs and embryos. Marine Biol. Lab., Woods Hole, Mass. 247 p.

Costlow, J.D., Jr., and C.G. Bookhout. 1968. The complete larval development of the land-crab Cardisoma guanhumi Latreille in the laboratory (Brachyura, Gecarcinidae). Crustaceana, Supplement 2:259-270.

Coutant, C.C. 1973. Effect of thermal shock on vulnerability of juvenile salmonids to predation. J. Fish. Res. Board Can. 30:965-973.

Coutant, C.C., and J.M Dean. 1972. Relationships between equilibrium loss and death as responses of juvenile chinook salmon and rainbow trout to acute thermal shock. U.S. At. Energy Comm. Res. Devel. Rep. BNWL 1520· 19 p.

Coutant, C.C., H.M. Ducharme, Jr., and J.R. Fisher. 1974. Effects of cold shock on vulnerability of juvenile channel catfish (Ictalurus punctatus) and large-mouth bass (Micropterus salmoides) to predation. J. Fish. Res. Board Can. 31:351-354.

Cushing, D. 1973. The Detection of Fish. Pergamon Press, Oxford. 200 p.

Davis, H.S. 1953. Culture and Diseases of Game Fishes. Univ. Calif. Press, Berkeley. 332 p.

Davy, F.B., H. Kleerekoper, and P. Gensler. 1972. Effects of exposure to sub-lethal DDT on the locomotor behavior of the goldfish (Carassius auratus). J. Fish. Res. Board Can. 29:1333-1336.

DeCoursey, P., and W.B. Vernberg. 1972. Effect of mercury on survival, metabolism and behavior of larval Uca pugilator (Brachyurs). Oikos 23:241-247.

_____ 1974. The effect of dredging in a polluted estuary on the physiology of larval zooplankton. (submitted to Water Research).

Dodson, J.J., W.C. Leggett, and R. Jones. 1972. The behavior of adult American shad (Alosa sapidissima) during migration from salt to freshwater observed by ultrasonic tracking techniques. J. Fish. Res. Board Can. 29:1445-1449.

Drummond, R.A., W.A. Spoor, and G.F. Olson. 1973. Some short-term indicators of sublethal effects of copper on brook trout, Salvelinus fontinalis. J. Fish. Res. Board Can. 30:698-701.

Dyer, D.L., and D.E. Richardson. 1962. Materials of construction in algal culture. Appl. Microbiol. 10:129-131.

Fox, H.M. 1936. The activity and metabolism of poikilothermal animals in different latitudes. I. Proc. Zool. Soc. London 1936:945-955.

Galtsoff, P.S., W.A. Chipman, Jr., J.B. Engle, and H.N. Calderwood. 1947. Ecological and physiological studies of the effect of sulphate pulpmill wastes on oysters in the York River, Va. U.S. Fish Wildl. Serv. Fish. Bull. 43:58-186.

Galtsoff, P.S., F.E. Lutz, P.S. Welch, and J.G. Needham (ed.) 1937. Culture Methods for Invertebrate Animals. Comstock Publ. Co., Ithaca, New York 590 p.

Gibson, R.N. 1973. Tidal and circadian activity rhythms in juvenile plaice, Pleuronectes platessa. Mar. Biol. 22:379-386.

Goddard, C.I., J.W. Lilley, and J.S. Tait. 1974. Effects of MS-222 anesthetization on temperature selection in lake trout, Salvelinus namaycush. J. Fish. Res. Board Can. 31:100-103.

Gooding, R.M., and J.J. Magnuson. 1967. Ecological significance of a drifting object to pelagic fishes. Pac. Sci. 21:486-497.

Goodyear, C.P. 1972. A simple technique for detecting effects of toxicants or other stresses and predator-prey interaction. Trans. Amer. Fish. Soc. 101:367-370.

Green, J.M., and M. Farewell. 1971. Winter habits of the cunner, Tautogolabrus adspersus (Walbaum 1792), in Newfoundland. Can. J. Zool. 49:1497-1499.

Groot, C., and W.L. Wiley. 1965. Time-lapse photography of an ASDIC Echo-Sounder PPI-Scope as a technique for recording fish movements during migration. J. Fish. Res. Board Can. 22:1025-1034.

Haefner, P.A., Jr. 1970. The effect of low dissolved oxygen concentrations on temperature-salinty tolerance of the sand shrimp, Crangon septemspinosa Say. Physiol. Zool. 43:30-37.

Hagen, W. 1970. Aquarium design criteria. Drum and Croaker, Special Edition. Nat. Fisheries Center and Aquarium, U.S. Dept. Interior Washington, D.C., 107 p.

Hansen, D.J. 1969. Avoidance of pesticides by untrained sheepshead minnows. Trans. Amer. Fish. Soc. 98:426-429.

_____ 1972. DDT and malathion: Effect on salinity selection by mosquito fish. Trans. Amer. Fish. Soc. 101:346-350.

Hansen, D.J., E. Matthews, S.H. Nall, and D.P. Dumas. 1972. Avoidance of pesticides by untrained mosquito fish, Gambusia affinis. Bull. Environ. Contam. Tox 8:46-51.

Hasler, A.D., E.S. Gardella, R.M. Horral, and H.F. Henderson. 1969. Open water orientation of white bass, Roccus chrysops, as determined by ultrasonic tracking methods. J. Fish. Res. Board Can. 26:2173-2192

Hatfield, C.T., and P.H. Johansen. 1972. Effects of four insecticides on the ability of Atlantic salmon parr (Salmo salar) to learn and retain a simple conditioned reflex. J. Fish. Res. Board Can. 29:315-321

Heimstra, N.W., D.K. Damkot, and N.G. Benson. 1969. Some effects of silt turbidity on behavior of juvenile largemouth bass and green sunfish. U.S. Fish Wildl. Serv. Tech. Paper 20:1-9.

Henderson, H.F., A.D. Hasler, and G.C. Chipman. 1966. An ultrasonic transmitter for use in studies of movements of fishes. Trans. Amer. Fish. Soc. 95:350-356.

Herting, G.E., and W. Witt, Jr. 1967. The role of physical fitness of forage fishes in relation to their vulnerability to predation by the bowfin (Amia Calva). Trans. Amer. Fish. Soc. 96:427-430.

Hobson, E.S. 1963. Feeding behavior in three species of sharks. Pac. Sci. 17:179-194.

_____ 1965. Diurnal-nocturnal activity of some inshore fishes in the Gulf of California. Copeia 1965:291-302.

_____ 1968. Predatory behavior of some shore fishes in the Gulf of California. U.S. Fish Wildl. Serv. Res. Rep. 73:1-92.

_____ 1971. Cleaning symbiosis among California inshore fishes. Fish. Bull., U.S. 69:491-535.

_____ 1972. Activity of Hawaiian reef fishes during the evening and morning transitions between daylight and darkness. Fish. Bull., U.S. 70:715-740.

Jacobson, S.M. and D.B. Boylan. 1973. Effects of sea water soluble fraction of kerosene on chemotaxis in a marine snail, Nassarius obsoletus. Nature London 241:213-215.

Jenkins, T.M., Jr. 1969. Social structure, position choice and microdistribution of two trout species (Salmo trutta and Salmo gairdneri) resident in mountain streams. Anim. Behav. Monogr. 2:1-123.

Johnson, J.H. 1960. Sonic tracking of adult salmon at Bonneville Dam, 1957. U.S. Wildl. Serv. Fish. Bull. 60:471-485.

Kania, H.J., and J. O'Hara. 1974. Behavioral alterations in a simple predator-prey system due to sublethal exposure to mercury. Trans. Amer. Fish. Soc. 103:134-136.

Keenleyside, M.H.A. 1962. Skin-diving observations of Atlantic salmon and brook trout in the Miramichi River, New Brunswick. J. Fish. Res. Board Can. 19:625-634.

_____ 1971. Aggressive behaviour of male longear sunfish (Lepomis megalotis). Z. Tierpsychol. 28:227-240.

Keenleyside, M.H.A., and W.S. Hoar. 1954. Effects of temperature on the responses of young salmon to water currents. Behavior 7:77-87.

Kinne, O., and H.P. Bulnheim (ed.) 1970. Cultivation of marine organisms and its importance for marine biology. Intl. Helgoland Symp. 1969. Helgolander wiss. Meeresunters. 20:1-721.

Kleerekoper, H. 1973. Effects of copper on the locomotor orientation of fish. Office of Research and Monitoring, Res. Rep. EPA-R3-73-045.

Kleerekoper, H., J.B. Waxman, and J. Matis. 1973. Interaction of temperature and copper ions as orienting stimuli in the locomotor behavior of the goldfish (Carassius auratur). J. Fish. Res. Board Can. 30:725-728.

Kleerekoper, H., G.F. Westlake, J.H. Matis, and P.J. Gensler. 1972. Orientation of goldfish (Carassius auratus) in response to a shallow gradient of sublethal concentration of copper in an open field. J. Fish. Board Can. 29:45-54.

Klein, M.L., and J.L. Lincer. 1974. Behavioral effects of dieldrin upon the fiddler crab, Uca, pugilator. (In press).

Lewis, W.M. 1963. Maintaining Fishes for Experimental and Instructional Purposes. So. Ill. Univ. Press, Carbondale. 100 p.

Lonsdale, E.M., and G.T. Baxter. 1968. Design and field tests of a radio-wave transmitter for fish tagging. Prog. Fish-Cult. 30:47-52.

Loosanoff, V.L., and H.C. Davis. 1963. Rearing of bivalve molluscs, p. 1-136. In F.S. Russell (ed.) Advances in Marine Biology, Vol. 1. Academic Press, London.

Magnuson, J.J., and D.J. Karlen. 1970. Visual observation of fish beneath the ice in a winterkill lake. J. Fish. Res. Board Can. 27:1059-1068.

May, R.C. 1971. An annotated bibliography of attempts to rear the larvae of marine fishes in the laboratory. NOAA Tech Rep. NMFS-SSRF-632, 24 p.

Mayer, A.G. 1914. The effects of temperature upon tropical animals. Pap. Tortuga Lab. 6:3-24. Carnegie Institution, Washington, D.C.

McCleave, J.D., and G.W. LaBar. 1972. Further ultrasonic tracking and tagging studies of homing cutthroat trout (Salmo clarki) in Yellowstone Lake. Trans. Amer. Fish. Soc. 101:4-45.

Myrberg, A.A. 1972. Using sound to influence the behavior of free-ranging marine animals, p. 435-468. In H.E. Winn and B.L. Olla (ed.) Behavior of Marine Animals, Vol. 2 Plenum Press, NewYork.

Myrberg, A.A., B.D. Brohy, and A.R. Emery. 1967. Field observations on reproduction of the damselfish, Chromis multilineata (Pomacentridae) with additional notes on general behavior. Copeia 1967:819-826.

Myrberg, A.A., Jr., S.J. Ha, J. Banbury, and S. Walewski. 1971. Effectiveness of acoustic signals in attracting "blue-water" sharks to the immediate vicinity of an underwater sound source. Office of Naval Research Tech. Rep., Dept. of the Navy.

Nakamura, E.L. 1966. Fiberglass tanks for transfer of pelagic fishes. Progr. Fish-Cult. 28:60-62.

_____ 1972. Development and use of facilities for studying tuna behavior, p. 245-277. In H.E. Winn and B.L. Olla (ed.) Behavior of Marine Animals, Vol. 2. Plenum Press, New York.

Neill, W.H., J.J. Magnuson and G.G. Chipman. 1972. Behavioral thermoregulation by fish: A new experimental approach. Science 176:1443-1445.

Nelson, D.R., R.H. Johnson, and L.G. Waldrop. 1969. Responses in Pahamian sharks and groupers to low-frequency, pulsed sounds. Bull. So. Calif. Acad. Sci. 68:131-137.

Newman, M.A. 1956. Social behavior and interspecific competition in two trout species. Physiol. Zool. 29:64-81.

Norris, K.S. (ed.). 1966. Whales, Dolphins, and Porpoises. University of California Press, Berkeley. 789 p.

Ogden, J.C., and N S. Buckman. 1973. Movements, foraging groups, and diurnal migrations of the striped parrotfish Scarus croicensis Bloch (Scaridae). Ecology 54:589-596.

Ogilvie, D.M., and J.M. Anderson. 1965. Effect of DDT on temperature selection by young Atlantic salmon, Salmo salar. J. Fish Res. Board Can. 22:503-512.

Ogilvie, D.M., and J.N. Fryer. 1971. Effect of sodium pentobarbital on the temperature selection response of guppies (Poecilia reticulata). Can. J. Zool. 49:949-951.

Olla, B.L. 1974. The effect of temperature of fish-to-fish attraction in an inshore schooling species, striped mullet, Mugil cephalus. In U.S. At. Energy Comm. Prog. Rep. AT (49-7) 3045. Unpublished manuscript.

Olla, B.L., and A.L. Studholme. 1971. The effect of temperature on the activity of bluefish, Pomatomus saltatrix L. Biol. Bull. 141:337-349.

Olla, B.L., A.J. Bejda, and A.D. Martin. 1974. Daily activity, movements, feeding and seasonal occurrence in the tautog, Tautoga onitis, Fish. Bull., U.S. 72:27-35.

Olla, B.L., W.W. Marchioni, and H.M. Katz. 1967. A large experimental aquarium system for marine pelagic fishes. Trans. Amer Fish. Soc. 96:143-150.

Olla, B.L., R. Wicklund, and S. Wilk. 1969. Behavior of winter flounder in a natural habitat. Trans. Amer. Fish. Soc. 98:717-720.

Paine, R.T. 1969. The Pisaster-Tegula interaction: prey patches, predator food preference, and intertidal community structure. Ecology. 50:950-961.

Poddubnyi, A.C., Y.I. Spektor, and S.M. Kidun. 1966. The results of preliminary experiments in tracking sturgeon carrying electronic tracer tags. Vop. Ikhtiol. 6:725-734.

Popper, A.N., M. Salmon, and A. Parvulescu. 1973. Sound localization by the Hawaiian squirrel fishes, Myripristis berndti and M. argyromus. Anim. Behav. 21:86-97.

Prentice, E.F. 1969. Gull predation in a reactor discharge plume. U.S. At. Energy Comm. Develop. Rep. BNWL 1050, Pt. 2:2.33-2.34.

Reese, E.S. 1973. Duration of residence by coral reef fishes on "home" reefs. Copeia 1973:145-149.

Reinboth, R. 1973. Dualistic reproductive behavior in the protogynous wrasse Thalassoma bifasicatum and some observations on its day-night changeover. Helgolander wiss. Meersunters. 24:174-191.

Reish, D.J., and J.L. Barnard. 1960. Field toxicity in marine waters utilizing the polychaetous annelid Capitella capitata (Fabricius). Pac. Nat. 1:1-8.

Rochelle, J.M., and C.C. Coutant. 1974. Temperature sensitive ultrasonic fish tag, Q-5099. Oak Ridge Nat. Lab. ORNL-TM-4438.

Saeki, A. 1958. Studies on fish culture in filtered closed-circulation aquaria. Bull. Jap. Soc. Sci. Fisheries 23:684-695. Trans. by E.R. Hope. 1974. Div. Sci. Info. Services, DRB, Canada.

Sale, P.F. 1971. Extremely limited home range in a coral reef fish Dascyllus aruanus (Pisces:Pomacentridae). Copeia 1971:324-32.

Sartori, J.D., and T.J. Bright. 1973. Hydrophonic study of feeding activity of certain Bahamian parrotfishes, Family Scaridae. Hydrolab J. 2:25-56.

Scheier, A., and J. Cairns, Jr. 1968. An apparatus for estimating the effects of toxicants on the critical flicker frequency response of the bluegill sunfish. Purdue Univ. Eng. Ext. Series No. 132, Part 2:849-855.

Scholz, A.T., J.C. Cooper, D.M. Madison, R.M. Horrall, A.D. Hasler, A.E. Dizon, and R.J. Poff. 1973. Olfactory imprinting in coho salmon: behavioral and electrophysiological evidence. Proc. 16th Conf. Great Lakes Res. p. 143-153.

Schuyf, A., and S.J. deGroot. 1971. An inductive locomotion detector for use in diurnal activity experiments in fish. J. Cons. int. Explor. Mer 34:126-131.

Sindermann, C.J. 1970. Principle Diseases of Marine Fish and Shellfish. Academic Press, New York. 369 p.

Smith, S.H. 1973. Application of theory and research in fishery management of the Laurentian Great Lakes. Trans. Amer. Fish. Soc. 102:156-163.

Sparck, R. 1936. On the relation between metabolism and temperature in some marine lamellibranchs, and its zoogeographical significance. Biol. Medd. 13:No. 5.

Sparks, R.E., W.T. Waller, and J. Cairns, Jr. 1972. Effect of shelters on the resistance of dominant and submissive bluegills (Lepomis macrochirus) to lethal concentrations of zinc. J. Fish. Res. Board Can. 29:1356-1358.

Spotte, S.H. 1970. Fish and Invertebrate Culture. Wiley-Interscience, New York. 145 p.

_____ 1973. Marine Aquarium Keeping. Wiley-Interscience, New York. 171 p.

Sprague, J.G. 1968. Avoidance reactions of rainbow trout to zinc sulphate. Water Research 2:367-372.

_____ 1971. Measurement of pollutant toxicity to fish - III. Sublethal effects and "safe" concentrations. Water Research 5:245-266.

Standard Methods for the Examination of Water and Wastewater. 1971. Amer. Pub. Health Assoc., Inc., New York. 874 p.

Starck, W.A., II, and W.P. Davis. 1966. Night habits of fishes of Alligator Reef, Florida. Ichthyologica 38:313-356.

Stasko, A.B. 1971. Bibliography of underwater telemetry in biological applications. Underwater Telemetry Newsletter 1(1). Fish. Res. Board Can., St. Andrews, New Brunswick.

Stasko, A.B., R.M. Horrall, A.D. Hasler, and D. Stasko. 1973. Coastal movements of mature Fraser River pink salmon (Oncorhynchus gorbuscha) as revealed by ultrasonic tracking. J. Fish Res. Board Can. 30:1309-1316.

Stevenson, R.A., Jr. 1972. Feeding behavior of the bicolor damsel-fish (Eupomacentrus partitus Poey) by environmental factors, p. 278-302. In H.W. Winn and B.L. Olla (ed.) Behavior of Marine Animals, Vol. 2. Plenum Press, New York.

Strasburg, D.W., and H.S.H. Yuen. 1960. Progress in observing tuna underwater at sea. J. Cons. int. Explor. Mer. 26:89-93.

Sylvester, J.R. 1972. Effect of thermal stress on predator avoidance in sockeye salmon. J. Fish. Res. Board Can. 29:601-603.

Takahashi, F.T., and J.S. Kittredge. 1973. Sublethal effects of the water soluble component of oil: Chemical communication in the marine environment, p. 259-264. In D.G. Ahearn and S.P. Meyers (ed.) The Microbial Degradation of Oil Pollutants. Louisiana State Univ. Press, Pub. No. LSU-SG-73-01.

Tardent, P. 1962. Keeping Clupeidae, Scombridae, and Scomberesocidae in the Naples aquarium. Bull. Inst. Oceanog. Monaco No. special 1A: 29-34.

Tavolga, W.N. (ed.). 1964. Marine Bio-Acoustics. MacMillan Co., New York. 413 p.

_____ 1967. Marine Bio-Acoustics, Volume 2. Pergamon Press, New York. 353 p.

Thorson, G. 1936. The larval development, growth and metabolism of arctic marine bottom invertebrates compared with those of other seas. Medd. Gronland 100: No. 6.

Todd, J.H., J. Atema, and J. Bardach. 1967. Chemical communication in social behavior of a fish, the yellow bullhead (Ictalurus natalis). Science 158:672-673.

Todd, J.H., D. Engstrom, S. Jacobson, and W.O. McLarney. 1972. An introduction to environmentalethology: A preliminary comparison of sublethal thermal and oil stresses on the social behavior of lobsters, and fishes from a freshwater and a marine ecosystem. U.S. At. Energy Comm. Prog. Rep. AT(11-1) 3567. Unpublished manuscript.

Tyler, A.V. 1971. Surges of winter flounder Pseudopleuronectes americanus into the intertidal zone. J. Fish. Res. Board Can. 28:1727-1732.

Verheijen, F.J. 1956. On a method for collecting and keeping Clupeids for experimental purposes, together with some remarks on fishery with light-sources and a short description of free cupulae of the lateral line organ on the trunk of the sardine, Clupea pilchardus Walb. Pubbl. Staz. zool. Napoli 28:225-240.

Vernberg, W.B., and F.J. Vernberg. 1972a. The synergistic effects of temperature, salinity, and mercury on survival and metabolism of the adult fiddler crab, Uca pugilator. U.S. Fish Wildl. Serv. Fish. Bull. 70:415-420.

_____ 1972b. Environmental Physiology of Marine Animals. Springer-Verlag, New York. 246 p.

Vernberg, W.B., P.J. DeCoursey, and J.O. O'Hara. 1974. Multiple environmental factor effects on physiology and behavior of the fiddler crab, Uca pugilator. In F.J. Vernberg and W.B. Vernberg (ed.) Pollution and the Physiology of Marine Organisms. Academic Press, New York (In press).

Vernberg, W.B., P. DeCoursey, and W.J. Padgett. 1973. Synergistic effects of environmental variables on larvae of Uca pugilator (Bosc). Mar. Biol. 22:307-312.

Weir, P.A., and C.H. Hine. 1970. Effects of various metals on behavior of conditioned goldfish. Arch. Environ. Health. 20:45-51.

White, A.Q. 1974. Effect of sublethal doses of cadmium and low dissolved oxygen levels on locomotion of adult Paleomonetes pugio. (In preparation).

Winn, H.E. 1964. Biological problems in acoustic systems (Behavior of fishes with special reference to their sensory capacities). Office of Naval Research. Final Report:104-489.

Wisby, W.J., and D.R. Nelson. 1964. Airplane observations of acoustic orientation in sharks (abst.). Amer. Fish. Soc. Conf., Session on Fish Behavior and Sensory Physiology.

Woelke, C.E. 1972. Development of a receiving water quality bioassay criterion based on the 48-hour Pacific oyster (Crassostrea gigas)embryo. Wash. Dept. of Fisheries, Tech. Rep. 9, 93 p.

Young, A.H., P. Tyler, F.G.T. Holliday, and A. MacFarlane. 1972. A small sonic tag for measurement of locomotion behavior in fish. J. Fish. Biol. 4:57-65.

Yuen, H.S.H. 1961. Bow wave riding of dolphins. Science 134:1011-1012.

_____ 1963. Schooling behavior within aggregations composed of yellowfin and skipjack tuna. Proc. World Scientific Meeting on the Biology of Tunas and Related Species, July 2-14, 1962, FAO Fish. Rep. 3:1419-1429.

_____ 1966. Swimming speeds of yellowfin and skipjack tuna. Trans. Amer. Fish. Soc. 95:203-209.

_____ 1970. Behavior of skipjack tuna, Katsuwonus pelamis as determined by tracking with ultrasonic devices. J. Fish. Res. Board Can. 27:2071-2079.