WATER QUALITY LABORATORY

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ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF RESEARCH AND DEVELOPMENT

NATIONAL MARINE WATER QUALITY LABORATORY

SEMI-ANNUAL REPORT * July-December 1974

AN ASSOCIATE LABORATORY OF

NATIONAL ENVIRONMENTAL RESEARCH CENTER, CORVALLIS, OREGON

This report does not constitute publication but is for information only. All data must be considered provisional.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL MARINE WATER QUALITY LABORATORY
SOUTH FERRY ROAD
NARRAGANSETT. RHODE ISLAND 02882

April 15, 1975

Enclosed is the Semi-Annual Report from the National Marine Water Quality Laboratory for June 1974 to January 1975. I regret the 3-month delay in getting this report out for final publication, but as you will see, it is a thorough compendium of our research activities during the 6-month period.

As I have stated earlier, the research activities and scientific programs at the National Marine Water Quality Laboratory are highly varied. We are constantly confronted with new problems which demand unique and innovative solutions. Our approach to these complex problems is maturing and the data that we are producing will serve both EPA program and regional offices as well as the scientific community.

The purpose of these reports is severalfold: First, they allow our scientific investigators to go through a thorough review of their work and formulate their thoughts and words with diagrams and graphs in preparation for formal publication in peer-reviewed articles, for EPA publications, or for criteria development and for legal cases.

Secondly, it allows our customers (those mentioned in the above paragraph) to take a preliminary look at our data, our experimental methods, and our scientific approach. In publishing such a report as this, we open ourselves to our critics as well as our proponents, as we attempt to discuss in an open forum the pros and cons, the scope and failures of the work we do in this facility.

Spring is on the verge of breaking here in Rhode Island, and the forthcoming months will be exciting times for our laboratory. The planned 20,000 ft^2 addition to our existing facility is moving along on schedule with the cooperation and hard work of many. We look forward to a ground breaking and initiation of construction sometime in mid-summer.

Other changes are on the horizon--such as the reorganization of EPA's research and development program.

Our past relationships with NERC, Corvallis, and our Washington counterparts have been productive and fruitful experiences. We look forward to the new directions suggested by EPA's new management structure and will work dilligently as a laboratory to fulfill the plans that they have proposed. It is our wish, as well as that of the new management structure in Washington, that our laboratory research programs proceed without interruption.

To those of you who have the opportunity to visit us in Narragansett in the following months, we welcome you. I feel that the true spirit, capability and capacity of this laboratory is best observed by meeting with the individual scientists, technicians and support personnel who carry out our work here.

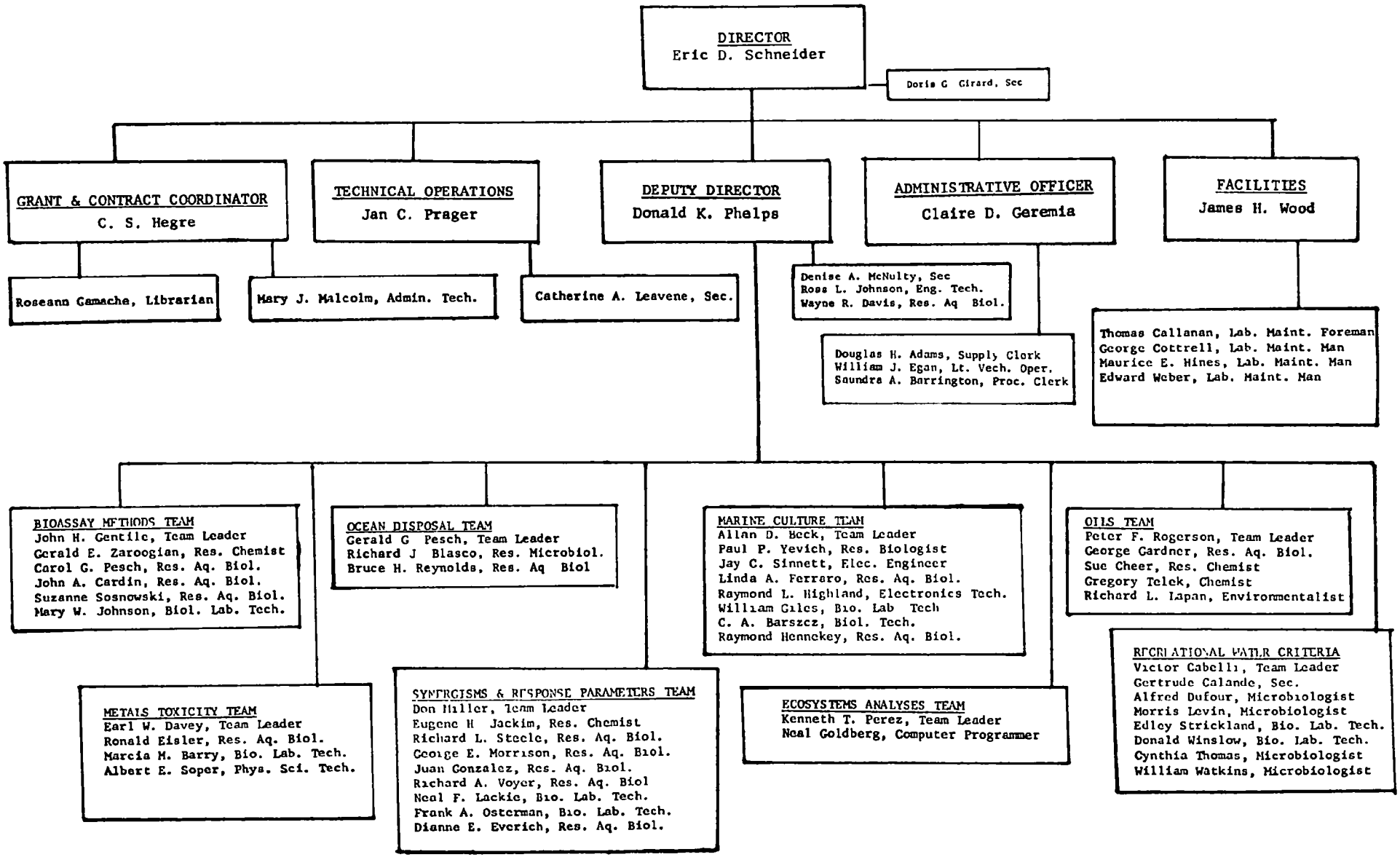
I encourage you to visit with us, review and critique our programs and suggest new solutions and experimental approaches to our problems. If you cannot visit us, feel free to call or write. Our phone numbers and a map of our facility are included at the end of this report.

If I can be of any help, please feel free to call me personally. If I cannot answer the problem, my secretary or I will put you in touch with a cognizant person in our laboratory that can be of help.

Sincerely,

Eric D. Schneider
Director

EDS:dgg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The Bioassay Team is responsible for the development of bioassay systems and techniques which permit the interaction of test organism and pollutant in a manner closely approximating their natural encounter. The program has and will continue to focus on developing methodology for a wide variety of estuarine species of both ecological and commercial importance. Species representing different communities, trophic levels, and feeding types are selected so as to permit evaluation of the widest possible impact. Previous emphasis of this program has been on short-term techniques; however, now we have expanded to include all life stages with a strong emphasis on the use of eggs and larvae. Our program utilizes species with short life histories. This permits evaluation and comparison of short-term as well as reproductive effects which are necessary for projections on population viability.

New trends in the bioassay program include pollutant bioaccumulation and its significance to both public health standards and possible effects on reproduction and population integrity. Most recently we have started to evaluate mixed effluents and their effect on planktonic communities. Bioassay designs are being developed that more closely reflect the actual exposure profile and thus permit a more realistic estimate of impact.

This report will cover three major tasks in our work plan: (1) the development and evaluation of short-term bioassay techniques for phytoplankton, zooplankton, fish larvae, and polychaetous annelid larvae (ROAP 21 AKK - Task 07); (2) the establishment of design criteria for long-term exposure systems and the fabrication and testing of these systems for compatibility with all life history stages (ROAP 21 AKK - Task 11); and (3) evaluate the performance of long-term bioassay systems (ROAP 21 AKK - Task 12).

During the period covered in this report we have completed a series of short-term bioassays on metals in cooperation with the

Metals Team (ROAP 16 AAT). The data from these studies will appear in the following sections.

ROAP 21 AKK - Task 07 -- Develop and Evaluate Short-Term Bioassay Techniques

Phytoplankton

Short-term bioassays were performed on both individual metals and in two instances on mixed waste materials. Individually tested metals included silver, chromium, nickel, lead, arsenic, cobalt, and selenium. Mixed wastes included arsenic waste and antimony wastes. Cyclotella nana (Thalassiosia pseudonana) and Skeletonema costatum were tested at 30 o/oo salinity and 20°C. Figures 1a and 1b are a summarization of this information. The graphs show the EC-50 (growth rate) response for a variety of test materials. The graph (courtesy of Neal Goldberg) shows that the mixed wastes are considerably less toxic than the individual metals. However, not having a compositional analysis it is difficult to evaluate the toxicity of the metal itself.

It is interesting to note that there were some obvious differences in sensitivity between the test species. Copper was considerably more toxic to C. nana while S. costatum was far more sensitive to silver. Thus when waste materials are being evaluated for their potential environmental impact it is necessary to look at more than one species whenever possible.

Of further interest is the mild stimulation of growth rate recorded for the metal selenium at concentrations as high as 33 ppm. Preliminary studies of selenium toxicity indicate Acartia tonsa is sensitive to selenium at concentrations below 1.0 ppm. If further studies warrant, a series of food chain studies involving Skeletonema costatum and Acartia tonsa will be initiated.

In order to streamline data reduction from algal assays a computer program was developed by Neal Goldberg of our Ecosystems Analysis Team. This program will accept either cell density or biomass input for each observation period and exposure level and cal-

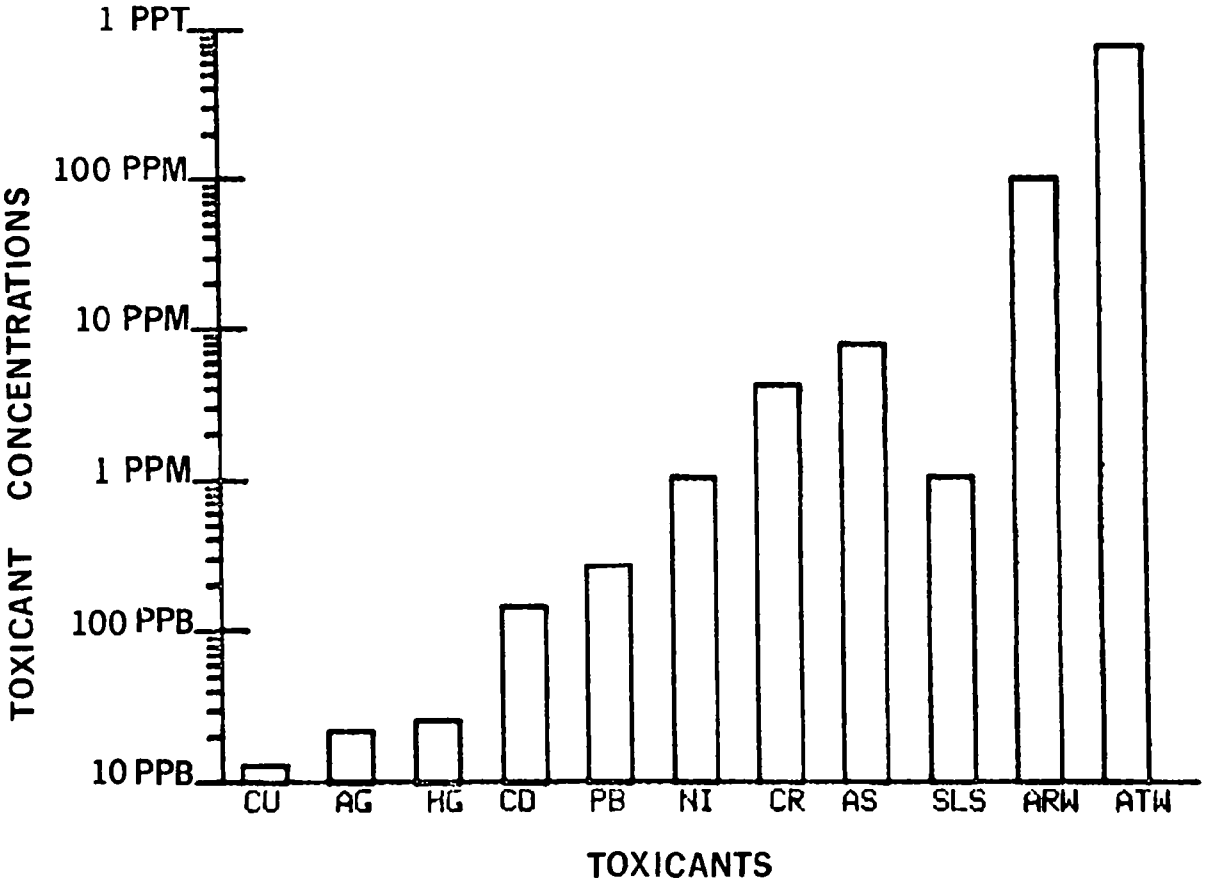


Fig. 1a Comparison of EC-50's for growth rate of Cyclotella nana exposed to individual heavy metals, sodium lauryl sulfate (SLS) and arsenic (ARW) and antimony (ATW) wastes.

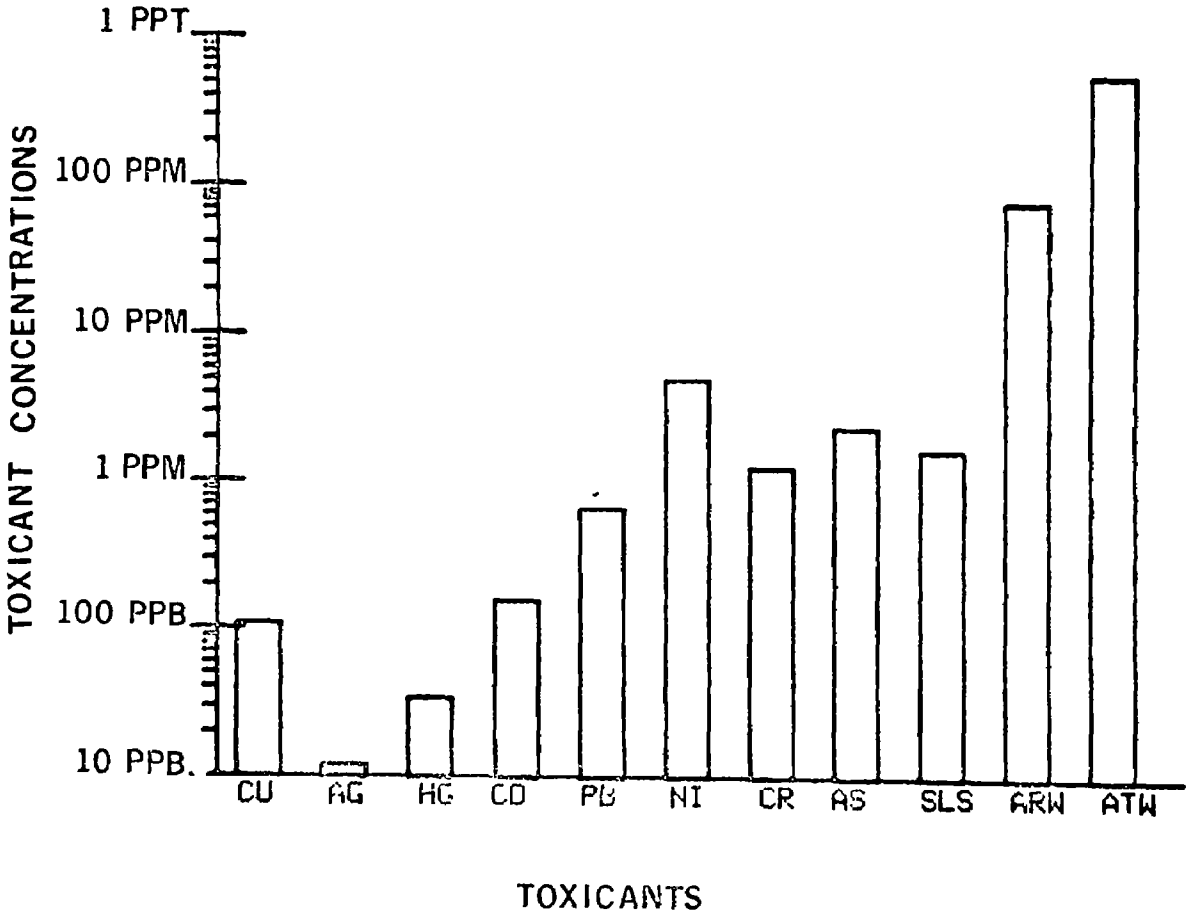
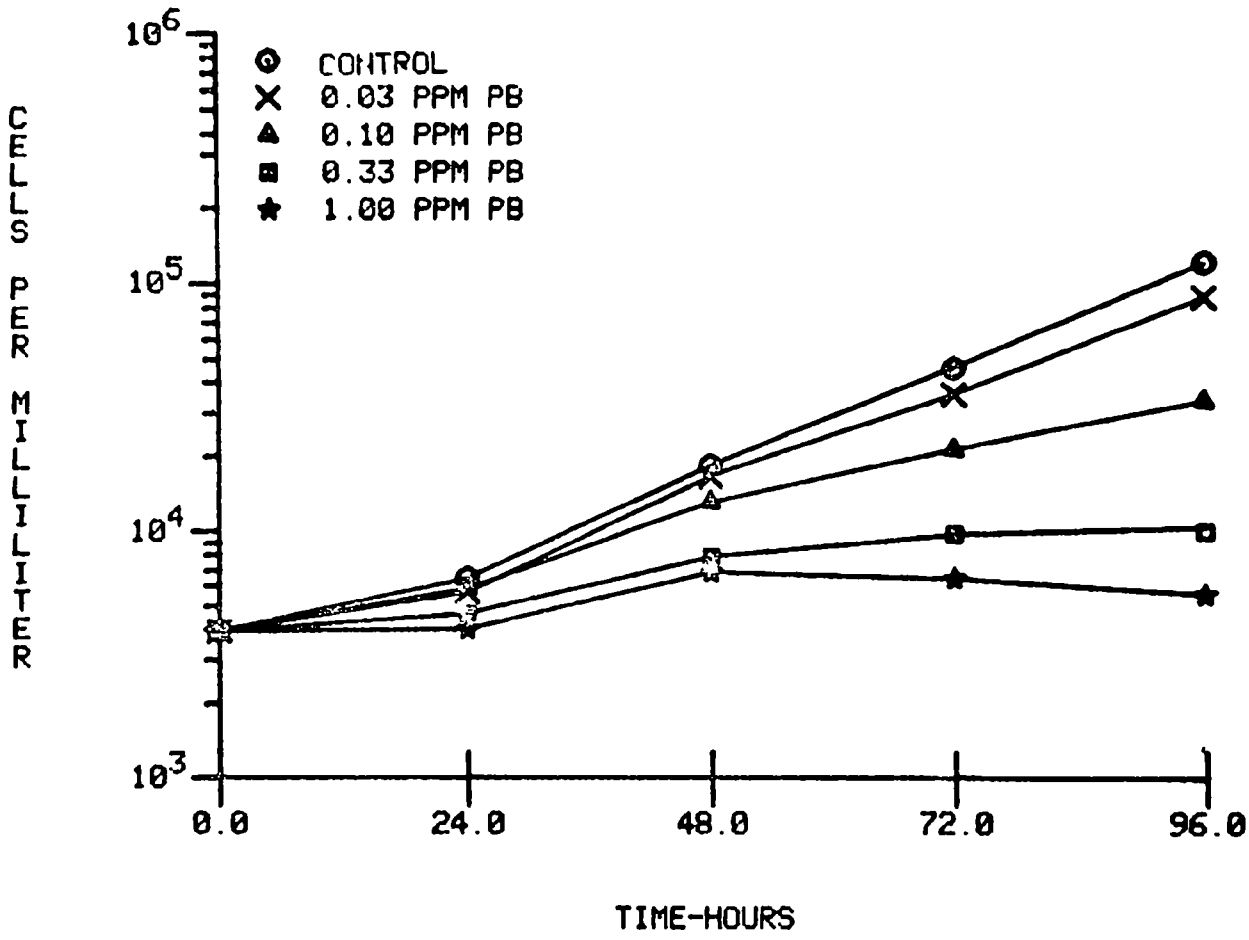


Fig. 1b Comparison of EC-50's for growth rate of Skeletonema costatum exposed to individual heavy metals, sodium lauryl sulfate (SLS) and arsenic (ARW) and antimony (ATW) wastes.



EXPOSURE TIME (HOURS)

	0.0	24.0	48.0	72.0	96.0
CNTL	3921.	6505. (0.73)	18373. (1.50)	46083. (1.33)	120999. (1.39)
0.03 PPM	3921.	5646. (0.53)	16698. (1.56)	35990. (1.11)	89373. (1.31)
0.10 PPM	3921.	5902. (0.59)	13200. (1.16)	21539. (0.71)	33960. (0.66)
0.33 PPM	3921.	4601. (0.23)	7907. (0.78)	9707. (0.30)	9965. (0.04)
1.00 PPM	3921.	4005. (0.03)	6890. (0.78)	6494. (-0.09)	5550. (-0.23)

Fig. 2 Computerized data presentation of algal assays for lead. Table values are cell densities with growth rates in parenthesis.

culate growth rates, correct for coincidence when appropriate, and plot the complete growth response curves. The data is stored on tape from which hard copies are made. An example of the data presentation capabilities of this program is shown in Figure 2. The table presents cell density/ml of culture with the growth rate for each incremental observation period in parenthesis. This capability has reduced our data reduction time in half.

ROAP 21 AKK Task 07

Lobster Larvae

Lobster larval assays were not emphasized this year due to problems associated with collection and more importantly holding. Laboratory facilities are now approaching the stage where ovigerous females can be maintained in a controlled temperature environment prior to hatching. Hatched larvae will be held in kriesels and used for short-term bioassays. A series of exposure chambers have been designed and fabricated and are currently being evaluated using other microcrustaceans and fish larvae as the test species.

A methods manual describing the year-round production of lobster larvae was prepared by Schlesser et al. under contract to EPA. A detailed critique was prepared and returned to the authors for revision. As a result of revisional problems and the availability of materials we will be unable to initiate validation of the methodology for several weeks.

In preparation for the validation, we have obtained a permit to collect ovigerous female lobsters from the Rhode Island Department of Natural Resources. Presently, the lobsters are being held in communal tanks at ambient seawater temperature since late September. The divided holding and brood tanks have been manufactured and we are awaiting delivery. Upon receipt of these tanks, we will initiate a program of gradually increasing the temperature of the seawater to accelerate maturation and hatching. The procedures

we will follow will be those described in the mannual. We have ordered the necessary kriesels for culture of the ensuing larvae.

ROAP 21 AKK - Task 07

Zooplankton

An attempt was made to evaluate the feasibility of using mixed indigenous zooplankton populations in routine bioassays. This type of assay is potentially useful in monitoring the effects of ocean dumping to offshore species which have not been cultured through a whole life cycle. A feasibility experiment was performed to evaluate this potential assay technique. We found that the high degree of expertise required in the design, performance, species identification and interpretation prohibit this assay from being used routinely. Further, there was evidence of interspecific predation which further complicated interpretation. The use of natural indigenous populations in bioassays becomes feasible only when essentially "monospecific" populations exist.

A culture status report on the availability of zooplankton species was completed and submitted to the Culture and Holding Team (ROAP 21 AKF).

At the request of the Metals Team (ROAP 16 AAT) information on the toxicity of heavy metals to zooplankton was compiled from our laboratory research program. The data consists of short-term bioassays on the species and metals listed below:

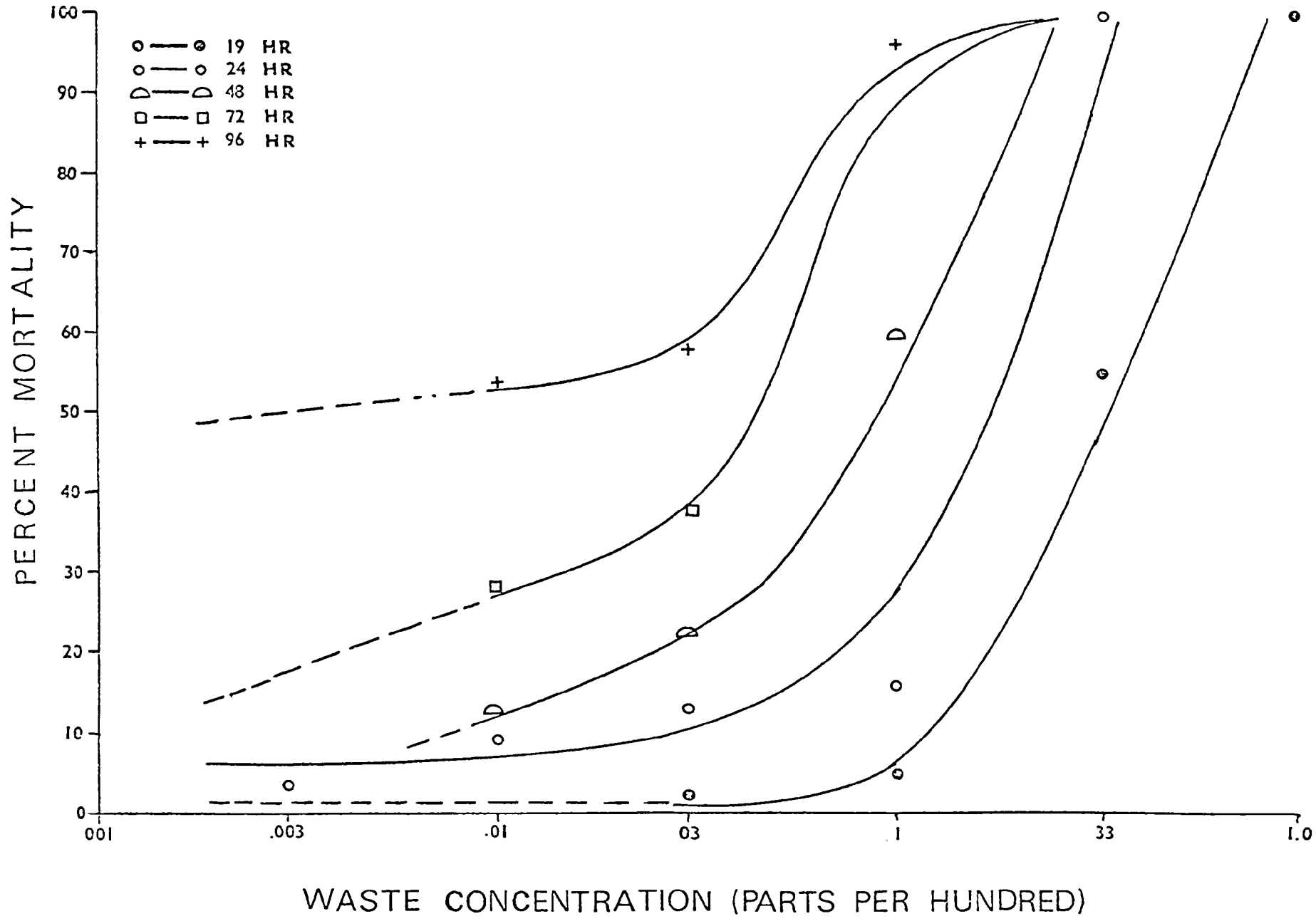
METALS MATRIX FOR COPEPODS

TEST ORGANISM	96 Hr. LC_{50} (in mg/l) of Toxicant					
	Cd^{+2}	Cu^{+2}	Hg^{+2}	Zn^{+2}	Cr^{+6}	Ni^{+2}
Acartia tonsa	.320	.023	.060	.400	7.20	.620
Pseudodiaptomus coronatus	1.50	.140	.070	1.75	7.40	12.0
Eurytemora affinis	1.00	.500	.080	1.30	---	8.00
Tigriopus japonicus	4.30	.880	.115	2.70	22.5	4.60
Acartia clausi	.230	.046	.011	1.40	6.5	2.85

In conjunction with the Metals Team (ROAP 16 AAT) a series of static bioassays were performed on Acartia tonsa from field tows. Results show that antimony waste has a very high oxygen demand. After twenty-four hours exposure, the dissolved oxygen levels were reduced to 1.5 ppm at 1.0% and .33% waste. This probably contributed to the 100% mortality noted at these levels. In an effort to rule out the dissolved oxygen effect, an air-water life system was devised to maintain the dissolved oxygen at 5.1 ppm in the .33% level for twenty-four hours. Mortality at .33% was about 35% with aeration, compared to 100% in unaerated cultures. Sixty percent mortality was observed after 72 hours. These mortalities were not related to dissolved oxygen levels (D.O. >5.1 ppm) (Fig. 3). The arsenic waste material was twenty times more toxic to Acartia clausi than the antimony waste. Of particular interest were a series of assays on the toxicity of Ag^{+2}. This metal has in the past received very little attention at this laboratory. The indications are that it is of comparable toxicity with Hg^{+2} and Cu^{+2} to the genus Acartia. We anticipate examining the chronic effects of this metal to Acartia tonsa.

The continuous flow culture system for calanoid copepods is now successfully rearing the F_4 generation from individual Acartia tonsa isolated from an October field collection. Automatic continuous feeding has been replaced by manual batch feeding with improved results. The continuous flow head tank is now heated providing 18°C filtered seawater to the culture system. Initial attempts to set up nine replicate continuous cultures of Acartia tonsa were complicated by repeated contamination with harpacticoid copepods that developed from eggs that passed thru the filter system. Work is now in progress on the installation of a multi-media seawater filteration system. This should allieviate problems with contamination. Experiments are now in progress to evaluate the feasibility of

Fig. 3 Response of Acartia tonsa to antimony waste.



using populations of Acartia tonsa for long-term bioassays. We are presently defining the degree of replicate variance we can expect from nine unstressed populations. Each replicate was started with 20 pairs of males & females and will run for 60 days. Qualitative and quantitative population characterization will then be performed on each replicate.

As part of developing assay parameters for such long-term bioassays (Task 11), studies on Acartia fecundity are in progress. The difficulty in using this parameter relates to its sensitive and positive correlation to nutrition. An initial study comparing females exposed and unexposed to cadmium, while not statistically significant, did show a definite decrease in fecundity in exposed females. However, handling and sampling problems remain to be resolved before consistent and reproducible information can be expected from this technique in an assay.

ROAP 21 AKK - Task 07

Polychaetes

A prototype culture system using flowing filtered seawater was set up. This system will support the complete life cycle of C. capitata if temperature control is available to maintain a water temperature warm enough for reproduction. The flow-through system is definitely superior to static cultures. This fall the static cultures became grossly contaminated with numerous cilliates, harpacticoid copepods etc.,and the remaining polychaetes looked unhealthy. The flow-through cultures were only slightly contaminated, and the polychaetes remained active and healthy. The reasons for the mortalities and unhealthy appearance in the static cultures are unknown.

A prototype flow-through chamber for exposing C. capitata to toxicants was devised. The chamber has not yet been tested.

Three more polychaetes were added to the culturing efforts. A

clone of local Capitella capitata collected from Charlestown Pond was established. A culture of polychaetes, tentatively identified as Polydora ligni, was started from planktonic larvae collected from Narragansett Bay. Ctenodrilus serratus, a minute polychaete which commonly reproduces by transverse fission, was brought from California by Donald Reish on December 6, 1974.

Short-term static screening bioassays of five metals were conducted on polychaete larvae (tentatively identified as Polydora ligni) collected from Narragansett Bay and Narrow River in November and December. No mortalities were seen with selenium or chromium^{+6} at 10 ppm which was the highest concentration used. LC-50 values for the other metals are as follows:

Metal	96 hr	192 hr
Ag	187 ppb	96 ppb
Hg	54 ppb	--
AsO_3	5.6 ppm	2.8 ppm

Concentrations for silver were verified by atomic absorption analysis. The mecury bioassay involved the daily changing of media and toxicant redosing.

ROAP 21 AKK - Task 12--Evaluate Performance of Long-Term Bioassay Systems

Long-term Exposure of Oysters to Sublethal Levels of Cadmium.

A study was started in November, 1973 to determine the kinetics of bioaccumulation of cadmium in oysters (Crassostrea virginica) during winter and summer conditions. In addition to the bioaccumulation of cadmium we wanted to determine 1) if any effect of accumulated cadmium on fecundity and/or viability of larvae occurs; 2) if depuration does indeed occur after termination of the cadmium dosing; and 3) if any histopathological damage could be detected. Since the oysters are laden with copper upon harvest, the copper content of the oysters was studied in conjunction with the cadmium study. Our intent here is to determine whether copper is depurated by the oysters

in seawater containing ambient levels of copper and if cadmium competes with copper for sites within the tissues.

Throughout the duration of the study, we are always in constant search for the occurrence of some phenomenon or criterion that would allow us to predict a detrimental effect to oysters or a population when monitoring oysters exposed to levels of cadmium in seawater not normally found in their natural environment.

A) Bioaccumulation - The oysters were continually exposed to 5 and 15 ppb cadmium in fiberglass troughs using a continuous-flow seawater system in the wet lab. Ambient seawater temperature and salinity were maintained throughout the entire study. The exposed oysters along with controls were sampled bi-weekly for the first 24 weeks and weekly for the remaining 16 weeks during which time the water temperature was higher resulting in greater amounts of seawater passing over the gills. The total soft parts of the oyster are removed from the shell, prepared for atomic absorption spectrometry and analyzed for cadmium and copper.

Cadmium addition was terminated after 40 weeks. Levels of cadmium in the troughs were monitored weekly using the Chelex resin technique developed by Dr. Davey of this laboratory.

Before exposure, cadmium levels in the total soft parts of the oysters was 2.72 ppm wet weight; whereas at the end of 40 weeks exposure to 5 and 15 ppb cadmium, mean cadmium levels of 13.57 ppm and 33.34 ppm were found respectively. After 40 weeks exposure to non-contaminated seawater the control oysters had a mean cadmium level of 1.69 ppm in the total soft parts.

Both exposed and control animals appeared healthy by gross examination. Also, the exposed oysters produced as much new shell growth as the controls. Some oysters in each group added as much as 1.8 cm new growth during the study.

B) Histopathology - Oysters were sampled for histopathological

examination concurrent with samples for bioaccumulation. Histopathological examination has shown that the reproductive tract of the oysters exposed to 15 ppb cadmium is slower in maturing than that of the oysters exposed to 5 ppb cadmium and the controls.

C) Depuration - After 40 weeks the addition of cadmium to the experimental troughs was terminated. The initial samples of oysters was taken 1 month after termination of exposure to cadmium and succeeding samples have been taken bi-weekly. The oysters are prepared for atomic absorption spectrometry and analyzed for cadmium and copper.

The depuration phase of the study is still in progress.

At this time it appears that neither cadmium nor copper is depurated by the oyster. However, it must be emphasized that this data is preliminary and the variation in copper and cadmium amongst the oysters within a sample is substantial; therefore, we cannot be certain as to exactly what is happening until the study is terminated and the data is analyzed statistically.

D) Viability of Gametes - Oysters were induced to spawn and the larvae from oysters exposed continuously to 5 and 15 ppb cadmium and from control oysters were incubated for 72 hours at 20°C in 22 o/oo seawater.

This phase of the study was performed to determine whether gametes from oysters exposed to cadmium are as viable as gametes from oysters not exposed to cadmium and to determine if the male and/or female gametes are effected differentially by cadmium. Also, we wanted to determine if the larvae from gametes of cadmium-laden oysters were as viable as those from controls.

If an effect on the gametes occurs when exposed to cadmium then this quite conceivably could have a detrimental effect on a natural oyster population.

In this study we encountered a problem not experienced before in that the oysters exposed to 15 ppb cadmium could not be induced

to spawn as readily as those exposed to 5 ppb cadmium and the controls. Therefore it was extremely difficult to synchronize spawning in order to get gametes to make the various crosses.

It appeared that the gonads took longer to mature in the oysters exposed to 15 ppb cadmium. Then when they did mature and started to spawn there was a shorter interval of time between the onset of spawning and when they were spawned out as compared to controls and the oysters exposed to 5 ppb cadmium. We observed in a similar study, where oysters were exposed to 10 ppb cadmium continuously for 40 weeks, that the control oysters spawned out sooner naturally than those exposed to cadmium. However, in this case we encountered no difficulty in synchronization of inducement to spawn.

Larvae produced from gametes from exposed parents showed normal development to the straight hinge stage. However, at the 48 hr observation time up to 53% of these straight hinge larvae were dead, as evidenced by empty shells. This did not occur when either of the gametes came from control oysters. This phenomenon was observed regardless of whether the larvae were incubated in cadmium-laden seawater (5 or 15 ppb Cd) or clean water.

Also, it is important to note that the gametes from one oyster in each treatment were used in the appropriate cross fertilizations.

E) Viability of Larvae - Larvae from oysters exposed continuously to 5 and 15 ppb cadmium for as long as 36 weeks were incubated in natural seawater with and without cadmium (5 and 15 ppb) for 4 weeks to determine if normal embryological development occurs under these separate conditions. These larvae were fed Isochrysis galbana every 3 days.

Larvae from control oysters grew better in seawater containing 5 ppb cadmium than in natural seawater and that containing 15 ppb cadmium.

Less mortality occurred in seawater containing 15 ppb cadmium than in seawater containing 5 ppb cadmium or no cadmium adds. Per-

haps the cadmium is exerting its bacteriostatic or fungistatic properties on the microflora.

The highest mortality amongst the larval treatments or crosses was observed when female gametes from oysters exposed to 15 ppb cadmium were crossed with male gametes from control oysters and reared in natural seawater with no cadmium adds. A 90% mortality occurred with this cross and treatment whereas a 40% mortality was observed when both gametes came from control oysters and reared in natural seawater.

A mortality of 75% occurred with the cross of female gametes from oysters exposed to 5 ppb cadmium with male gametes from control oysters and reared in natural seawater. Also interesting is the fact that these larvae measured the largest of any other treatment (122μ).

Long-term Exposure of Quahaugs to Sublethal Levels of Cadmium.

A study was started in December to determine the kinetics of bioaccumulation of cadmium in the mahogany quahaug (Arctica islandica). In addition to the bioaccumulation we are monitoring the toxicological, biological, and histopathological effects of cadmium on this animal.

The same system which was used for the oyster study above is being used for this study. The addition of 10 cm of sand to the troughs to permit the quahaugs to burrow was the only alteration. The quahaugs are being exposed to 5 and 15 ppb continuously and the exposure should continue for 12-15 weeks. The quahaugs are sampled bi-weekly to determine cadmium body burdens and for histopathological examination. The total soft parts are used for atomic absorption spectrometry of Cd.

The study is proceeding well as was anticipated. At the time of the next report we should have completed this study.

Publication

Drs. Zaroogian and Cheer prepared a manuscript on cadmium accumulation in oysters.

ROAP 21 AKK - Task 07

Ichthioplankton

Short-term static bioassays were performed using the Atlantic silverside (Menidia menidia). The four-spined stickleback (Apeletes quadracus) and embryonic summer flounder (Paralicthys dentatus) as test species. Bioassays were conducted to quantify the toxicity of silver, arsenite, selenium, chromium, lead, and zinc. Several other metals (barium, vanadium, beryllium, etc) were omitted from study either because of their insolubility or their potential hazard to laboratory personnel.

Results of these assays are summarized in Table 1 and generally represent a single assay. While repetition of these assays can be expected to refine the data, present results permit ranking of the tested metals as highly toxic (Ag, Hg, Cu); moderately toxic (Cd, As, Se, Cr^{+6}) and low toxicity (Ba, Zn, Pb). It is interesting to note that if we were to use other bioassay data from this report somewhat different patterns would emerge in terms of ranking the toxicity of these metals. This re-inforces our contention that until more data is available which will allow us greater predictability, an array of species should be tested whenever possible. Future studies will focus on long-term exposures to one or two of the above metals in an effort to obtain data on incipient lethal levels, rates of bio-accumulation and potential effects on reproductive success.

In cooperation with the Culture Team, we have been trying to expand our larval assay capabilities. In our geographical area, the potential exists for working with flounder larvae for 9 months of the year. For the first time we had the opportunity to study the summer flounder larvae, Paralicthys dentatus which were successfully spawned by Grace MacPhee of our staff. Unlike the winter flounder larvae, this species was extremely sensitive to handling and temperature variation. We did perform a very limited number of assays on both

the eggs and larvae for comparative purposes. Both the handling and temperature control problems previously mentioned will be resolved so that a more definitive study can be performed during the next spawning season.

Short-term static bioassays were performed on two mixed industrial wastes. One waste was classified as an arsenic waste while the second was not identified chemically. The results of these assays, summarized in Table 2, indicate a relatively low toxicity compared to heavy metals. However, long-term chronic exposures that concentrate on bioaccumulation, reproductive, growth and other sub-lethal parameters must be evaluated before the potential environmental impact of these wastes can be ascertained. Further since these wastes are in a dilute aqueous state the concentration of an active ingrediant is also diluted. Without proper chemical characterization the true hazard of these materials is difficult to assess.

A considerable amount of biological testing for ocean disposal permits involves the use of the brine shrimp, Artemia salina. While offering many obvious advantages relating to ease of storage, hatching, and holding, assays using this organism are subject to a wide range of criticisms. Principal among them are: it is not an species indigenous to estuarine and coastal waters (except San Francisco Bay); it has, at best, limited ecological importance and significance in marine food webs. Since most of our assay work involves indigenous marine species, we assembled some comparative short-term assay data (Table 3). It is obvious that there is wide variation between organisms when compared to one toxicant and between various toxicants on the same species. This brief comparison points out that Artemia salina can not be used as the sole indicator of toxicity. For that matter, no one organism is always more toxic than any other. Yet there are those that display a consistently high sensitivity to many compounds and it is these species that are currently being recommended

for marine assays. They include the phytoplankton, the zooplankton Acartia tonsa and Acartia clausi; the oyster larvae (Crassostrea virginica), and fish and larvae from Menidia menidia and the flounder larvae (Pseudopleuronectes). The polychaete worms are best suited for long-term studies involving toxicant accumulation and mobilization.

At the request of Linda Ferraro of the Culture Team, acute bioassays were conducted on five groups of Menidia menidia. The first group was field animals, a second was held in natural unfiltered seawater, and the remaining three were held in filtered seawater and exposed to different dietary regimes. The unfed group showed widespread mortality at all toxicant concentrations and were extremely sensitive to handling. Interestingly sensitivity to cadmium of each group of diet fish did not differ significantly from field animals (Table 4). Interpretion is complicated by the presence of two distinct size groupings- 3.5 cm and 7.8 cm fish. When examined with respect to each of these size groups the lab diet fish exhibit more tolerance to cadmium, (Table 5). There is the possibility that field animals were stressed from collecting which might account for their greater susceptibility. This study will be repeated using larger numbers of same size organisms but exposed continuously to the toxicant permitting evaluation of an incipient lethal concentration.

Four continuous exposure systems have been fabricated by R. Johnson and are being tested. Initially this system will be used to provide short-term continuous exposures for comparison to the statics. Chronic long-term exposures on toxicants chosen with regard to their potential as marine pollutants, detectability by current analytical capabilities, and low hazard human risk will be initiated this spring. The embryo's and larvae of winter flounder, and atlantic silverside; lobster larvae, and other species will be studied in this system. This system has been designed for maximum flexibility and may be used for a wide range of organisms, singly or in combination.

TABLE 1

	Menidia menidia juveniles 3-5 cm		Apletes quadracus	
	48 hr LC_{50}	96 hr LC_{50}	48 hr LC_{50}	96 hr LC_{50}
Silver	.43 ppm	.43 ppm	.55 ppm	.55 ppm
Mercury	.66	.072 ppm	1.2 ppm	.45 ppm
Copper	---	---	---	---
Arsenite	17 ppm	16.5 ppm	17.5 ppm	16 ppm
Selenium	17 ppm	14.5 ppm	41 ppm	18.5 ppm
Cadmium	22 ppm	10.5 ppm	---	---
$Chromium^{+6}$	greater than 30 ppm	19 ppm	greater than 30 ppm	greater than 30 ppm
Barium	greater than 100 ppm	greater than 100 ppm	greater than 100 ppm	greater than 100 ppm
Lead	---	---	---	---
Zinc	---	---	---	---

Table 2: Toxicity of two industrial waste products to various organisms

	Arsenic Waste	Mixed Effluent
Menidia Menidia juv. 3.5 cm.	96 hr TL_{50} Greater than 300 ppm	----------
Apeltes quadracus	144 hr TL_{50} Greater than 300 ppm	----------
Paralichtys dentatus embryos	120 hr TL_{50} Greater than 30 ppm	----------
Fundulus heteroclitus larvae (newly hatched)	----------	96 hr TL_{50} 260 ppm
Artemia salina	----------	96 hr TL_{50} 580 ppm

Table 3: Comparative Toxicity of *Artemia salina* with Indigenous Marine Organisms. Tabular Values are 96 hr LC-50 in mgs/l.

SPECIES	SLS	CU^{+2}	Cd^{+2}	Hg^{+2}	KCN	Effluent
Artemia salina	2.3	2.2	51.0	0.6	0.06	580
Phytoplankton						
Thalassiosira pseudonana	1.5	0.015	0.10	0.015		100
Skeletonema costatum	2.0	0.10	0.33	0.03		
Zooplankton						
Acartia tonsa		0.023	0.32	0.06	0.09	120
Acartia clausi	0.2	0.064	0.52	0.04		
Eurytemora affinis	5.0	0.50	1.0	0.08		
Pseudodiaptomus coronatus	0.2	0.14	1.5	0.07		
Tigriopus japonicus	5.8	0.88	4.3	0.12		
Tisbe furcata	1.2					
Molluscs						
Crassostrea virginica (larvae)	1.0	0.10	3.8	0.006		
Polychaetes						
Neanthes arenaceodentata		0.30	12.0	0.03		
Capitella capitata		0.20	7.5			
Ophryotrocha		0.40				
Fishes						
Fundulus heteroclitus (adult)	4.5	10.0	55.0			
Fundulus heteroclitus (larvae)		0.64	11.0	.16		260
Pseudopleuronectes americanus (larvae)		0.22		.75	0.48	
Menidia menidia (larvae)			1.2	0.12		
Apeltes quadracus (adult)				.45		
Menidia menidia (juvenile)			10.5	.072		

Table 4: Toxicity of Cadmium to Various Groups of Menidia menidia (3-8 cm) 96 hr LC-50 Values.

Unfed Group	Undeterminable. High mortalities at all levels
Diet I	10 ppm
Diet II	12 ppm
Diet III	9 ppm
Field Population	10 ppm

Table 5: Toxicity of Cadmium to Various Groups of Menidia menidia

Size	48 hr LC_{50}	96 hr LC_{50}	48 hr LC_{50}	96 hr LC_{50}
3-5 cm	17.5 ppm	11 ppm	greater than 30 ppm	35 ppm
7-8 cm	4.7 ppm	------	17 ppm	

Dr. Gentile and Mrs. Johnson prepared a manuscript on phytoplankton bioassay methodology for the ocean dumping manual.

Dr. Gentile, S. Sosnowski, and J. Cardin prepared a manuscript on zooplankton bioassay methodology for the ocean dumping manual.

Dr. Gentile represented the NMWQL at a conference on Ocean Disposal Bioassay Methods, July 6-8 at Atlanta, Ga.

Dr. Gentile presented a paper "Power Plants and Estuaries" at the 104th Annual Meeting of the American Fisheries Society in Honolulu, September 9-11.

Drs. Gentile and Cheer presented a workshop at ATP methodology and application to power plants at NFIC, Denver, Colorado, October 5-8, 1974.

The research activities of this team currently include considerations of the effects of pollutants on organisms in a dynamic environment. Some environmental factors can influence rates of toxicant uptake by organisms. Elevated temperature, for example, which will increase both metabolic and whole organism activity, can also result in increased uptake of metals. Low salinity can have the same effect. We are interested in defining patterns and quantifying the changes in uptake rate following environmental alterations. We are also interested in understanding the mechanisms underlying observed changes in uptake in order to ascertain whether they are applicable for a variety of metals and a diversity of organisms. In considering environmental interactions, we should also know the normal adaptive range of our principle experimental organisms regarding temperature, oxygen and salinity. This can be elucidated in a preliminary fashion by multivariate studies which determine the tolerance limits for each of these factors, singly and in combination.

The team is also concerned with methods development in order to identify and measure the effects of sublethal stress on organisms. This question is currently being addressed at several levels of biological organization--from schooling behavior of fishes to subcellular indicators, such as ATP analysis to indicate viability of the bacterial flora of marine sediments.

I. Environmental Effects Studies

A. Influence of Temperature, Salinity and Substrate on Cadmium Uptake by Marine Organisms (ROAP 21 AKF)

Task 067--Determine the influence of environmental and physiological variables on uptake of specific pollutants.

A project was designed and conducted by E. Jackim, R. Steele, G. Morrison and F. Osterman to elucidate the effects of selected environmental factors on uptake of a metal. This project will also provide background information contributing to development of bioassay systems

which more closely resemble the field situation.

Radio-cadmium (Cd-109) was used as a tracer to evaluate the effects of temperature, salinity, and presence of sediment on uptake rates of this metal by acclimated and non-acclimated organisms in a static system. Stacking dishes containing the desired organisms and/or bottom substrate were placed in 40 liter aquaria containing 20 liters of filtered seawater of known salinities. These were held in light and temperature controlled boxes with a magnetic stirrer slowly circulating water. Sufficient cadmium was added throughout the run to each tank to maintain a concentration of 20 micrograms/liter. Likewise, sufficient tracer (Cd-109) was added to maintain the radioactivity to about 300 CPM/ml.

Nine species of animals and eight macroalgae were utilized in preliminary studies (Table 1). The * indicates four animals and three macroalgae which were selected for further study.

Table I - Experimental Organisms

Yoldia limatula	Common Name
*Mya arenaria	soft shell clam
*Mytilus edulis	bay mussel
*Mulinia lateralis //	coot clam
*Nucula proxima	pea clam
Podarka obscura	small polychaete
Strongylocentrotus drobachiensis	common sea urchin
Menidia menidia	silverside fish
Palaemonetes pugio	grass shrimp
Laminaria saccarina	kelp
Grinellia americana	red alga
*Codium fragile	antler weed
Rhodomenia palmata	red alga
Ulva lactuca	sea lettuce
Enteromorpha intestinalis	sea lettuce
*Ascophyllum nodosum	sea wrack
*Fucus vesiculatus	rock weed

The first series of studies was conducted without any sediment in the test container. Rates of cadmium accumulation by seven species

of macroalgae and invertebrates, when exposed at 20°C and 20% salinity, are illustrated in Fig. I. The bay mussel, Mytilus edulis, had the greatest and most rapid uptake of cadmium at all temperature/salinity combinations. None of the three filter feeding bivalves appeared to have reached equilibrium. The deposit feeding pelecypod, Nucula proxima, accumulated relatively little Cd and was the only animal that appeared to reach a state of equilibrium with respect to cadmium concentration. The macroalgae tested are not major accumulators of the metal when compared to Mytilus. Ulva uptake reached an equilibrium concentration of Cd in 14 days. The maximum exposure period was 26 days, although water quality in the static experimental systems deteriorated somewhat in runs of this duration. A biological filter will be used in future work to assure good water quality throughout the study.

All bivalves, (Mya, Mulinia, Nucula and Mytilus) exposed to cadmium showed greater accumulation rates of the metal at the lower salinity (20 °/oo) (Table 2). Cd uptake in the macroalgae, Codium fragile, Ascophyllum nodosum, and Fucus vesiculatus also followed this pattern. Temperature had a direct effect on cadmium accumulation rates in the four molluscan bivalves, with greater uptake at the higher temperature (20°C). In contrast macroalgae showed no appreciable difference in cadmium uptake between 10 and 20°C. Table 2 summarizes the Q_{10} values for Cd uptake observed for each species between 10 and 20°C in both 20 and 32 °/oo salinity. The possibility of temperature/salinity interaction affecting cadmium accumulation are presently being evaluated.

Table 2: Cadmium Uptake Q_{10} for Marine Organisms Exposed at 10 and and 20°C. Quotient represents mean uptake over three successive weeks. N=10 for the animals.

Species	20 °/oo	32 °/oo
Mya	-------	1.88
Mytilus	1.17	1.66

Mulinia	1.23	1.68
Nucula	2.30	---
Palaemonetes pugio	2.56	1.94
Codium	1.83	1.65
Ascophyllum	1.89	2.31
Fucus	1.22	1.21

The influence of temperature and salinity acclimation on cadmium uptake was initiated to determine whether previous environmental history affects uptake. Experimental organisms were acclimated at two salinities (20 and 32 °/oo) and two temperatures (10 and 20°C) for two weeks. These organisms were then placed in cadmium-dosed water at seven temperature and salinity combinations and the cadmium accumulation recorded. Acclimation did not appreciably alter the pattern or rates of cadmium uptake in Mya, Mulinia, and Codium. Acute transfer of Mytilus edulis from 20°C acclimation temperature to 10°C caused only 15% initial reduction in cadmium uptake relative to mussels acclimated and exposed at 10°C.

The interaction between sediment, test organisms, and cadmium added to the water was also explored. It is recognized that the behavior of burrowing organisms in a container without any sediment could be quite atypical relative to that of the field. This could influence metal uptake in a number of ways. Further, marine sediments are recognized to act as a reservoir of heavy metals which can be released by natural processes which alter the oxidized state of sediment surface layers. This was demonstrated in the laboratory by stirring cadmium-contaminated natural silty-clay sediments in an aerated beaker for 36 days. Approximately 26% of the bound cadmium was released back into the water.

Natural sediments were found to be highly variable with regard to cadmium uptake. Over 50 cores and sediment dredgings from Narragansett Bay and open coastal beaches were examined. The observed uptake ranged from 4.4 mg Cd/gm dry sediment in dark reducing sediments

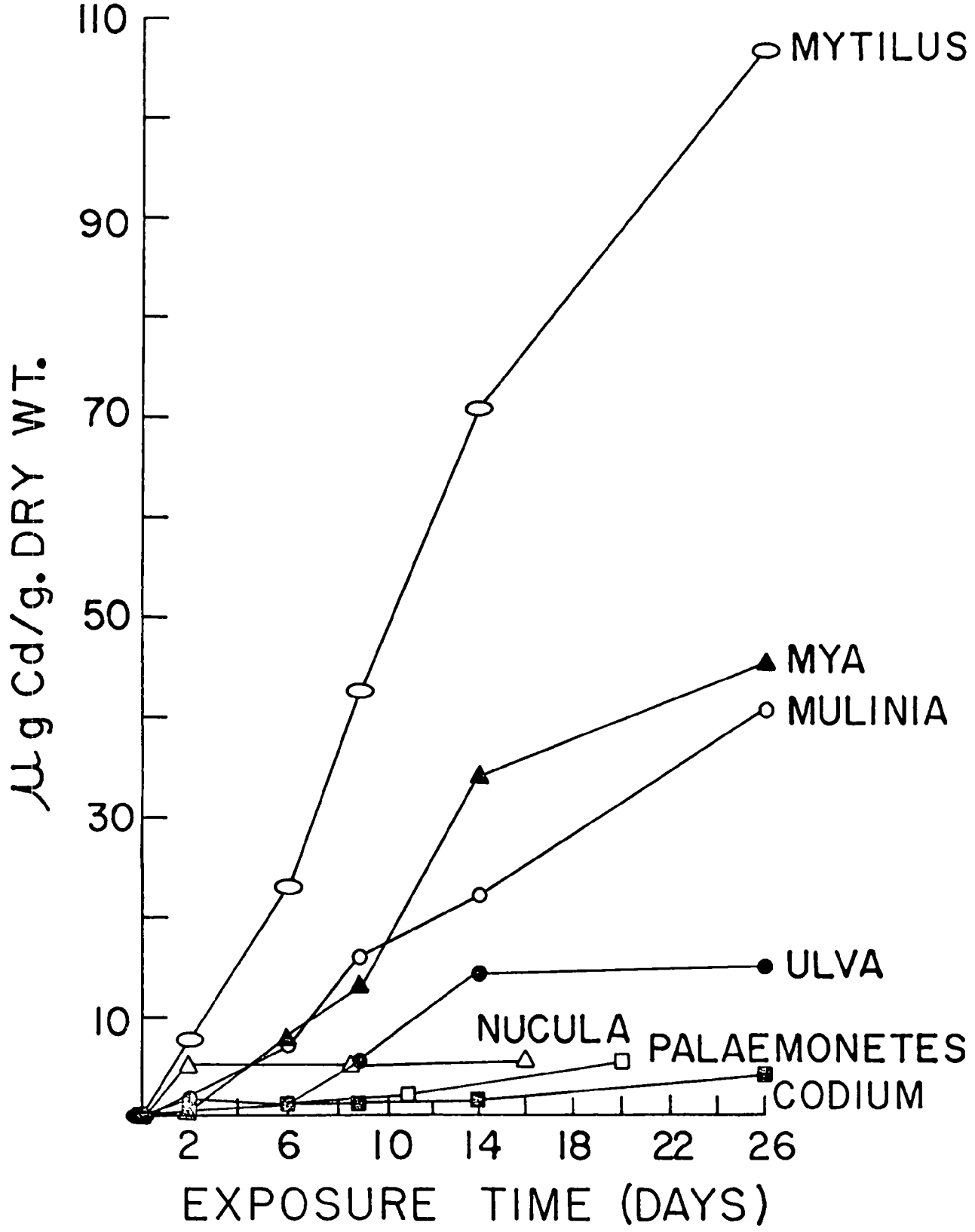

110
90
70
50
30
10
µg Cd/g. DRY WT.
MYTILUS
MYA
MULINIA
ULVA
NUCULA
PALAEMONETES
CODIUM
2
6
10
14
18
22
26
EXPOSURE TIME (DAYS)

FIGURE 1

to less than 1 mg Cd/gm in beach sand. Because of this variability, a standard synthetic sediment was made up which would have a predictable cadmium uptake capacity as well as some compatability for benthic organisms. After testing many mixtures, one consisting of diatomaceous earth containing 6% FeS was selected. FeS was included because of its presence in natural bottom sediments and its demonstrated affinity for cadmium.

Experiments with natural, artificial, and no sediment showed about 25% less uptake of Cd in Mulinia and Mya (filter feeding bivalves) when held in either sediment. This work is being continued with other types of organisms.

B. Effect of Cadmium on Juvenile Stages of Macroalgae (ROAP 21 AKF):

Task 069--Determine the influence of multiple environmental stress on marine organisms previously exposed to specific pollutants.

Little is known of the sensitivity of the early stages of macroalgae to pollutant stress. There is the potential of greater sensitivity of eggs and young sporophytes. Also such material is attractive as research material as a large number of specimens, all of the same developmental stage and representing similar genetic makeup are available for study.

Research has been undertaken to study the effect of toxicants, in this case Cd, on viability of eggs and sporophytes of Fucus vesiculatus. Techniques are being developed to elucidate which parts of plants collected in the field are most likely to contain fertile gametes. It appears that a turgor shock is necessary for expulsion of the eggs and sperm from their receptacles. The best method yet found for optimum production of gametes is initial wetting with distilled or deionized water, air drying for 15-45 minutes, and then reimmersion into seawater.

Optimum conditions are being determined for growth of zygotes

and juvenile stages as a preliminary to determining the effects of stressful conditions and toxic substances.

C. Temperature, Dissolved Oxygen and Salinity Requirements of a Marine Amphipod (ROAP 21 AKF):

Task 070--Development of biological criteria in support of legal standards for dissolved oxygen, temperature, and salinity, singly and in combination.

Mr. Neal Lackie is conducting a factorial study on the marine amphipod, Jassa falcata. The 24-hr. TLM was determined for a wide range of dissolved oxygen (D.O.), temperature and salinity conditions. Animals were acclimated to 20°C $\pm$ 1 and 30 °/oo salinity $\pm$ 2 for one week in flowing seawater. One hundred animals were run at each experimental combination in a static system which controlls temperature and D.O. Results are presented graphically for temperature vs. D.O. (Fig. 2) and temperature vs. salinity (Fig. 3). The mortality curve for most stress conditions is sharply sigmoid, which suggests death results from a single threshold phenomon. Temperature is limiting at 28 to 29°C when oxygen and salinity are at normal levels (i.e. D.O. at saturation and 32%). Further, there is no reduction of this limiting temperature when D.O. was reduced to 6 or 4 ppm, or salinity reduced to 20%. This suggests these lower oxygen and salinity levels do not exert any additional stress. When salinities and D.O. were reduced further, reduced thermal tolerance did result showing the effect of multiple stresses.

Two flow-through experimental systems controlling D.O. and temperature are being developed and tested for future factorial studies with small invertebrates. These systems will permit observation of motility, which will be used to indicate sublethal stress. Two different electronic systems are being considered to quantify motility.

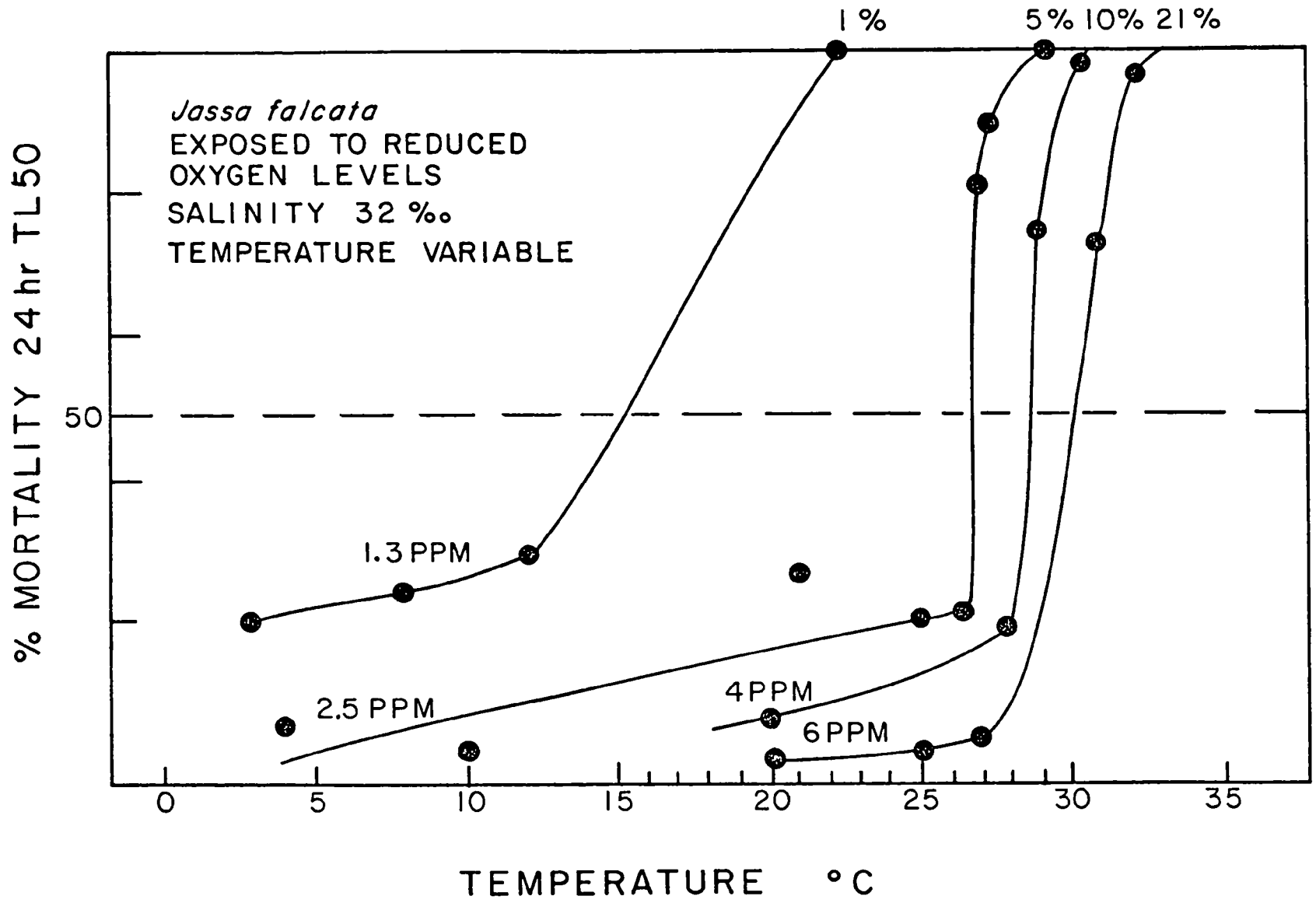

1%
5% 10% 21%
Jassa falcata
EXPOSED TO REDUCED
OXYGEN LEVELS
SALINITY 32 ‰
TEMPERATURE VARIABLE
% MORTALITY 24 hr TL50
50
1.3 PPM
2.5 PPM
4PPM
6PPM
0
5
10
15
20
25
30
35
TEMPERATURE °C

FIGURE 2

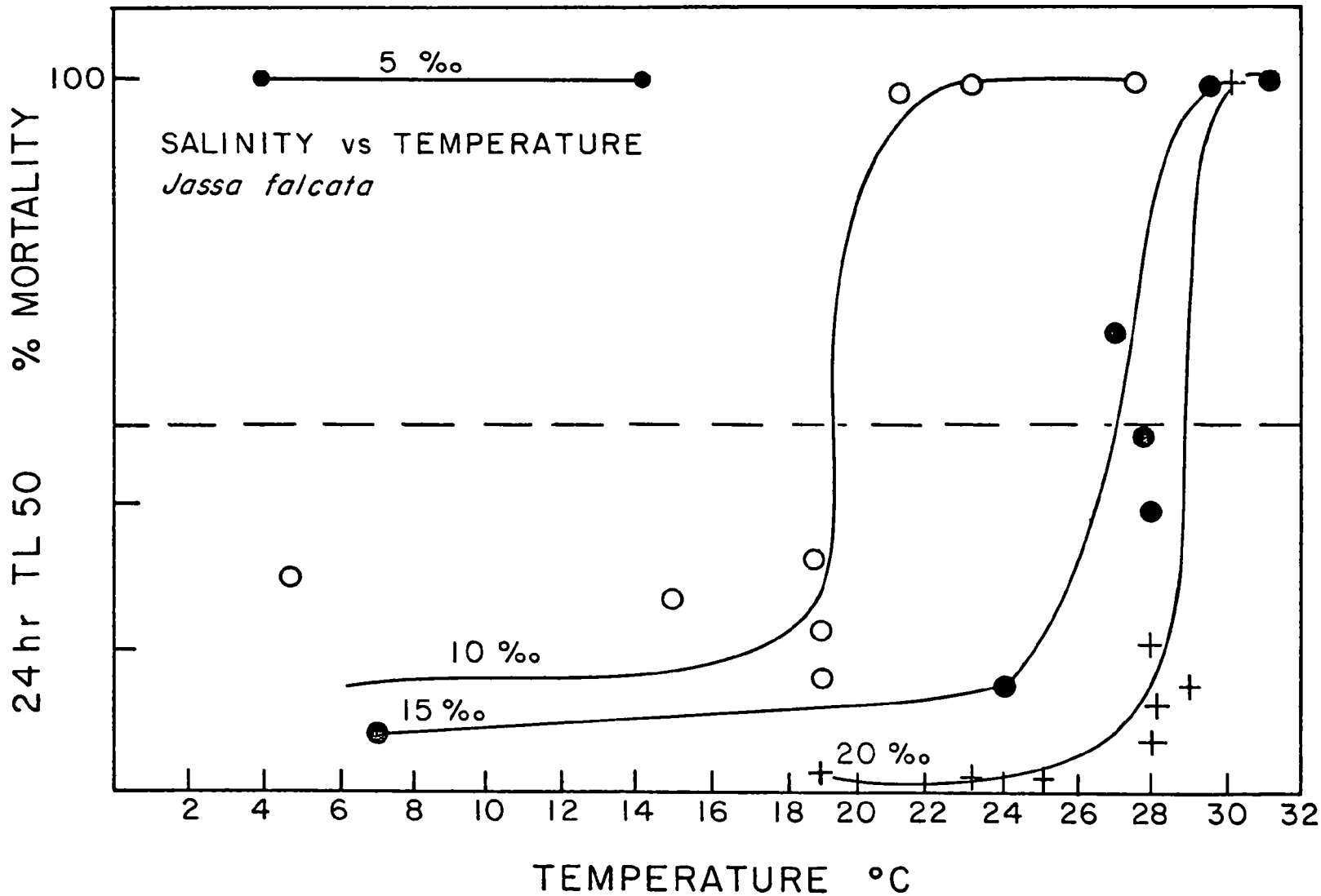

5 ‰
100
SALINITY vs TEMPERATURE
Jassa falcata
% MORTALITY
24 hr TL 50
10 ‰
15 ‰
20 ‰
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
TEMPERATURE °C

FIGURE 3

D. Influence of Reduced Dissolved Oxygen and Salinity on Growth in Marine Fishes (ROAP 21 AKF):

Task 070--Development of biological criteria in support of legal standards for dissolved oxygen, temperature, and salinity, singly and in combination.

A number of investigators have shown various physiological processes of fishes to be highly sensitive to alterations in environmental conditions. For instance, rates of growth of laboratory-held fishes is impaired by decrease in D.O. concentrations to levels only slightly below saturation. Reduction in growth rates have also been correlated with changes in levels of salinity beyond the "optimal" range of the variable. Effects of each of these two factors on growth of fishes have been studied independently of one another. Surprisingly, no information is available to describe the impact of both factors acting in concert despite the influence of each on energy expenditures associated with osmoregulation. Consequently a project was initiated by Dick Voyer to study the combined effects of D.O. and salinity on growth patterns of marine fish. The working hypothesis of the project is that maximal growth occurs at a salinity isotonic with blood of the fish since minimum osmotic work is required at that point.

The experimental system developed for the project is presented schematically in Fig. 4. Basically, the system can provide several levels of salinity by combining of seawater and tap water in varying proportions, resulting in final solutions of seawater at 10, 20 and 30 °/oo salinities. Overall three contrasting D.O. concentrations for each of the salinities are provided by bubbling nitrogen gas upward against the flow of water downward through stripping columns.

The system was initially tested with juvenile Atlantic silversides (Menidia menidia). A total of 540 fish were exposed to 9 D.O. and salinity combinations, with three replicates per level. Test

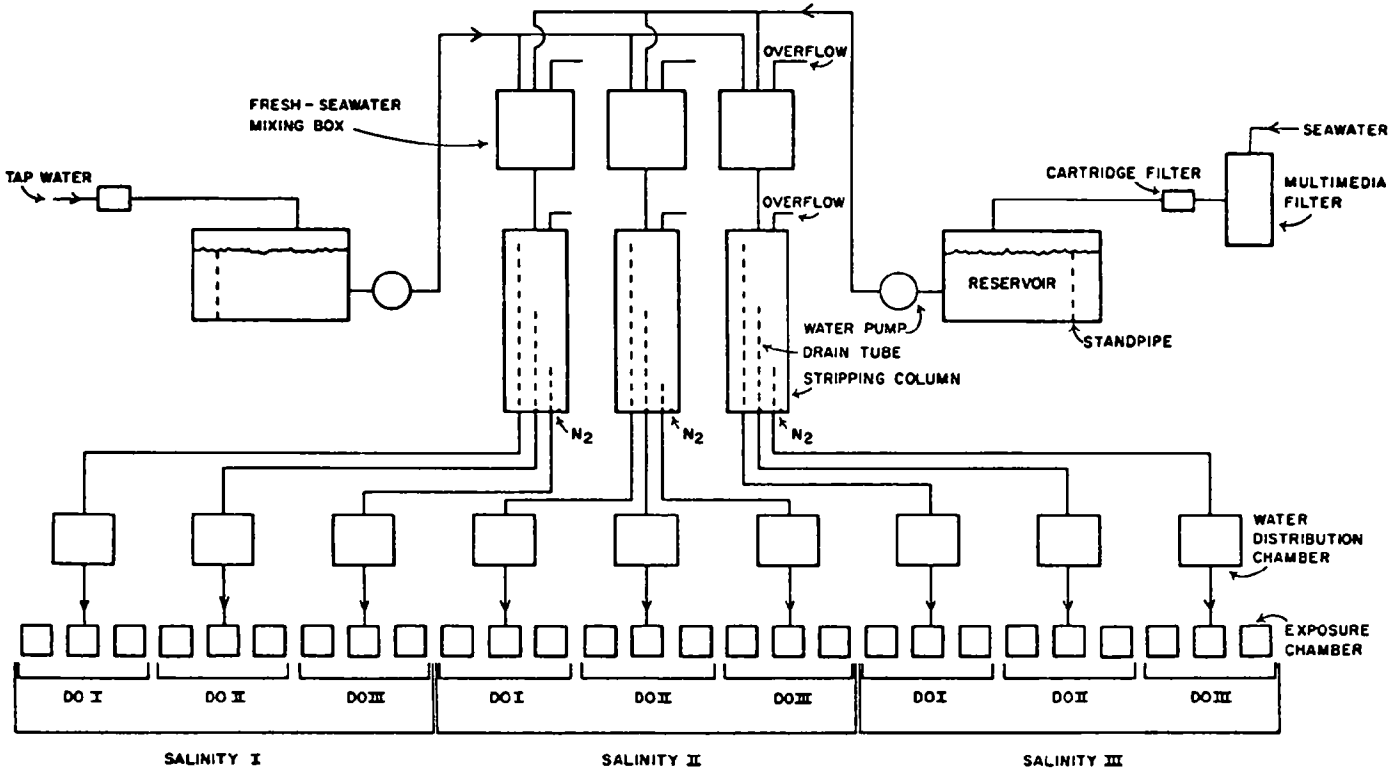


FIGURE 4. Schematic of experimental apparatus

levels of salinity were 10, 20, and 30%; for D.O., 4.0, 5.5 and 7.7 mg/l. Fish were sampled at regular intervals over a 52-day test period, with 9 fish randomly selected from each replicate and liver-body weight ratios, RNA-DNA ratios, and the relationship between body weight and body length of each fish estimated. Analytical assistance was provided by Drs. S. Cheer and E. Jackim.

No clear-cut effects of D.O. and salinity stress were discerned at these exposure levels. Both liver-body weight ratios and RNA/DNA ratios were highly variable and inconclusive. Body length-body weight relationships of all fish remained constant throughout the test. Mathematically the curve can be described by the equation, $\hat{y}=2.53x-3.49$. It was expected alteration in growth patterns would be reflected in difference in slope coefficient and ordinate intercepts.

Randomness of test results signifies that, if RNA-DNA and liver-body weight ratios are to be used as valid response parameters, large sample sizes (larger than 9 in case of silversides), will be necessary. However, in studies based on factorial designs, such as the one reported here, large samples may pose a problem in view of extensive replicates required in this type of study.

A cooperative study with the Culture Team on effects of salinity and D.O. concentration on development of eggs and larvae of winter flounder was subsequently initiated using this experimental system. The general purpose of the study was to assess effects of various combinations of D.O. and salinity on hatchibility, growth and survival of selected stages of winter flounder. Results will be of value in establishing oxygen-salinity optima for culturing the species and in elucidating oxygen-salinity requirements of the species.

A continuous flow system was developed and attached to the oil-exposure system managed by the Oils Team to study effects of continuously renewed oil solutions on embryological material, larvae and small

invertebrates. Embryos of the winter flounder (Environmental Effects Team) and polychaete worms (Bioassay Team) are presently being exposed. With regard to the flounder embryos, response parameters selected for study include hatchability of embryos, larval size, and yolk sac volume.

Plans for a system permitting delivery of toxicant solutions on a continuous basis to test chambers were provided to the Bioassay Methods Team. The apparatus outlined is now being put to use.

E. Effects of Power Plant Thermal Discharges in Tropical Waters (ROAP 21 AKF, Task 070).

Dr. Juan Gonzalez is conducting research to evaluate the effects of thermal discharges on biota of the south coast of Puerto Rico. Field studies have been initiated at a newly operating power-plant at Guayanilla. The thermal plume is being mapped in the cove affected, and the hydrography and biota of this immediate region described. The copepod, Acartia tonsa is found in the heated water, but it appears to be maintained there primarily by recruitment from adjacent coastal waters. During summer months, cove temperatures exceed maximum levels permitting growth and reproduction.

One of the most common macro-invertebrates inhabiting the anoxic mud of the mangrove zone in this region is the mud clam, Phacoides pectinatus, a mollusc used for food by local fishermen. This bivalve is found in the mud at a depth of between 25 to 45 cm. During the course of this research the temperature in their habitat has been between 25.4°C to 29.5°C; the oxygen concentration has ranged from 0 to 1.9 ml/l; pH values have varied from 6.8 to 7.5; and salinity from 31.2 to 35.4 °/oo. The physical and chemical characteristics of the water overlying the mud are different to those in the mud proper (Table 3). For instance, oxygen is in higher concentration and salinity is lower. However, we suspect water temperature

Table 3 Physical and chemical factors associated with the mangrove-mud environment in Parguera, Puerto Rico

	October			November			December			January		
	Water	Mud	Tube	Water	Mud	Tube	Water	Mud	Tube	Water	Mud	Tube
ml/l O_2	2.6		1.5	2.5		0.2	2.6		0.1	3.8		0
Ph	7.8	7.5	7.8	7.9	7.4	8.0	7.9	7.3	7.9	7.8		7.9
+°C	29.5	29.5	29.5	27.4		27.5	27.0		26.8	24		26
S‰	31		35.4	30.8		31.2	32		32.5	34		35

to be more variable throughout a 24 hr period based on a concurrent temperature survey being undertaken in another, but similar, area.

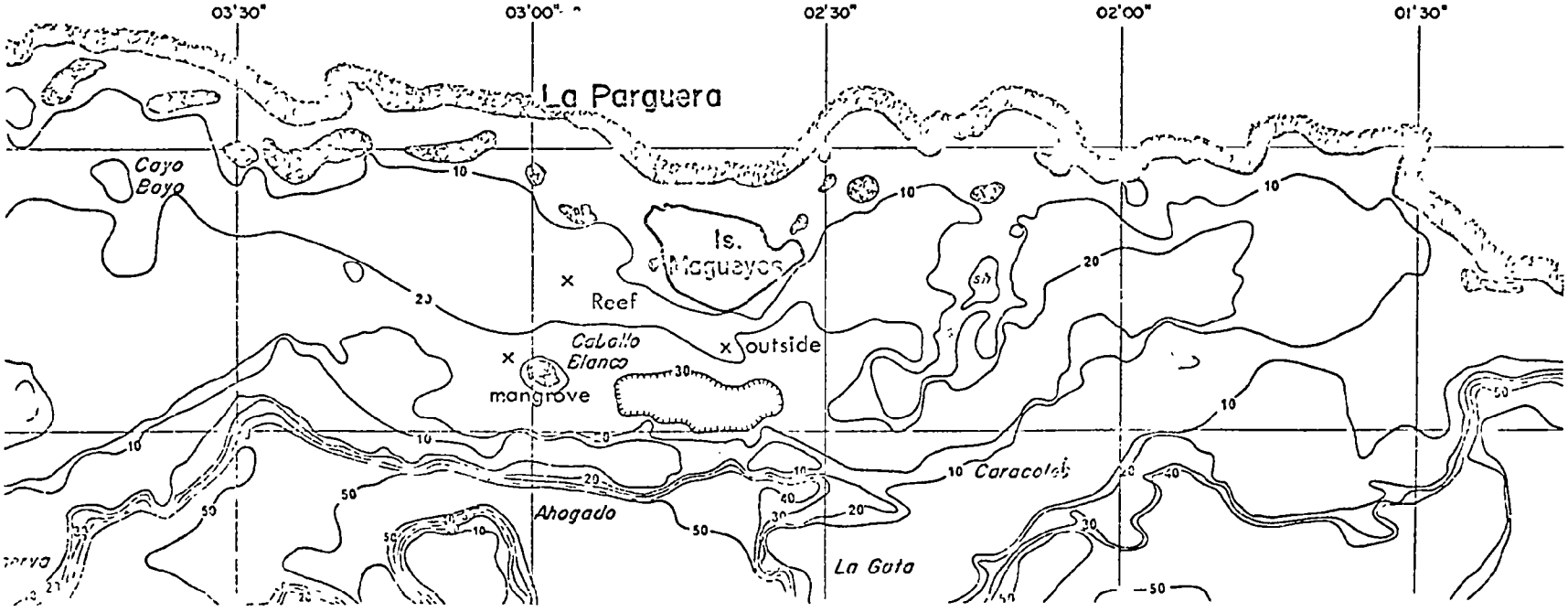
Another benthic species being studied now is the mangrove oyster Crassostrea rhizophorae. This species, although strongly associated with mangroves, has a habitat different from that of the mud clam. Crassostrea lives attached to mangrove roots at the intertidal zone. It is a filter feeder and, therefore, is not directly dependent on the detritus coming from mangroves. However, it depends on recycled nutrients from the mud which keep phytoplankton populations available.

Temperature has been monitored hourly over a 24-hour period monthly at 3 coastal stations since last November. Station 1 (Fig. 5) is on a reef that gets exposed during low tide. Consequently, when lows occur at night during the winter, the temperature decreases to values close to that of air temperature. The lowest temperature recorded during this period was 21.8°C. When low tides occur during day time in the summer the temperature raises to values above air temperature due to the effects of radiation. The highest temperature recorded last summer during one of the 24-hour stations was 31.5°C in one of the shallower spots; this was in March 1974. Reports of higher temperature have been published elsewhere by Glynn (1968). The diurnal temperature range of the reef water is often as great as 5 to 7°C.

Station 2 is located off the influence of reefs and mangroves. There the temperature is more stable throughout the 24-hr period. Temperature has ranged between 24.9 in the winter to 29.4 in the summer.

Station 3 at the mangrove islet. Here the temperature oscillates considerably during the 24-hr period and throughout the year. The temperature has ranged from 24.9 in the winter to 30.8 during the summer. Oysters are exposed to such a range of water temperatures,

FIGURE 5. Area in southwest coast of Puerto Rico where 24 hr stations are occupied once a month



as well as being exposed to a wide range of air temperatures during low tides.

One of the results of high temperatures on the reefs is the "browning" of Thalassia plants, to the point that only the "rhizomes" are left. Many sea urchins, including Lytechinus variegatus and Tripneustes esculentus are killed during summer low tide periods. Another sea urchin, Diadema antillarum "walks out" of these conditions during day time.

The thermal tolerance limits of two bivalves common to this coastal zone were determined in the laboratory.

1. The clam, Phacoides pectinatus, was collected and maintained in holding "cages" near the mangrove forest located close to the marine field station of the University of Puerto Rico. Subsequently, three or four dozen clams were brought to the laboratory and held in a tank with sufficient aeration. A thick concentration of Chlorella sp was dispensed to each tank for food. Temperature in the tanks varied from 19-21°C during a holding period of one week.

The upper thermal tolerance for this clam was examined by acute exposure of 20° $\pm$ 1 acclimated animals to 28 and 35°C. Only 25% mortality occurred in the 28° group within five days. At 35°C, the TL_{50} occurred in 62 hours and all clams died by 129 hours. Mean shell length of these experimental animals was 6.38 cm. Another group of smaller clams (5.6 cm mean shell length) exposed to 35°C, had a only slightly shorter TL_{50} of 60 hours. At 40°C, TL_{50}=3.5 hrs; at 43°C, TL_{50}=2.5 and 3 hours in two replicate runs.

2. Similarly, over 200 oysters, (Crassostrea rhizophorea) were picked from the roots of the red mangrove and held in wire "baskets" in the mangrove forest near the marine station. Several dozen were brought to the laboratory and held in a room at a temperature of 19-20°C. Aeration was provided and Monochrysis lutheri

was supplied as a food source.

Thermal tolerance studies were followed the same routine as for mud clam. Oysters acutely exposed to 35°C showed a TL_{50} at 72 hrs of exposure. At 40°C, the TL_{50} occurred in 35 hours; at 43°C, at 2 3/4 hours.

This work will be repeated after acclimating the animals to temperatures comparable to those of their habitat in order to determine if they are able to compensate (shift physiologically) to higher temperatures.

F. Extramural Research Supporting Environmental Effects Studies

1. ROAP 21 AKF, Task 031 - Develop biological criteria for NE crustacea in support of legal standards for temperature 800981.

Grant to Dr. A.N. Sastry, University of Rhode Island--Effect of Thermal Pollution on Pelagic Larvae of Crustacea (D. Miller, Project Officer)

Larvae of five benthic crustacea from New England region, Homarus americanus, Cancer irroratus, Palaemonetes pugio, Rhithropanopeus harissi and Pagurus pollicaris have been cultured and their best temperature and salinity conditions for survival have been determined. The optimum combination for larval development of each species are: 30 °/oo and 15 and 20°C, H. americanus (spring); 30 °/oo and 15°C, C. irroratus (spring); 30-35 °/oo and 25°C, P. pugio; 25 o/oo and 25°C, R. harissi and 30 °/oo and 25°C, P. pollicaris. The optimum combination for larvae of the two subtidal species shifts for summer hatches increasing from 10-20°C to 25° in H. americanus and 15° to 20°C for C. irroratus. Detailed studies on the development and survival of larvae of C. irroratus and H. americanus under cyclic temperature regime are in progress.

Tolerance of larvae to acute temperature and low dissolved oxygen has been determined for C. irroratus, H. americanus and P. pugio. Larvae of estuarine species proved to be more tolerant

than those of subtidal species. Variation in the tolerance of different larval stages within each species was also observed. Metabolic-temperature response patterns have also been determined for C. irroratus and P. pugio larval stages. Larvae of the subtidal species are relatively stenothermal and showed significant shifts in the pattern of response during development. The relatively eurythermal larvae of estuarine species showed no such shift in their metabolic temperature response patterns although the upper temperature limits have shifted to more normal temperature (30-35°C) for the later stages.

ROAP 21 AKF, Task 035--Determine effects of temperature and current on survival and behavior of larval marine and estuarine fishes. #R-801032. Grant to Dr. R. Stevenson, Univ. of Miami (Fla.).

Testing of larval fishes occupied the period from June through December, 1974. A total of 450 Archosargus rhomboidalis (sea bream) and Cynoscion nebulosus (spotted trout) were tested for their ability to swim for one hour against current velocities of 1.0, 2.5, and 5.0 centimeters/second. Tests were run at ambient temperatures and four degrees above (26 and 30° for Archosargus and 28 and 32° for Cynoscion). These species were tested during the period that they were naturally spawning in the area. Larvae were sacrificed, dried, and weighed after testing.

As yet, there are insufficient data to make statistically valid statements about the effects of temperature and current on the swimming ability of larvae. Attempts to relate the data in a number of ways have not yet shown significant trends. Analyses by length classes and weight suffer because not enough data points are present in each class. The data show high variability both within and among classes of larvae. Although rearing methods were standardized in so far as possible, large batch differences were seen in swimming stamina of the larvae. It appears that such culture problems must be resolved

before swimming stamina can be used effectively as a method to evaluate sublethal stress.

ROAP 16 AAT, Task 007--Grant for study of toxicity of metals in larval arthropods 801305

Grant to Dr. J.D. Costlow, Jr. Duke University (D. Miller, Project Officer)

Efforts this period have continued to consider which stages in the larval development of the xanthid crab, Rhithropanopeus harrisii, are the most sensitive to exposure to sublethal doses of mercuric chloride. The response to mercury of the megalopae from different mother crabs proves to be replicable. However, a considerable degree of variability has been noticed in the response to mercury of the more sensitive zoeal stages with various genotypes. Further replications, in which descreet developmental stages were subjected to several different sublethal levels of mercury, suggest that the ecdysis period between the fourth zoeal stage and the megalopae represents the more sensitive time in the development of the crab to mercury exposure.

Work on the protein complements of control and mercury exposed larval crabs has been continued. No differences have yet been found in control and experimental animals at exposure levels lower than those producing obvious morphological or viability changes.

Study began on the effects of mercury on the oxygen-transporting protein, hemocyanin. The oxygen-binding properties of hemocyanins from Callinectes sapidus and Limulus polyphemus as a function of pH were measured. The effects of varying amounts of sodium chloride on the oxygen affinity are also being explored. Initial observations indicate that sodium chloride will have a significant regulatory effect and that chloride ions are the active component. The effects of varying amounts of mercury will be studied next.

II. Response Parameter Development

A. The Sub-Lethal Effects of Toxicants on the Schooling Behavior in Juvenile Menidia menidia (ROAP 21 AKF): Task 072

Experiments to determine the sub-lethal effects of toxicants on the behavior in the silverside, Menidia menidia, were continued through this fall by K. Koltes Robinson. The experimental system, fully described in the previous semi-annual report, consists of four circular chambers, 2 1/2 feet in diameter by 6 1/2 inches deep, each with a 12 inch opening to a central chamber.

After investigating the effects of 5 sub-lethal levels of cadmium on group behavior in M. menidia during early summer, difficulties arose in determining base-line behavior in subsequent studies. Schooling bonds attenuated during control studies which had not occurred with schools used during the winter and spring. This could be attributed to one or both of the following factors. There may be a seasonal variability in the schooling bond, similar to that found in birds, operates in these fish. This is manifest by a tight, organized school during the colder months giving way to a loosely structured aggregation during the warmer months. Breder (1959) and Atz (1953) have reported the occurrence of this seasonal variation in other schooling fish. Breakdown of schooling bonds can also occur with prolonged laboratory holding, as reported by McFarland and Moss (1970) with northern anchovies. Yet this was not observed in a school of M. menidia held for an extensive period during the winter months, lending support to the idea of a seasonal variability. None the less, the experimental procedure for the present study was altered to accommodate the possibility of a laboratory artifact. Thus, during the later summer months as the school became increasingly more dispersed, experimental fish were held not more than 48 hours after collection. The period for behavioral accommodation for a school after transfer into the experimental system was also shortened

to two hours. This appeared sufficient for the fright response to disappear. If the fish were not swimming normally through the tanks, a longer acclimation period was allowed.

Data is collected according to a Markovian Chain whereby a measurement of the position of the school is recorded every 10 seconds. Since the nature of the schooling bond is of particular concern, a "school" is defined as not exceeding 1/2 the area of any one tank. A normal school is usually well within these limits. The same fish are used for the control and experimental run, each of which is of 30 minutes duration. A short interval between these two runs is required for the system to come to equilibrium with the toxicant - about 10 minutes. Transition probabilities are then computed for each matrix and the two matrices compared. At least five replicates at each concentration level are run. At the completion of an experiment, a sample of water is withdrawn from each chamber and the toxicant concentration is verified on a Perkin-Elmer Atomic Absorption Spectrophotometer. The fish are removed and the entire system is cleaned with a 5% solution of nitric acid followed by rinsing with de-ionized water.

Studies of 5 sub-lethal levels of cadmium (15 ppb - 1 ppm) and 5 levels of copper (50 ppb - 1 ppm) have been completed, but the statistical analysis of this data is still in progress. Preliminary results have indicated detection of at least 30 ppb cadmium by schooling fish. In contrast, Menidia appeared less sensitive to copper, with 100 ppb Cu required to produce a mild stress response.

Generally, Cd caused the school to be dispersed in about 10 minutes after introduction of the toxicant. Individual swimming speed also slowed. These studies were conducted in the colder months when normal responses to stress situations, such as fright, resulted in tightening the school. Hence, breakdown of the schooling bond upon exposure to cadmium is considered an atypical behavior. School

response to copper was of a different nature. Rather than a disorganized slowing of movement, the fish responded by increasing their speed in what appears to be exploratory behavior. This response would clearly be of adaptive value in that it would contribute to the fish leaving a contaminated area more rapidly by orthokinetic analysis. Cadmium on the other hand, appears to elicit a non-adaptive response. These studies with cadmium and copper should be repeated during the same season to consider whether seasonal variability in schooling could have influenced the contrasting behaviors observed upon exposure to these two metals.

B. Development of a method for studying the behavioral responses of larval and juvenile fish to toxicants (ROAP 21 AKF):
Task 021--Develop and modify technology necessary for verifying physiological state, nutritional state, histopathology, and recent history.
Task 072--Select parameters for detection of response on chronic exposure, changes in which could alter ability to compete and survive in an ecosystem. Develop and evaluate techniques for measuring response of selected organisms.

Experiments were conducted by Dianne Everich to develop a method for assessing the effect of cadmium on the swimming behavior of juvenile Menidia menidia. Swimming speed was measured while fish were exposed to 2 sublethal cadmium concentrations: 10 and 100 ppb. Controls were in filtered sea water. Fish were transferred from the rearing tanks into 4 circular polyethylene tanks, 5 fish per tank. Each experimental tank had a grid of 2 cm squares drawn on the bottom and contained 1 liter of toxicant solution in filtered sea water. The tanks were housed in an environmental-control box to reduce noise and control water temperature and photoperiod. Observations were made directly or with a closed-circuit video system 1 hour, 24 hours and 48 hours after transfer of fish to the observation tanks.

Cadmium dosing was initiated at the time of transfer. Swimming speed was estimated by counting the number of 2 cm grid lines crossed during a 15 to 30 sec. time period.

The results of two preliminary experiments are summarized in Table 4 and Figure 6 and 7. Fish size was similar between test groups of each run, but not between runs. This precludes comparison of these two experiments, for size variations lead to marked differences in mean swimming rate. Differences in swimming speed of Cd exposed fish vs. controls was tested for significance with "Students" t-test. In each experiment, there was no significant difference between controls and 10 ppb Cd fish after 24 or 48 hours. Fish exposed to 100 ppb did show a reduction in swimming speed. In Run #1, there was about 32% reduction after 24 and 48 hours. In #2, a 17% reduction was observed after 24 hours exposure; 22% by 48 hours.

C. ATP Analysis Methodology (ROAP 21 AKF): Select parameters for detection of response on chronic exposure, changes in which could alter ability to compete and survive in an ecosystem. Develop and evaluate techniques for measuring response of selected organisms.

Dr. Sue Cheer has been conducting an extensive literature search concerning the applications and improved methodologies for ATP analysis. The major emphasis of this search was on potential techniques for the analysis of ATP in marine sediments. A bibliography of relevant publications is now available. This survey is being continued. In addition, experiments were carried out to verify that some organisms (algae) are subject to lysis and subsequent loss of ATP when improperly sampled. Also, it was verified that room temperature, pre-extraction thawing of quick-frozen (dry ice or liquid nitrogen) filtered field samples resulted in no loss of ATP for at least five minutes. Findings from these studies and the current literature review were utilized in an ATP Techniques Short

TABLE 4. Swimming speed (cm/sec) of juvenile Menidia menidia exposed to cadmium (mean ± standard error).

Experiment	Cd^{++} Concentration	Exposure Time (hrs)		
		1	24	48
1	control	0.67 ± 0.05	0.72 ± 0.04	0.93 ± 0.06
	10 ppb	0.80 ± 0.06	0.81 ± 0.04	0.88 ± 0.04
	100 ppb	0.61 ± 0.13	0.49 ± 0.04	0.62 ± 0.08
2	control	0.73 ± 0.05	0.66 ± 0.03	0.82 ± 0.03
	10 ppb	0.02 ± 0.07	0.69 ± 0.03	0.76 ± 0.05
	100 ppb	0.86 ± 0.05	0.53 ± 0.02	0.64 ± 0.02

FIGURE 6

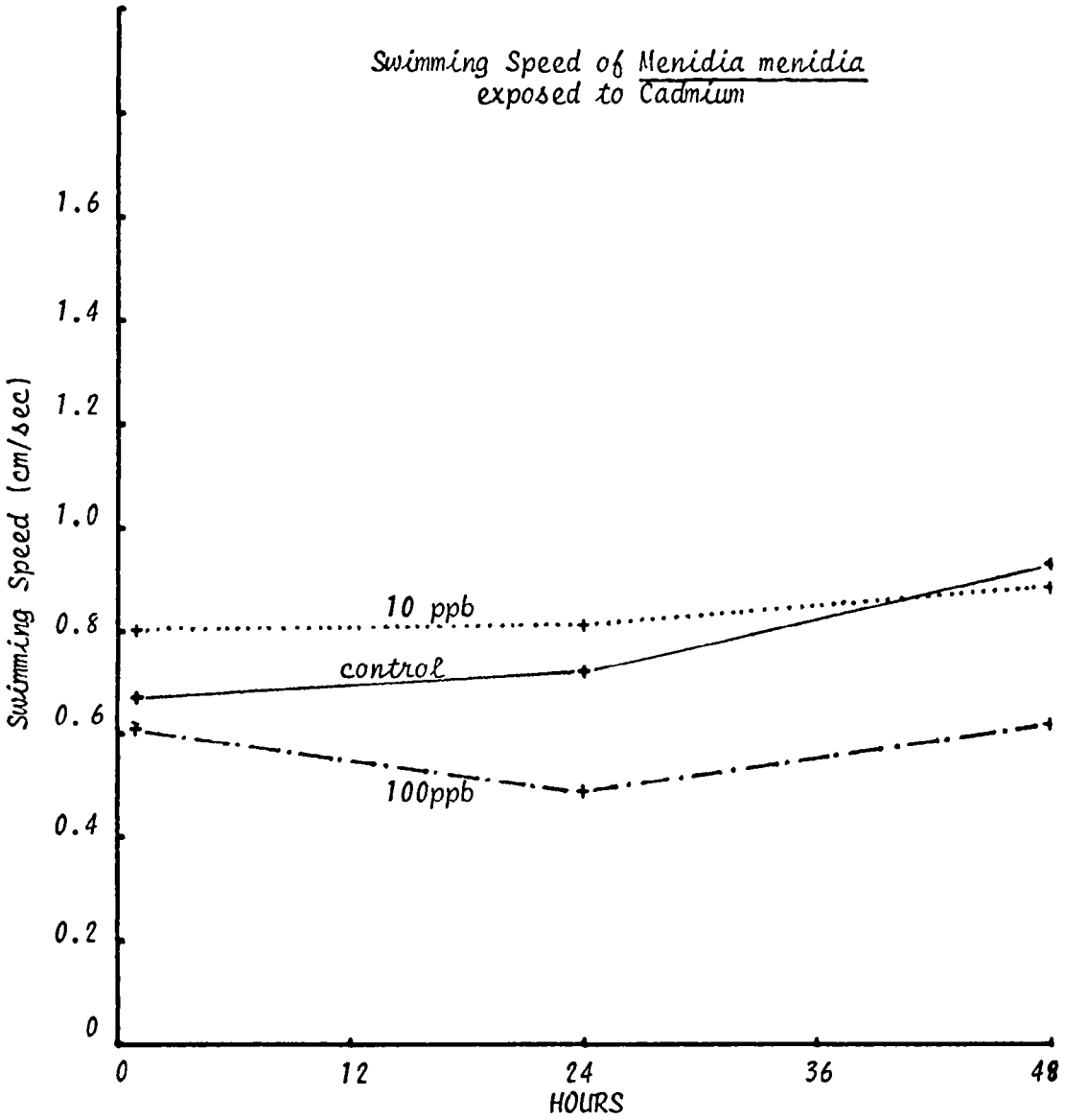
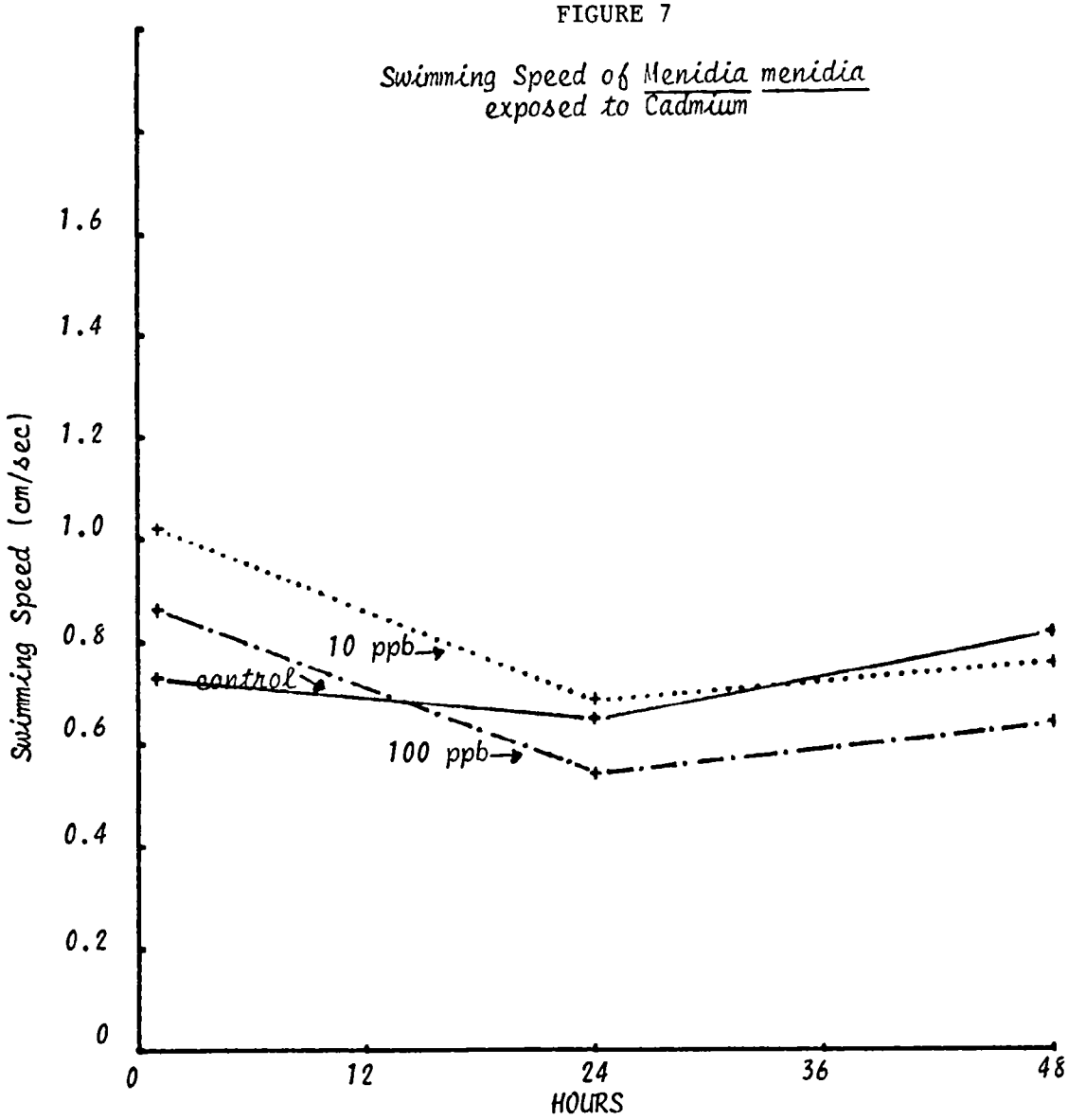

Swimming Speed of Menidia menidia
exposed to Cadmium
Swimming Speed (cm/sec)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
10 ppb
control
100ppb
0
12
24
36
48
HOURS

FIGURE 7


Swimming Speed of Menidia menidia
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1.6
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0.6
0.4
0.2
0
10 ppb
control
100 ppb
0
12
24
36
48
HOURS

Course taught in Denver (NFIC) by Drs. Cheer and Gentile in November.

D. Extramural Research Supporting Response Parameter Development.

1. ROAP 21 AKF, Task 027--Develop and modify technology necessary for verifying physiological state, histological history, and environmental history through research grant (Neomysis americana, Acartia tonsa, Uca pugnax, Menidia menidia). Grant to Dr. S. Barbar, Lehigh University (D. Miller, Project Officer).

All work is now focused on the effects of temperature and cadmium exposure, singly and in combination, on the mysid shrimp, Neomysis americana. Research has progressed in three areas of mysid biology: natural history, laboratory maintenance and culture, and adult histology. Biweekly field sampling for a full year has contributed information on such aspects of population dynamics as abundance, size distribution, sex ratios and reproductive conditions. Hourly collections over complete tidal cycles has contributed information on tidal migrations. Problems of extreme sensitiviey to mechanical damage in transport and handling were overcome. In the laboratory, best survival was obtained in continuous flow trays. Studies to elucidate a good laboratory feeding regime and optimal temperature conditions are underway.

Histological studies of serial sections of the mysid were undertaken to provide an understanding of normal structural details. Three methods of fixation have been explored for electron probe analysis in order to locate and quantify cadmium uptake by the mysids. Other methods to be employed to localize cadmium is histochemistry and autoradiography.

2. ROAP 21 AKF, Task 024--Development of biomedical procedures for characterizing physiological state of test organisms 800831. Grant to Dr. Donald Horton, TRIMGOM, Portland, Maine (E. Jackim, Project Officer).

Studies relating hematological changes to temperature changes in winter flounder were completed. Several differences in the hematology and cardiac response between naturally summer acclimatized (c.a. 15°C) fish and 20°C stressed fish were apparent.

Whole animal respiratory responses of fish exposed to Cd are also being assessed.

Electrophoretic studies on fish blood are being developed to the point at which quantification of individual proteins can be realized.

3. Task 040--Vital microscope development, software modification. Grant to Dr. J.O.B. Greaves, Southeastern Massachusetts University, North Darmouth, Mass., "The Development of an Interactive System to Study Sub-lethal Effects of Pollutants on the Behavior of Organisms." (D. Miller, Project Officer.)

This project is for development of a system for the acquisition, analysis and display of behavioral data from both micro-and macroforms. The system includes a video-to-digital processer to convert images of organisms to a reduced information form readable by a digital computer. This device is now being interfaced to other system components, which include a 16-bit minicomputer. Software development, which is progressing as scheduled, is in FORTRAN to provide a system which is as machine independent as possible to enhance system exportability to other laboratories.

III. Team Publications:

Jackim, E. 1974. Enzyme Responses to Metals in Fish. In "Pollution and Physiology of Marine Organisms" Edited by John and Winona Vernberg. Academic Press, N.Y.

Miller, Don Curtis and Allen D. Beck, 1975. Development and applications of criteria for marine cooling waters. In: Symposium On The Physical And Biological Effects On The Environment Of Cooling Systems And Thermal Discharges At Nuclear Power Stations, Int'l Atomic Energy Agency. (in press).

Miller, Don C., Workshop Participant for "Behavioral Bioassays", in Marine Bioassays, Workshop Proceedings, Marine Technology Society, 1974.

We are attempting to define the limits a total system can be disturbed such that when the disturbance or stressor is relaxed the system will return back to its original structural and functional state. The approach being used is the microcosm method i.e., a miniature of the real world ecosystem is stimulated physically in the laboratory. Such a miniaturization is possible for a marine system because (1) most of the biotic components are small and (2) it appears simple i.e., there are only two major elements interacting together, a benthic phase and a pelagic phase. The types of stressors and disturbances used in the study are artificial sewerage and various biotic manipulations (e.g., species removals, additions, etc).

To date, we have made one "dry run" to test (1) how well our physical system mimics the real world and (2) how the biotic portion of the system responded. It was found that number of modifications in the system had to be made and that original computations for simulating certain conditions were incorrect. Some of the modifications, computations and results of this first run are described below.

Initially, the imposed light levels and spectra chosen for the microcosms were based upon the following conditions and assumptions:

(1) conditions -

a. average depth of Narragansett Bay is 9 meters

b. almost complete oxygen water saturation of the bottom layer and only 1-2°C difference between top and bottom

c. given extinction coefficients (k) and mean daily and monthly surface radiation levels (I_m)

(2) objective-wanted average intensity and spectral composition found in Narragansett Bay for a uniformly mixed water column of 9 meters for a given season of the year.

(3) assumptions -

a. because of $(1)_b$, assume mixing between top and bottom is complete and fairly rapid.

b. rate of exchange between top and bottom and, therefore, the rate of light exposure is much less than the rate of change in light intensity with season.

c. rate of change in light intensity over 9 meters of water has no biotic effect.

Using the above, we computed the daily average light intensity/month, $\bar{I}_m$ with the formulation $\bar{I}_m=(I_m/kz)\ (1-e^{-kz})$. The seasonal $\bar{I}_m$ values for k=1.0, a predetermined extinction coefficient, are presented in Figure 1. We did not have extinction coefficients for different intensities/wave length so that it was not possible to make the same computation for the average spectral composition in a uniformly mixed 9 meter water column as done for light intensity. Instead, we calculated the depth ($z\bar{I}_m$) at which $\bar{I}_m$ would occur in a 9 meter water column and then compared the known spectra for coastal waters at that depth to the light spectra used on the microcosms (Fig. 2). Since the longer wave lengths and ultraviolet components would be attenuated rapidly and absorbed in the surface, respectively, the "cool-white flourescent" bulbs were quite satisfactory. We, therefore, assumed that the average spectral composition in a uniformly mixed 9 meter water column was the same as the spectral composition at the depth computed above for the average light intensity, $\bar{I}_m$. It was found that such a light regime was too high. A major bloom occurred seven days after the water was initially added to the 12 microcosms. The resulting die-off was too great an organic load for the benthic system to handle which brought about anaerobic conditions. Direct measures are now being made to correct this problem.

All the microcosms were mixed equally with a rotating plastic shaft. After comparing the dissolution rates of plaster-of paris blocks and "sour-ball" candy in Narragansett Bay to three of our microcosms, it was found that the former was approximately two times higher than the latter. The necessity for obtaining equivalent mixing rates other than for water column motion is that gas diffusion

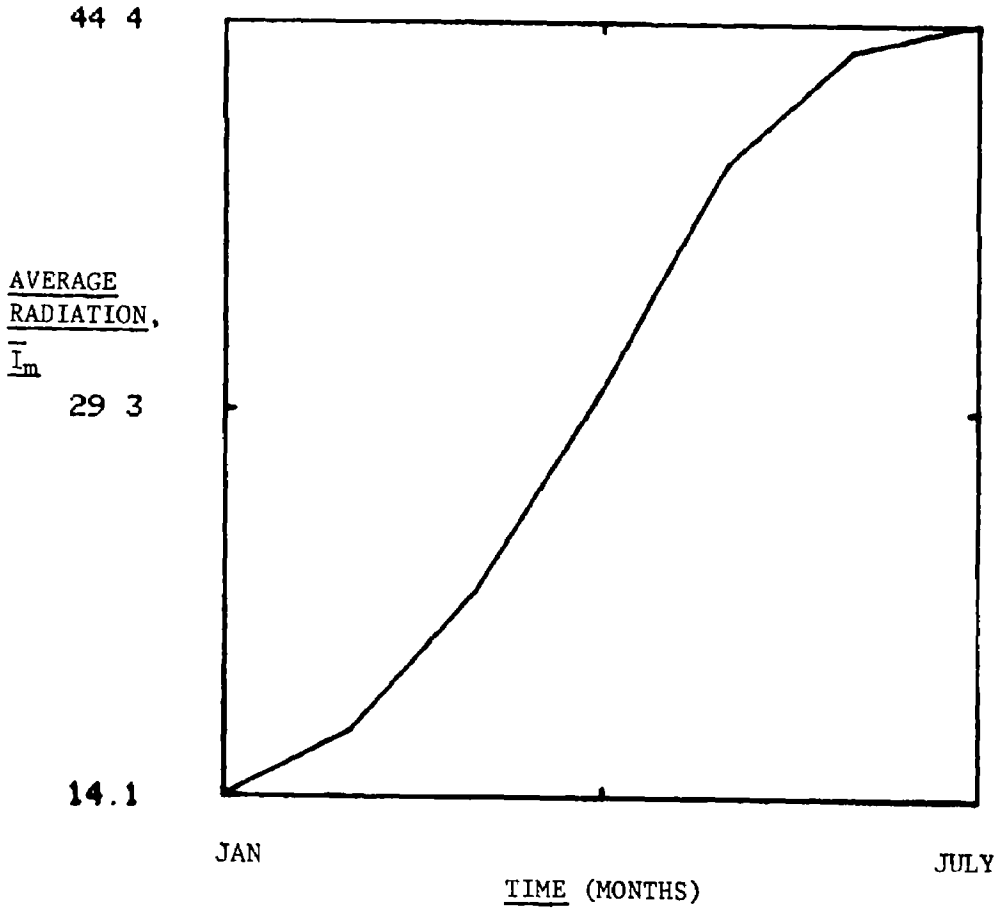
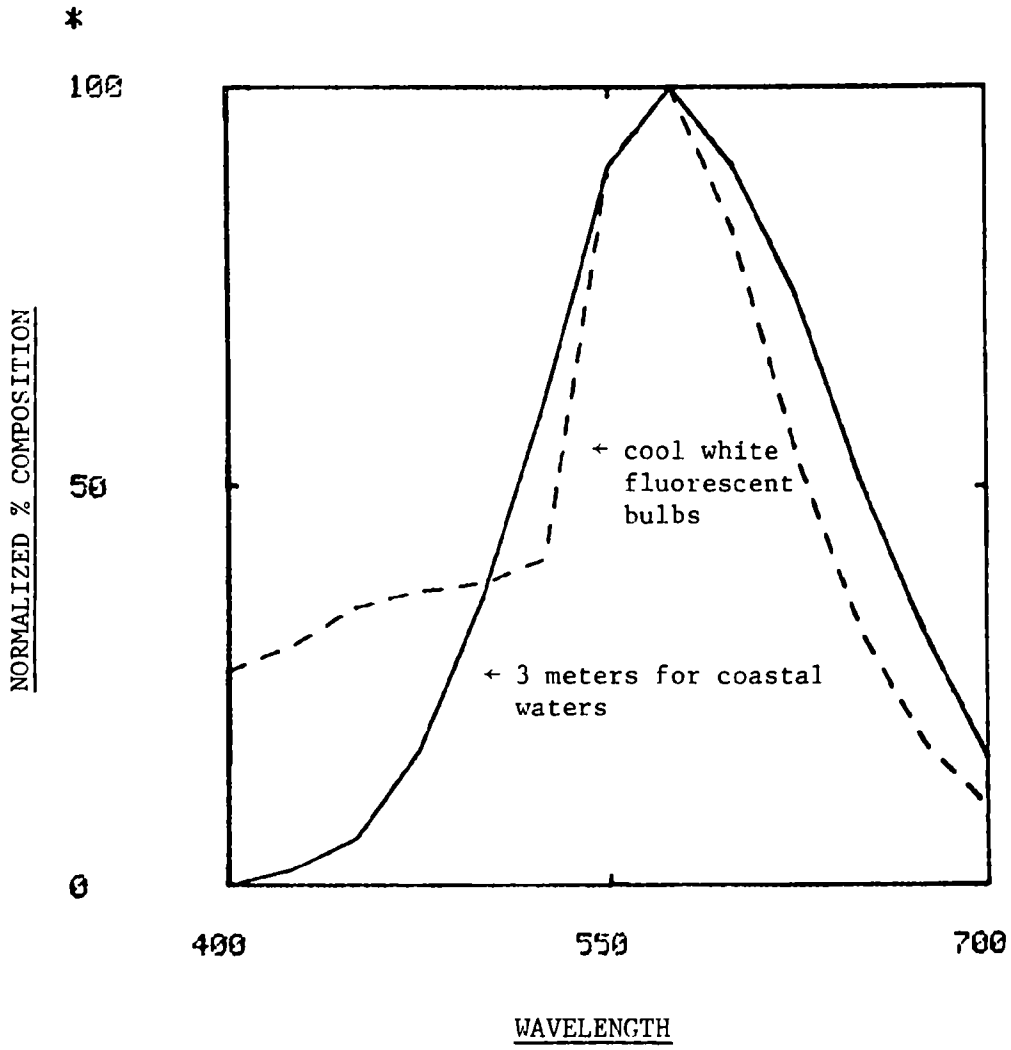


FIG. 1. Average seasonal light intensities in a 9 meter water column.

FIG. 2 Spectral composition for coastal waters and Sylvania cool white fluorescent bulbs.



coefficients must be similar so as to insure realism in the total metabolism measurements. A new system of stirring is being designed to produce a more turbulent mixing.

The stressor used in the study, an artificial sewerage has been produced. However, the form, species and quantity of metals to be added to the sewage has not been decided due to a lack of data. We have recently sampled two local sewerage inputs for metals and expect the results soon.

ROAP 21 AKF/029--A Demonstration of Sublethal Effect Due to Pollution Stressors on Corals and Associated Biota. J.E. Bardach and S.V. Smith, University of Hawaii.

This study is working with total but somewhat manipulated tropical marine microcosms. The microcosms were exposed to different stresses such as salinity temperature, nutrients and species manipulations and the resulting response of total metabolism and structure was observed. General relationships and conclusions are forthcoming.

A major expansion in fish culture capabilities occurred during the past half year. Grace MacPhee, Fish Physiologist, GS-9, joined the staff. She brought with her, considerable expertise in flounder culture.

Considerable study was given to indigenous fish species, amenable to laboratory culture and potentially acceptable subjects for experimental projects of the various NMWQL research teams. A supply of fish embryos and larvae is desired on a year-round basis. Because of limited wet lab space, and no large capacity sea water temperature control systems, it is not possible to provide any one single species throughout the year. However, working within ambient water temperatures an array of species can be provided during the annual cycle, with embryos and larvae of one or two different species continuously available. To achieve this, the following representative important species were selected for in-house culture to provide the necessary experimental organisms in the early life stages:

Pseudopleuronectes americanus (winter flounder)	December-March
Limanda ferruginea (Yellowtail flounder)	April-June
Menidia menidia (Atlantic silversides)	May-July
Tautogolabrus adspersus (cunner)	May-August
Scopthalmus aquosa (windowpane flounder)	May-August
Paralichthys dentatus (summer flounder)	October-December

Implementing this yearly plan, Grace MacPhee successfully spawned and cultured embryos and larvae of the summer flounder. Substantial numbers were provided for experimental use to several NMWQL teams. This was followed by culture of the winter flounder, still in progress.

Other Marine Culture Team projects are in the area of characterizing condition of animals provided for experimental use, nutrition studies, invertebrate larvae culture and development of field collection techniques. These, and other subjects are detailed in the following pages.

For research management purposes, tasks accomplished by this team are identified under ROAP 21 AKF "Ecological Requirements for the Protection of Estuarine and Marine Life". This report is organized by tasks under this ROAP.

ROAP 21 AKF - Task 02--Collections

Collections: January 1, 1974 thru December 31, 1974

TABLE 1 - COLLECTIONS

Fish:	No. Collected	No. Collections
Menidia menidia, (Atlantic silversides)	24,285	19
Fundulus heteroclitus, (mummichog)	1,825	10
Gasterosteus aculeatus, (3-spine stickleback)	2,000	2
Pseudopleuronectes americanus, (winter flounder)	226	5
Alosa pseudoharengus, (Alewife)	120	2
Paralichthys dentatus, (gravid adults)	12	2
Crustaceans:		
Homarus americanus, (lobster)	12	1
Carangon septemspinosa, (sand shrimp)	200	1
Paleomonetes vulgarus, (grass shrimp)	4,210	5
Shrimp spp	1,000	1
Cancer irroratus, (rock crab)	82	3

Shellfish:

Nassarius obsoleta, (mud snail)	1,000	2
Artica icelandica, (ocean quahaug)	550	3
Crassostrea virginica, (oyster)	4 bu.	3
Mya arenaria, (clam)	765	15
Mercenaria mercenaria, (quahaug)	1,500 plus	7
Argopecten irradians irradians, (scallop)	400	*
	TOTAL	80

*Purchased from local supplier

Worthy of special notice were the success of Menidia collections. Unprecedented low mortalities resulted from use of new handling techniques in the field and in transport. Considering the susceptibility of this species to handling, the techniques used could be easily adapted to other delicate fishes thus expanding our total capabilities for new species.

Collection Equipment: Several new seines were purchased this year. They incorporate a unique "deep bag", designed by Ray Hennekey. This has proved most effective and of considerable help in making better handling-transfer techniques possible.

A new fish-sorting device is in the design-construction stage. Hopefully, this device will replace more traditional sorters because of its capability to sort delicate species with minimal handling and damage. The return of Menidia in sizeable numbers will allow for final testing and modification of the device in the spring.

A "German" style "otter" trawl has been procured and is used for benthic fish and invertebrate collections from the 45 foot chartered fishing vessel.

Fish Culture - Task 02G2(a)

Laboratory cultured embryos and larvae were supplied for experimental use:

SPECIES	USER
Winter flounder, Pseudopleuronectes americanus	Bioassay, Oil, Synergisms
Summer flounder, Paralichthys dentatus	Bioassay, Oil
Atlantic Silversides, Menidia menidia	Bioassay, Oil, Response Parameters

Major NMWQL fish culture activities in addition to supply included (1) development and application of materials, methods, and techniques for summer flounder, Paralichthys dentatus embryo and larvae culture and (2) preparation for and collection of gravid adults for winter-spring spawning and culture of Winter flounder, Pseudopleuronectes americanus.

Summer Flounder Culture (by Grace MacPhee)

Holding and maintenance of adult summer flounder was accomplished as follows. Adults were collected in Narragansett Bay from several stations in the West Passage, during July 29-October 18, 1974. Fish were maintained in 250 gallon tanks in a flow through raw seawater system at ambient temperature. The fish were fed live quahogs silversides, mummichogs, and sheepshead minnows.

Hormone injections to induce spawning were started on 10/15/74, and the first results were obtained 10/16/74 when two males produced milt. The first eggs were produced 10/22/74. A spontaneous tank spawning took place 10/29/74 with very low fertilization resulting (0.5%), presumably because the males produced very little milt. Table I. gives dates of successful stripping, amounts of eggs produced, and per cent fertilization.

TABLE I

Date	Amount of Eggs	% Fertilization	No. of fish Spawning
11/3/74	50,000	80%	1 female, 2 males
11/10/74	80,000	95%	1 female, 2 males
11/17/74	100,000	65%	2 females, 2 males
11/24/74	10,000	80%	1 female, 2 males

In all, seven fish produced sexual products (3 males, 4 females). The other fish were probably immature females. At present it is impossible to sex summer flounder externally.

The breeding stock was returned to original collection sites in Narragansett Bay on 11/26/74 because lack of water temperature control made it impossible to keep them any longer.

Some difficulties encountered in maintaining the fish in the present seawater system were: outbreaks of disease, parasitic invasions, and crowding due to small tank size. Fish which were injured in the trawl developed vibriosis and fin rot. The disease was diagnosed by William Watkins of the Recreational Water Quality Team at Dr. Richard Wolke's (Animal Pathologist, University of Rhode Isalnd) laboratory (see Linda Ferraro's section for further comments). The fish were treated with the drug Furanace, by bathing for an hour in a concentration of 10 g/ml water. Noticable improvement was shown within three days. Fin rot was also a problem. It occurred in trawl damaged fish and also in intact fish after a month in captivity. Topical treatment with malachite green 2% solution and malachite green-formalin solution did not produce noticible improvement. This was probably because histological grade malachite green was used. This contains zinc which prevents the dye from working.

Collection injury was significantly reduced. Allan Beck devised a sorting tray for use on the boat with fishes collected by otter trawl.

The net was emptied into a large shallow fiberglass tank 4'x8'x1' which was filled with water. The fish were not out of the water except when hoisted over the side. They did not contact the deck during sorting and consequently received minimal physical abuse and damage.

Summer flounder eggs and larvae were supplied to the bioassay team for heavy metal toxicity studies. Some larvae were supplied to the response parameters team to see if their behavior could be monitored by remote T.V. camera.

TABLE 2

People supplied	Number of eggs	Number of Larvae	Dates
Gentile & Cardin (Bioassay team)	4,500	600	11/5-11/26
Everich (Response parameters)		6*	11/20

* To evaluate behavioral video recording system.

The rate of development of summer flounder eggs was dependent on water temperature. Because there is no water temperature control, quantitative data was not available but estimates of developmental time were obtained (Figure a). An important difference to note is the delay between embryonic Phase II (Stages taken from Smith, W.G. and M.P. Fahay. 1970. Description of eggs and larvae of the summer flounder Paralichthys dentatus.(Bur. of Sport Fisheries & Wildlife Res. Dept. 75)

Further data on summer flounder is detailed in MacPhee, Grace, K., 1975. Synopsis of biological data on the summer flounder. Fisheries Bulletin, NOAA. National Marine Fisheries Service Publ. In press.

Invertebrate culture--ROAP 21AKF--Task 2G3

Culture facilities have been designed and fabricated in the Narragansett wet lab by Bill Giles. Previously this culture effort was accomplished at the Research Barge, Jerusalem, by George Morrison. All future invertebrate culture will now be at Narragansett.

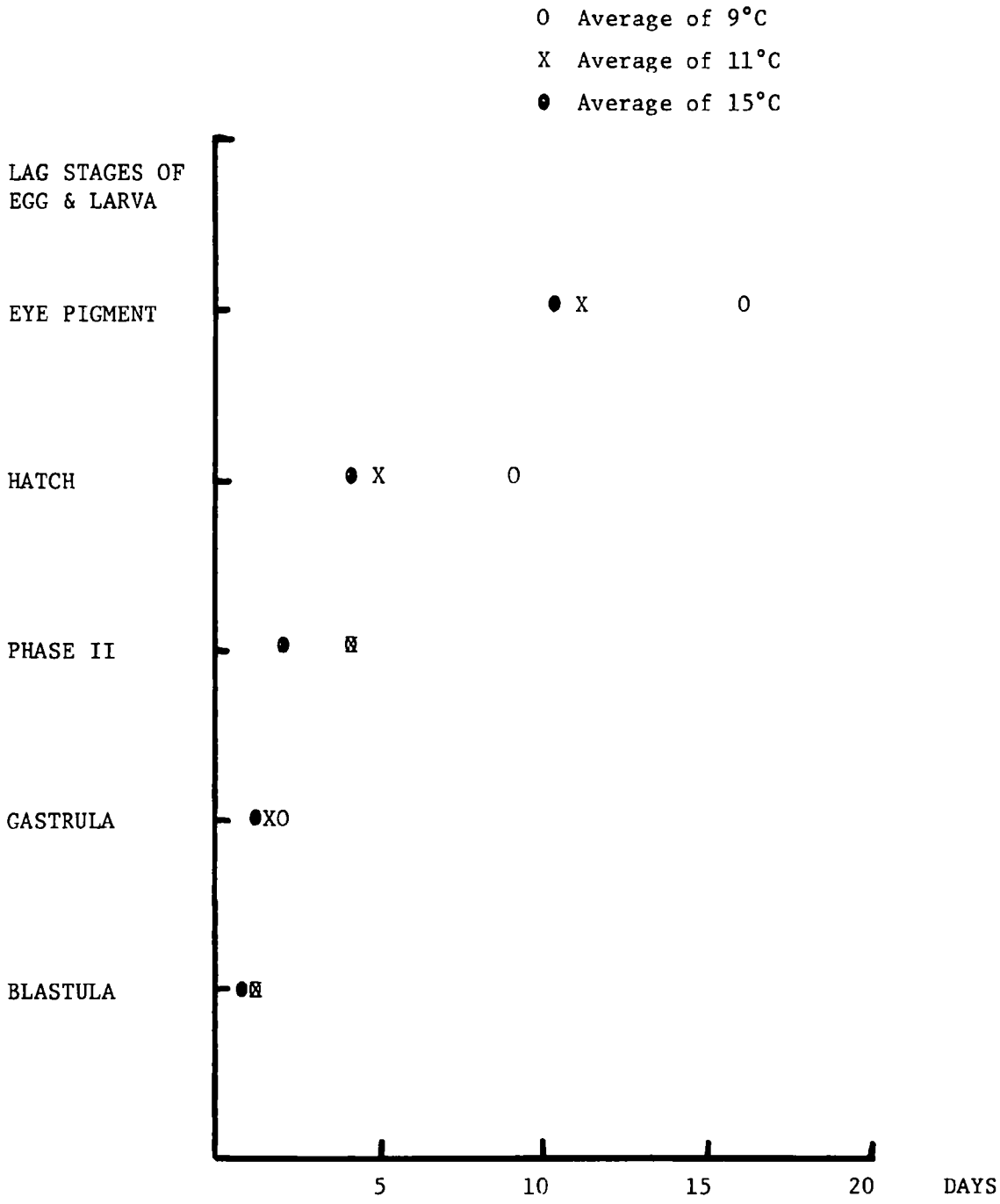


FIGURE A. DEVELOPMENT TIME-TEMPERATURE - SUMMER FLOUNDER

Presently available facilities will support the culture of the following invertebrates:

marine rotifer - Brachionus plicatilus

brine shrimp - Artemia salina

barnacle - Balanus improvisus, spp.

mussel - Mytilus edulis

coot clam - Mulinia lateralis

scallop - Argopecten irradians irradians

oyster - Crassostrea virginicus

quahaug - Mercenaria mercenaria

ROAP 21 AKF - Task 2G4--Culture of marine polychaete Capitella capitata

The culture methodology provided by Dr. Donald Reish under EPA Grant 800962 was validated in NMWQL facilities by Carol Pesch. The organism is now available for experimental use. Some difficulty was encountered in transferring Dr. Reish's successful methodology to NMWQL facilities. Ms. Pesch prepared a written evaluation of the methods manual provided. The main criticism was lack of specificity in the routine daily physical procedures involved in culture maintenance. The manuals should be sufficiently detailed to permit successful culture by a competent biological technician with no previous experience in polychaete culture. A culture and bioassay methods manual is due to be published and will include input from Ms. Pesch and other members of the Bioassay Methods Team.

Task 2G4--Validation of EPA Grant 800962 polychaete culture methodology is considered substantially complete.

ROAP 21 AKF--Task 09--Production of Larval Lobsters

The American Lobster (Homarus americanus) culture methods manual was provided under EPA Grant 802494 by Dr. Schleser, Univ. of California, Davis. The Marine Culture Team has neither the facilities nor personnel, so validation of culture methodology

combined with bioassay experiments will be accomplished by personnel of the Bioassay Methods Team and progress detailed in another section of this report.

Nutrition--21AKF--Task 2E-By Leslie Richardson

The determination of the nutritional chemistry of winter flounder (Pseudopleuronectes americanus) is now being carried out by Leslie Richardson. The emphasis is presently on the precise proportions of proteins and the amino acid profiles for the eggs, yolk-sac larvae, post larvae, juveniles and adults of the flounder. Potential foods for the various life stages are also being analyzed beginning with wild plankton tows from the Narragansett bay.

The accompanying flow sheet Fig. B describes the laboratory approach to determine the nutritional characteristics. The results are expressed on a moisture-free and ash-free basis. The nitrogen characterization determines the percentage recovery of the total nitorgen of each sample as free amino acid nitrogen. Both acid and alkaline hydrolyses are carried out on the samples for complete amino acid profiles. Tryptophan and cystine assays are included. Analyses for mineral and fatty acid content are being incorporated into the total laboratory design.

FIGURE B. NUTRITIONAL CHEMISTRY ANALYSES

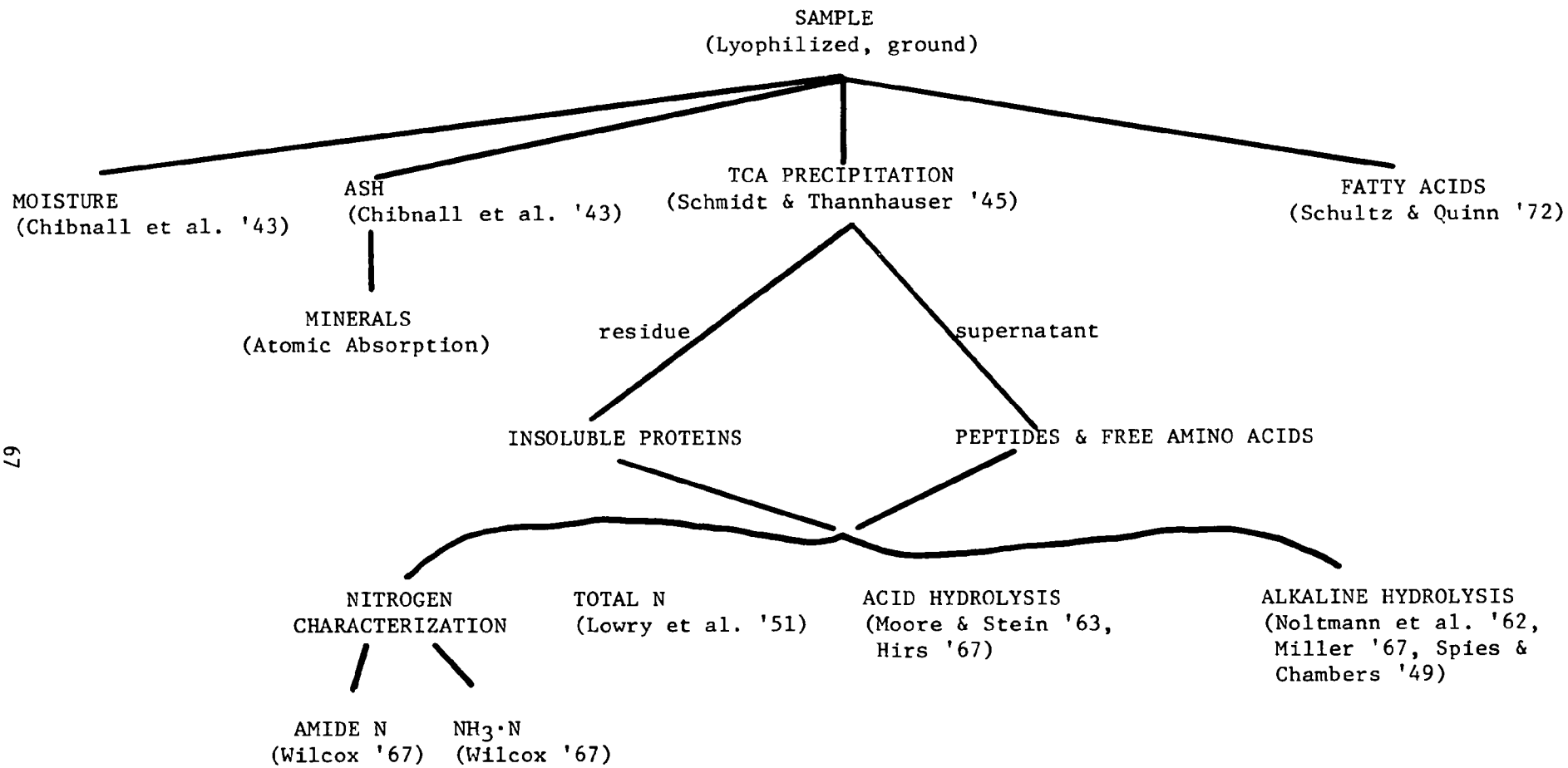


Figure B
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Similar studies will be conducted on Atlantic silversides, Menidia menidia.

As previously stated (NMWQL Semi-Annual Report January 1, 1974 thru June 30, 1974) the goal of nutrition studies is to develop a series of artificial diets or natural diets which can be qualitatively and quanitatively standardized, and which are capable of providing the necessary nutrients at the various life stages of the organism. Ideally, the developed diets should promote normal growth, survival, and physiology of test organisms as compared to field populations. Genetic variability between various fish of the same species will result in a range of "normal" values for selected condition indices. Hopefully, optimal laboratory culture and nutritional regimes will provide experimental animals within the normal range. Until this is achieveable emphasis will be on standardizing, if not normalizing, condition of test organisms.

Results of the past six months nutritional studies with Atlantic Silversides, accomplished by Linda Ferraro are as follows:

Menidia menidia Nutrition Experiments--Feeding Study No. 2
By Linda A. Ferraro--Marine Culture Team, NMWQL, January, 1975

I. INTRODUCTION

Preliminary nutrition experiments were conducted one year ago in autumn, 1973, on Atlantic silversides, Menidia menidia, in which laboratory fed and unfed test animals were subjected to histological examination and biochemical analysis for comparison with field-colelcted Menidia. The object of this work was two-fold: (1) to test a simplified method for ascertaining biochemically the nutritional status of the animals, and (2) to give baseline data on how closely lab-fed fish approximated the condition of field animals. Comparative evaluation parameters included laboratory survival, histological examination, hepatosomatic index (HSI or liver-to-body weight ratio), and biochemical analysis for glycogen, lipid and protein synthesis

(RNA:NDA ratio). The experiment was conducted in an open, raw-seawater system at ambient temperatures (18°-15°C), and the test diet consisted of 6 g. Biorell flake food and 16 g. thawed Artemia salina brine shrimp per 100 fish per day. Data from the 1973 experiment indicated the biochemical methods to be sensitive and suitable, and that histological examination supported the biochemical findings. The results indicated that the starved group approximated the 1973 field animals more closely than the laboratory fed group, while the laboratory fed group showed significant increased in liver glycogen and HSI with time. The conclusions drawn at that time were that histological and biochemical methods used for analysis were sensitive enough to detect nutritional differences even after one week of laboratory holding. It was demonstrated that the simple use of HSI alone paralleled or reflected the glycogen balance quite closely, and thus could be used as a reliable indicator of nutritional status with respect to energy storage capacity.

Since the laboratory fed animals received a generous diet initially calculated to supply them with as much as they would eat, the possibility of overfeeding vs. dietary imbalance may have been a factor in the analytical differences they displayed from the field animals, coupled with their reduced energy expenditure due to lack of activity required for predator-avoidance and food search. It was not established whether the high glycogen contents of laboratory fed fish were due to dietary imbalance (excess caloric content) or merely due to overfeeding and/or lack of exercise comparable with that of field animals. In addition, despite analytical differences, no data was generated indicating whether or not laboratory fed fish respond the same as field animals to conditions of stress and/or toxicant exposure. No comparative bioassays were conducted. And lastly, there was no certainty that the field "controls" were truly representative of all field Menidia. Therefore, it was desirable

to repeat and modify the experiment, both to confirm the reliability of the analytical methods and to resolve the questions left unanswered in the preceding experiment.

The purposes of the 1974 nutrition status experiment were fourfold: (1) to validate the reliability of the HSI as an indicator of nutritional (energy) state of the fish, at the same time comparing the condition of Menidia collected in 1974 vs. 1973, (2) to determine whether a reduced level of feeding of the same (1973) lab diet would more closely approximate field conditions, (3) to test a higher protein diet to see if a possible dietary imbalance was responsible for biochemical differences found previously, and (4) to utilize a portion of the test groups for comparative bioassays to determine differences in response to a toxicant, in effect assessing the effects of different laboratory diets on the fishes' performances in a bioassay, as compared with the responses of newly-collected field fishes.

The ultimate objective of this work is the development of a capability to hold Menidia menidia of varying sizes for extended periods and culture from egg to egg. Such a capability would lend itself to the conductance of long-term chronic bioassays, also requiring long periods of laboratory holding and feeding.

II. MATERIALS AND METHODS

The 1974 experiment was conducted at the same time of year (October) as the 1973 experiment, to insure some comparability of data between the two year-populations. All experimental work was conducted in aerated, open-system, filtered seawater round aquaria of 230-liter capacity. Uniform flow rates were established by means of a constant diameter inflow opening of one (1) mm. The entire experiment was conducted at ambient temperatures ranging from 16.4°C to 9.0°C.

The initial collection of Menidia menidia, from which all

experimental fish were taken was made on October 11, 1974. Approximately 1,000 juvenile and adult Menidia were collected by beach seine from Narrow River, Middlebridge, Rhode Island and held overnight in a large round tank of 578 liter capacity. Immediately upon collection, 15 fish were placed in Dietrich's fixative and furnished to Mr. Paul P. Yevich for histological examination, and 25 fish were submitted for body length and weight determinations, dissection, liver weight determinations, HSI calculations, and subsequent freezing for later biochemical analysis, if warranted. This aspect of the research was accomplished with the cooperation and advisement of biochemist, Dr. Sue Cheer, who participated in this research during the 1973 experiment. Both initial histological and analytical samplings comprised the initial field "control" data groups, which were used as an indicator of the fishes' condition prior to being placed on laboratory diet regimens.

On the following day, October 12, 1974, (Day 1 of the experiment), 800 Menidia were randomly distributed into eight 230-liter round aquaria (100 animals per tank). Both large and small fish were distributed into each tank. Each tank was assigned to one of four experimental diet groups, resulting in two replicates, A and B, or 200 fish, per diet. Experimental feeding groups are listed according to the schedule presented in Table 1. Feeding began on Day 1. The time of feeding and amounts fed refer to food administered per tank of 100 fish. Biorell and pulverized prior to feeding to ensure a particle size palatable to the fish. Frozen brine shrimp (Artemia salina) were fed after thawing.

Table 1: Daily Feeding Regimen -- *Menidia menidia* 1974 Comparative Feeding Study

Time	Group I (= 1973 diet)	Group II (Half-1973)	Group III (high-Protein)	Group IV (unfed)
A.M.	6 g. Biorell	3 g. Biorell	15 g. *Artemia*	0
P.M.	16 g *Artemia*	8 g. *Artemia*	15 g. *Artemia*	0

All tanks were cleaned at weekly intervals using a siphon modified at the intake end with the attachment of a plastic bristle vacuum-cleaner brush head, for dislodging and removing sedimented particulate from the sides and bottom of each tank. Tanks were monitored daily for the following parameters: water temperature, fish behavioral aberrations, moribundity and mortalities. Dead animals were removed immediately upon discovery and preserved in Dietrich's fixative for histopathological examination. Dead fish which had whitish gills and cloudy eyes were not preserved, as these criteria were considered evidence of advanced post-mortem autolysis, making histological examination fruitless. This was usually seen in conjunction with deaths occurring overnight.

During the course of the experiment, three samplings of each diet group, including equal numbers of A and B tanks, were taken for comparison with field fish collected on similar dates. These samples were submitted for biochemical, HSI and histological analysis. Sampling dates corresponsed with one week, three weeks and six-and-a-half weeks of laboratory feeding. Both large and small *Menidia* were included as equally-distributed as possible in these samplings. With the exception of Group IV (Unfed) animals, all of which had succumbed to starvation by

Day 46, all diet groups were represented, along with field-collected fish, at each sampling interval. Following the final sampling on Day 46, the experiment was officially terminated.

Comparative acute cadmium toxicity bioassays were conducted on three separate groups of newly-collected field Menidia, followed by bioassays on each of the four feeding groups, to ascertain comparative responses attributable to nutritional status. Ninety (90) Menidia were exposed in each bioassay, 15 controls plus 15 exposed to each of five concentrations of cadmium. Concentrations used were 30, 10, 3, 1 and 0.3 PPM of cadmium, and the bioassay was conducted using filtered seawater at 14°C. Both large and small fish were represented in the bioassays in all cases, both field and experimental. The bioassay was conducted by Mr. John A. Cardin of the Bioassay Methods Team.

A timetable of all fish sampling and bioassay assignments is presented in Table 2. Large and small fish are indicated as L and S, respectively, when measured. Animals with a total body weight of 0.14-0.80 g were classified small, and those whose body weight fell between 1.21-4.78 g were designated large. Total body length measurements corresponded well and supported such a size classification.

The fish were collected from a different location (Narrow River, Middlebridge, Rhode Island), which proved to be the only feasible site which provided enough Menidia at this time of year. The two size groups were intermixed in all field collections made to provide animals for this experiment. Statistical analysis of the data, when compared with respect to fish size, revealed significant differences in the nutritional condition of the animals. These differences are reflected both in HSI and in the cadmium bioassay responses, appearing in both field and lab-fed animals.

Table 2. Timetable of Sampling Data for 1974 Menidia menidia Comparative Feeding Study -- Numbers of Fish Sampled.

Date	Day	Analysis	Field	I	II	III	IV
10-11-74	0	Histology	15 (L)	--	--	--	--
		HSI	25 (L)	--	--	--	--
10-17-74	6	Histology	10	10	10	10	9
		HSI	25 (5L, 10S)	15 (14L, 1S)	15 (12L, 3S)	15 (8L, 7S)	15 (10L, 5S)
10-24-74	13	Cd bioassay	90 (L)	--	--	--	--
		Cd bioassay	90 (S)	--	--	--	--
10-31-74	20	Cd bioassay	90 (S)	--	--	--	--
		Histology	10	10	10	10	10
		HSI	20 (10L, 10S)	15 (7L, 8S)	15 (9L, 6S)	15 (8L, 7S)	15 (8L, 7S)
11-18-74	38	Cd bioassay	--	90 (L+S)	--	--	90 (L+S)
11-19-74	39	Cd bioassay	--	--	90 (L+S)	--	--
11-20-74	40	Cd bioassay	--	--	--	90 (L+S)	--
11-25-74	45	Histology	11	--	--	--	--
		HSI	20 (10L, 10S)	--	--	--	--
11-26-74	46	Histology	--	10	10	3	--
		HSI	--	15 (10L, 5S)	15 (10L, 5S)	14 (10L, 4S)	--

III. RESULTS

A. Laboratory Feeding -- Gross Observations and Pathology

During the first week of laboratory feeding, a number of deaths occurred amont all diet groups from 5 to 7% of total number by group. Their distribution was evenly scattered throughout all eight tanks. The last of these mortalities occurred on Day 7, after which no further mortalities occurred in Groups I, II and III. These initial deaths were attributed to stress factors due to collection, transfer and failure to acclimate. Interestingly, all of these were fish of the larger size range, suggesting that larger Menidia are more sensitive to handling than smaller ones.

A consistent pattern of gross lesions occurred in these fish, generally preceded by a uniform body hypermelanism, usually visible the day before the animal was found dead or moribund. These lesions consisted of fin necrosis, commencing with the caudal fin and progressing anteriorly, sometimes including dorsal and pectoral fins. Ventral and opercular subcutaneous ecchymoses were often present in conjunction with the fin lesions.

Vibrio spp. in an opportunistic pathogen sometimes present in the raw sea water supply pumped from Narragansett Bay. There may have been a possibility that the more severely stressed Menidia were more susceptible to the invasiveness of Vibrio anguillarum, resulting in their ultimate death by vibriosis. Since Vibrio is invasive through a dermal route of entry, slight handling abrasions would favor such an infection. However, no bacteriological isolations were made on affected Menidia to recover the organism, a positive diagnosis of death by vibriosis can not be made, but it is a logical supposition.

Following the initial mortalities, which totaled 48, no further mortalities occurred during the entire experimental period in any of the lab-fed groups, I, II and III. Behavioral observations revealed them to be active and healthy-looking, with retention of

schooling behavior. No gross lesions of any kind were observed. Both large and small fish appeared equally healthy on visual inspection.

Beginning on Day 31, the Group IV fish, which were unfed, began a gradual, consistent pattern of mortality, displaying characteristic gross signs of piscine starvation. These signs included a general appearance of emaciation, a sunken-in antero-dorsal region, and a pinched, jaundiced abdomen. Concurrent with these signs of malnutrition were hypermelanism and fin necrosis. Ulcerative lesions were absent.

A distinct behavioral change was noted in the Group IV animals by the fourth week. Schooling behavior of the fish had disappeared, even after attempts to deliberately startle them. The animals swam slowly and sluggishly in random directions, and some were observed to "rest" on the bottom at times, until prodded.

It was observed that the larger Menidia were the first to die, followed by the smaller ones. Termination of the experiment on Day 46 was mandated by the virtual complete mortality of Group IV animals. A total of 47 Menidia in Group IV died during this period.

Table 3: Mortality and Temperature Daya -- 1974 Menidia menidia Feeding Study (October 12, 1974 to November 26, 1974).

Day	Ambient Seawater Temperature	DIET GROUPS I	II	III	IV
1	15.0°C	--	--	--	1
2	15.0	5	2	--	1
3	14.0	5	6	6	5
4	16.0	1	3	1	2
5	16.4	2	1	--	2
6	15.5	--	1	2	1
7	15.0	1	--	--	--

TABLE 3 (Cont'd)

Day	Ambient Seawater Temperature	GROUP DIETS I	II	III	IV
8	14.5°C	--	--	--	--
9	14.0	--	--	--	--
10	13.5	--	--	--	--
11	15.0	--	--	--	--
12	13.5	--	--	--	--
13	13.7	--	--	--	--
14	12.5	--	--	--	--
15	12.0	--	--	1*	--
16	12.5	--	--	--	--
17	12.0	--	--	--	--
18	13.0	--	--	--	--
19	14.5	--	--	--	--
20	15.4	--	--	--	--
21	15.5	--	--	--	--
22	12.5	--	--	--	--
23	12.5	--	--	--	--
24	13.0	--	--	--	--
25	13.0	--	--	--	--
26	12.8	--	--	--	--
27	12.5	--	--	--	--
28	12.0	--	--	--	--
29	12.0	--	--	--	--
30	12.0	--	--	--	--
31	12.0	--	--	--	3
32	12.0	--	--	--	1
33	12.0	--	--	--	5
34	12.0	--	--	--	6
35	12.0	--	--	--	3

TABLE 3 (cont'd)

Day	Ambient Seawater Temperature	DIET GROUPS I	II	III	IV
36	11.5°C	--	--	--	14
37	11.0	--	--	--	5
38	11.8	--	--	--	4
39	10.8	--	--	--	2
40	10.8	--	--	--	--
41	11.0	--	--	--	1
42	10.8	--	--	--	--
43	9.5	--	--	--	--
44	9.0	--	--	--	--
45	9.2	--	--	--	--
46	9.0	--	--	--	3
Cumulative Mortality		14	13	10	59
Time of Occurrence		Week 1	Week 1	Week 1	Week 1 (12)

*This Group III animal was found dead next to an unidentified sharp metal object on the tank bottom. A sharply-defined, acute, peduncular, ulcerative lesion, accompanied by swelling, tissue edema and hemorrhage (ecchymosis) supported the diagnosis of accidental death resulting from peduncular concussion, ruling out a nutritional or infectious etiology.

B. Hepatosomatic Index (HSI) and Other Fish Measurements

The results of the 1973 experiment indicated that for that population of Menidia, four parameters measured gave a sensitive and consistent indication of nutritional status, these being total body weight, liver weight, HSI and liver glycogen determination. In 1973, these parameters followed the same pattern and could be correlated directly with the animal's mode of feeding. Laboratory-

fed Menidia showed considerably higher measurements for all four parameters with time, followed by field-collected fish and then laboratory-unfed fish. Surprisingly, among the 1973 animals, the lab-unfed fish approximated the field- collected fish more closely than the lab-fed fish.

Measurements conducted on the 1974 fish showed quite the opposite results among field and lab-fed fish, both in overall comparative dietary evaluations, as well as stress response patterns apparently correlated with the size of the fish studied. Table 4 lists the mean measurements and HSI values for the 1974 field and experimental Menidia. A graphical plotting of field animal total body weights vs. time, comparing 1973 and 1974 field Menidia, is presented in Figure 1. These data demonstrate the three distinctly separate aize groups represented over the course of the two studies. It can be seen that the 1973 field Menidia were of an intermediate size range from the two Menidia sizes collected in 1974. Figure 2 is a graphical plotting of 1973 and 1974 field Menidia liver weights vs. time. Figure 3 represents comparative 1973 and 1974 liver-to-body weight ratios (HSI) vs. time, and it demonstrates that both sizes of Menidia collected in 1974 had much higher HSI values than the 1973 Menidia. The 1974 HSI values for large and small fish were both increasing gradually with time, compared to those of the 1973 fish, whose HSI values remained relatively static. All the 1974 field and experimental animal and 1973 field animal HSI values are plotted similarly in Figure 4. Relatively higher HSI values are noted in all groups among the smaller fish, when contrasted to those of the larger fish. It is also apparent that, when both 1973 and 1974 field-collected Menidia are considered, the HSI's of all three experimental diet groups fall within the range of the HSI values exhibited by the field Menidia (excluding the unfed groups, particularly the large animals, whose HSI values

Table 4. Mean Measurements and HSI Values -- 1974 *Menidia menidia* Feeding Study -- 6½ Weeks' Duration

Time (Weeks)	Sample	Total Body Wgts(g.) Large	Total Body Wgts(g.) Small	Liver Wgts.(mg.) Large	Liver Wgts.(mg.) Small	HSI(%) Large	HSI(%) Small
0	Field control	2.87	--	46.9	--	1.66	--
1	Field control	3.18	0.39	60.8	18.0	1.93	4.57
↓	Diet I	2.66	--	42.6	--	1.66	--
↓	Diet II	2.53	0.49	34.8	15.7	1.38	3.26
↓	Diet III	2.75	0.43	42.0	14.0	1.56	3.25
↓	Gp. IV Unfed	2.27	0.46	31.1	11.1	1.37	2.46
3	Field control	2.18	0.61	66.7	32.5	3.07	5.33
↓	Diet I	3.05	0.48	54.1	22.0	1.84	4.50
↓	Diet II	2.88	0.42	41.3	15.8	1.44	3.79
↓	Diet III	2.78	0.34	63.0	13.8	2.26	3.86
↓	Gp. IV Unfed	2.71	0.28	29.2	5.1	1.06	1.89
6½	Field control	2.84	0.44	114.0	25.8	4.05	5.81
↓	Diet I	3.05	0.61	50.3	31.1	1.64	3.37
↓	Diet II	2.75	0.41	31.5	13.9	1.14	3.37
↓	Diet III	2.74	0.55	67.1	27.1	2.48	4.93
↓	Gp. IV Unfed	All dead by this time ----------→					

FIGURE 1. 1973/74 MEAN TOTAL BODY WEIGHTS

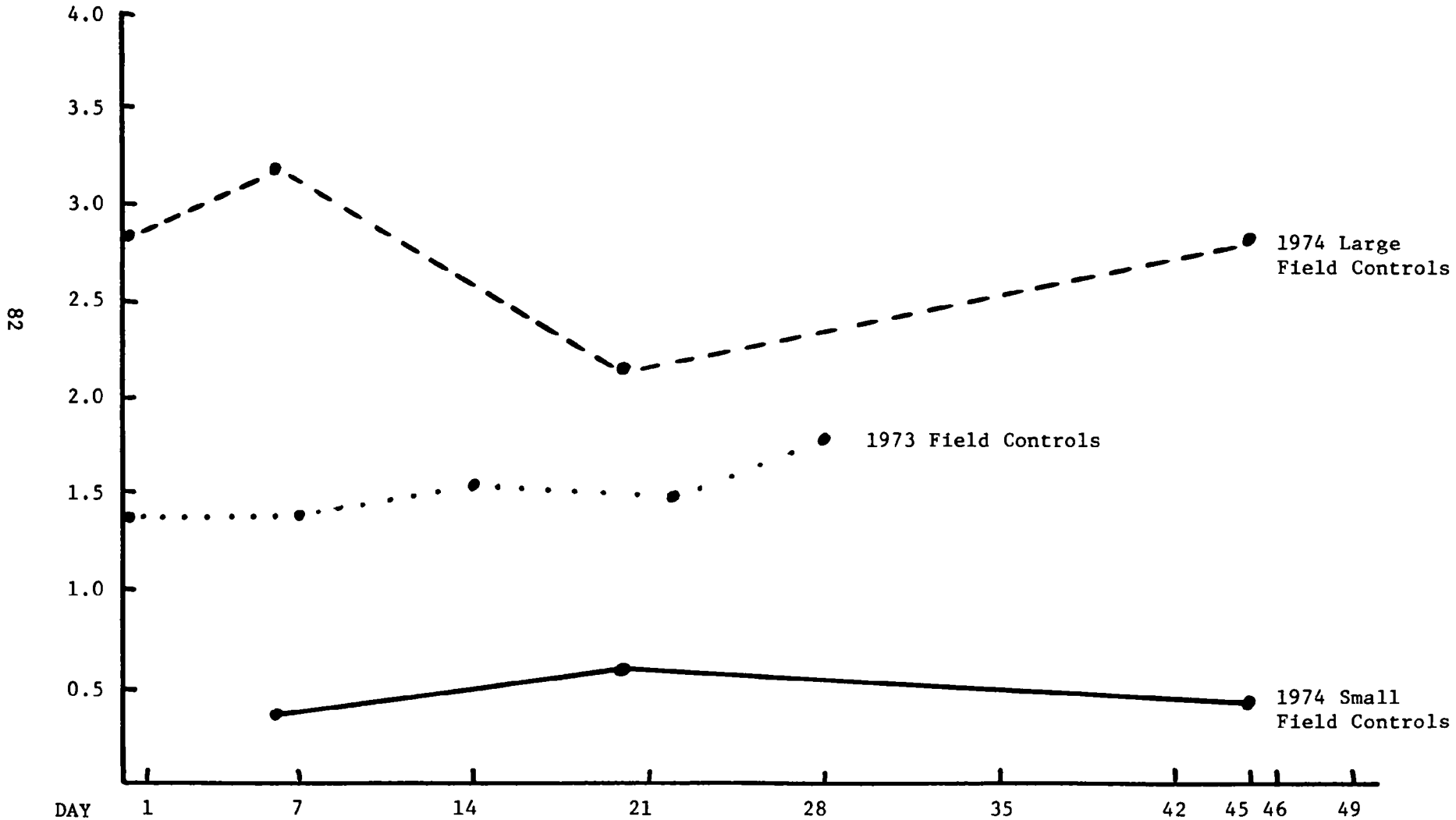


FIGURE 2. FIELD CONTROLS 1973 and 1974 - MEAN LIVER WEIGHTS (mg)

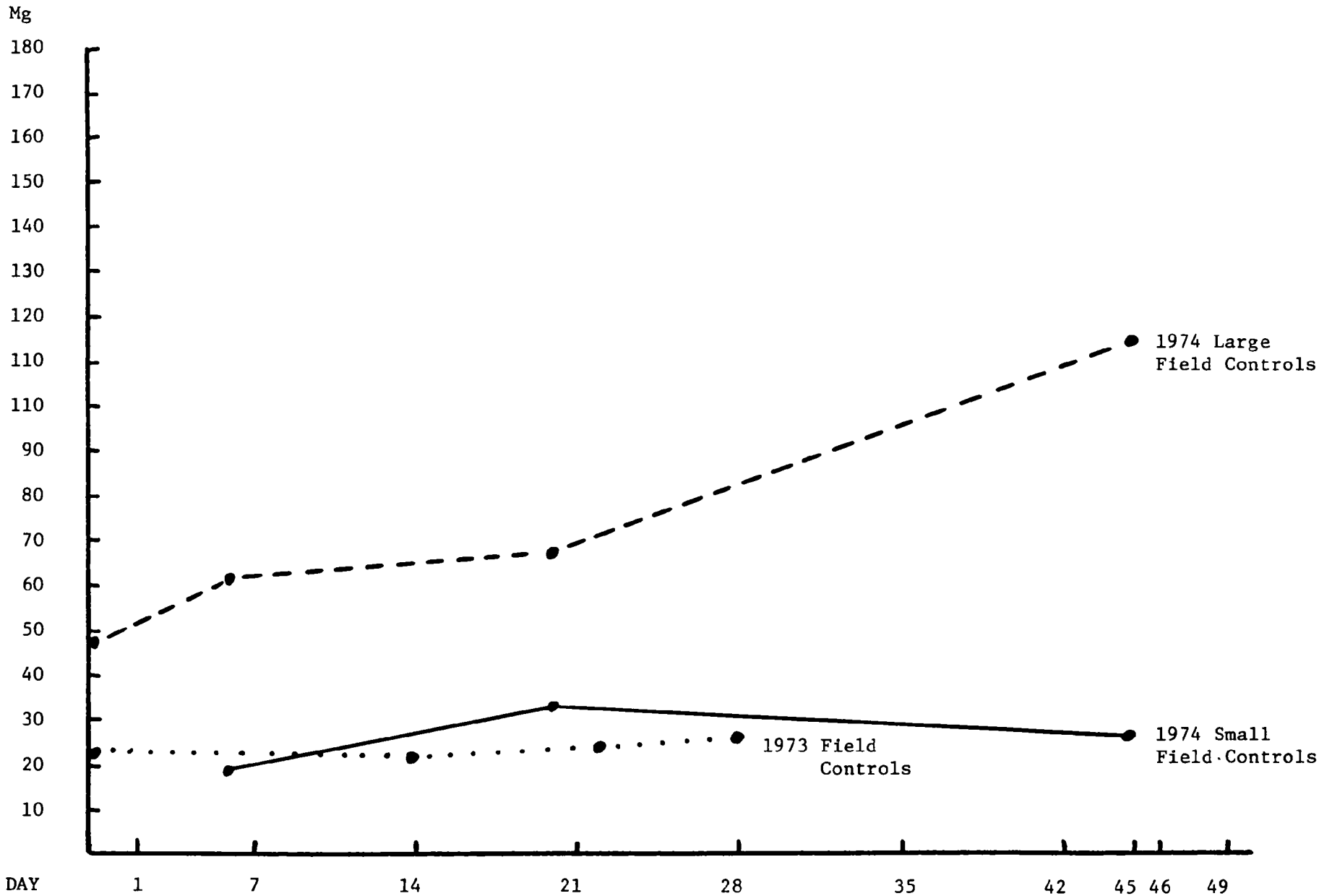


FIGURE 3. FIELD CONTROLS 1973, 1974 - MEAN LIVER/BODY WEIGHT RATIOS (%)

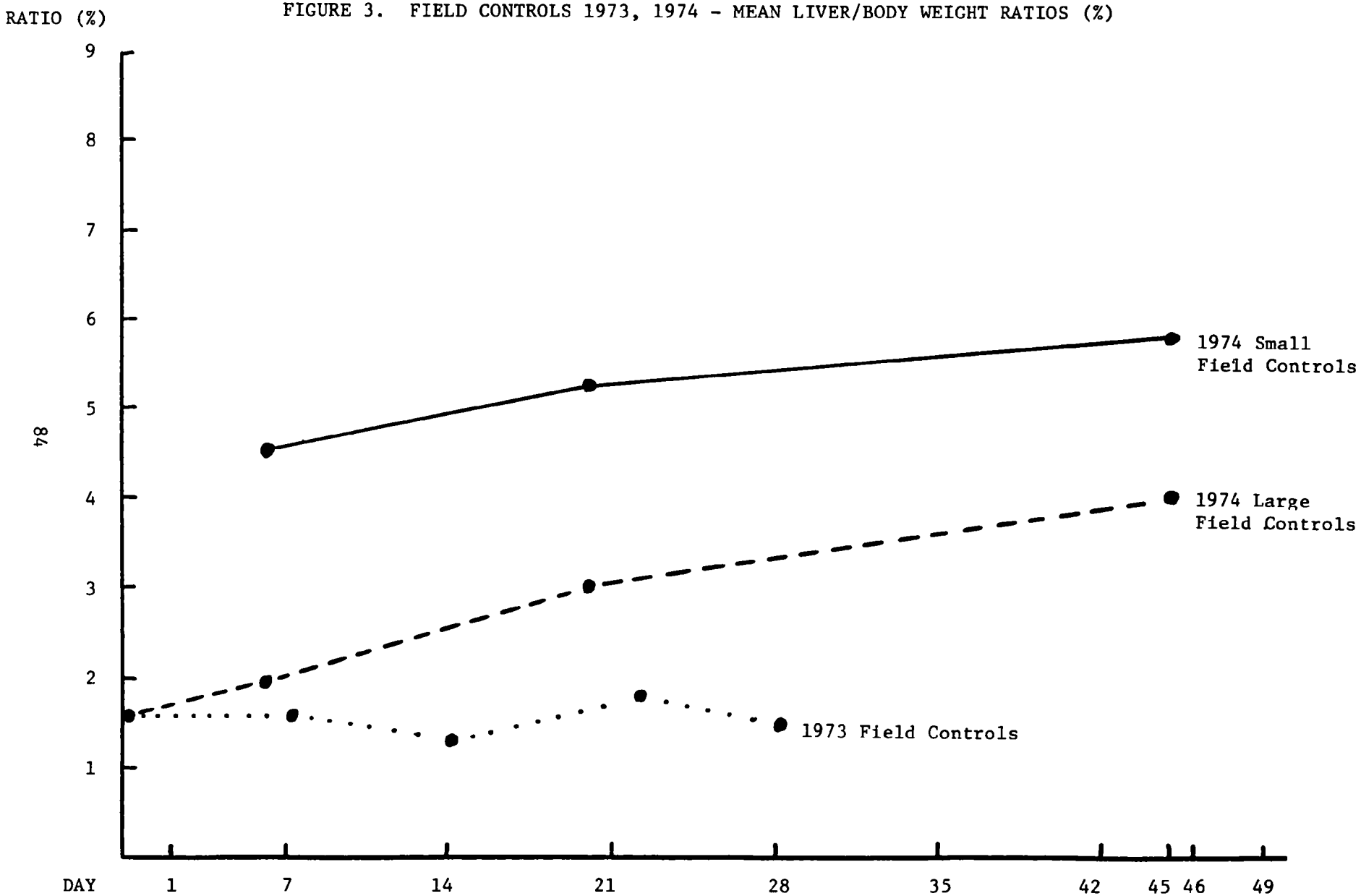
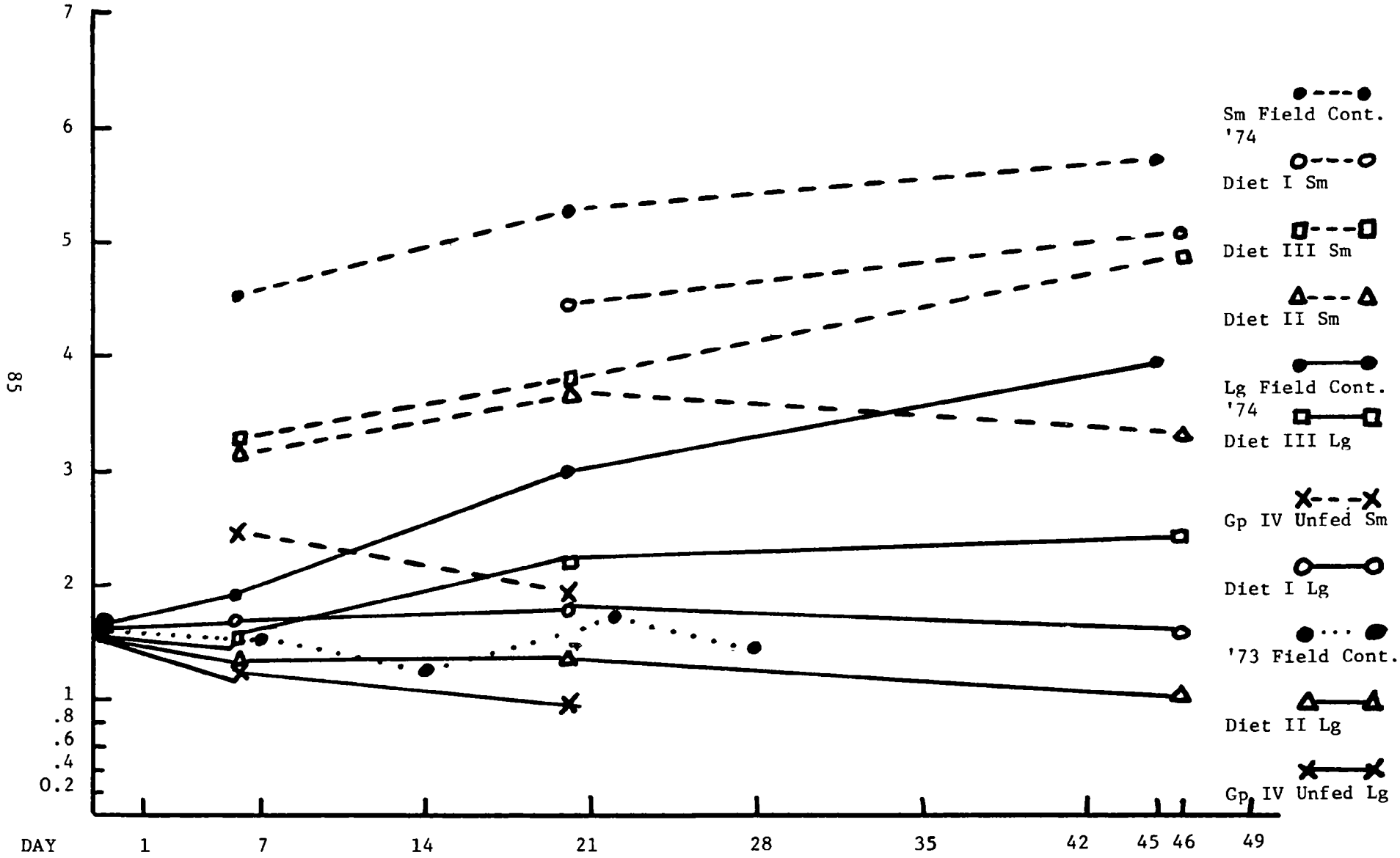


FIGURE 4. ALL 1974, 1973 FIELD CONTROLS (MENIDIA) - MEAN HSI (LIVER/BODY WEIGHT RATIOS)



would continually decline with starvation).

The striking differences in field animals HSI's for 1973 and 1974 may be readily contrasted in Table 5 and indicate rather marked variation in nutritional status of field animals alone, manifested both in different year-values and among different sizes of Menidia. With such a marked variation in the naturally occurring field animal HSI, it is difficult to assess exactly the nature of a standard HSI range for laboratory-fed animals, since some field animals could conceivably be undernourished in their natural habitat. This condition appears probable for the 1973 field Menidia.

TABLE 5: Mean Liver: Body Weight Ratios (HSI) for 1973 and 1974 Field Animals -- 1974 Menidia menidia Feeding Study

Time (weeks)	1973	1974 Small	1974 Large
1	1.6	4.57	1.93
2	1.3	--	--
3	1.8	5.33	3.07
4	1.5	--	--
6-1/2	--	5.81	4.05

1973 Week 0
1974 Week 0.

C. Acute Cadmium Toxicity Bioassays

Detailed results of the acute cadmium toxicity bioassays are reported elsewhere by Mr. John A. Cardin of the Bioassay Methods Team.

D. Histological Examination

The results of the histological samplings are pending completion of these examinations by Mr. Paul P. Yevich of the Histopathology

Unit.

IV. DISCUSSION

The results of this study are incomplete at this time, but several points merit discussion, and some conclusions may be drawn on the basis of what is presently known.

The initial feeding study observations indicate that a period of seven days elapsed before handling mortalities ceased, suggesting a one-week acclimation period is desirable prior to experimentation with Menidia. In addition, the larger-sized Menidia appear more sensitive to handling stress than the smaller Menidia. This finding is supported by Cardin's personal observations during the cadmium bioassay, in which he found the larger Menidia to be less adaptable to the experimental conditions, among both field and control fishes.

The use of HSI values as a measurement for condition shows a striking range among field animals alone. Reference to Figure 3 reveals a field variation in mean HSI ranging from a low point of 1.6 in 1973 to 5.81 in 1974, with smaller Menidia exhibiting consistently higher HSI values than larger Menidia, both in the field and under experimental conditions. Comparative HSI values depicted in Figure 4 indicate all laboratory-fed groups to fall well within this range. It has yet to be established definitively which HSI indicates a negative energy status, but it would appear to be in the range of 1.0 or thereabouts.

Acute cadmium toxicity bioassays were conducted by John Cardin of the Bioassay Methods Team. Results of his work, pertinent to culture, include (1) varying response differences, particularly among different sizes of Menidia, (2) laboratory-fed and field fish were comparable in responses, (3) the group of larger Menidia appeared to be more sensitive, whether laboratory-fed or field, and (4) within a particular size grouping, field fish were more

sensitive than laboratory-fed fish. The Bioassay Methods Team plans further bioassays to correct for possible handling mortalities. Current findings indicate this may have influenced toxicity results. While the laboratory-fed fish had been laboratory-acclimated for approximately one month prior to their bioassay, the field-collected Menidia used were bioassayed virtually immediately upon collection. Therefore, what appears to be a lower resistance to cadmium among field fish may in fact be a manifestation of failure to acclimate. It is proposed to correct for this by holding both field and laboratory-fed fish without feeding for a period of seven days prior to their bioassay, to eliminate acclimation stress among the field populations tested.

The results of the histological examinations may indicate subtler differences in condition among laboratory-fed and field animals, particularly among different diet groups. Hopefully they will be supportive of the conditions implied by the comparative HSI values, particularly with respect to liver and pancreas morphology, as well as the occurrence of visceral fat.

Electronics Group

Jay Sinnett, Electronics Engineer, GS-12 joined the NMWQL staff in November. An Electronics Group was formed and Ray Highland assigned with Jay as Electronics Technician.

Major duties of this group include (1) maintenance and repair of existing electronic equipment in the laboratory and (2) design and construction of specialized equipment for unique applications in biological experimentation. This includes:

21AKF Task 02--Invertebrate culture-Temperature control unit

21AKF Task 12--Behavioral studies--Automated positioning

21AKF Task 61--Microcosm studies--Multiple bath temperature controller

Other projects in progress include:

21AKF Task 02G(2) Winter flounder culture - Automated light control and dimmer switch

21AKF Task 72--Behavioral studies--Light control integrated with TV camera positioning.

21AKF Task 19-- Contract 14-12-872

Through both contract and in-house work, a capability is progressing for unmanned detection and telemetering of certain marine parameters to a laboratory minicomputer, which in turn will be capable of controlling these same parameters in experimental tanks at a remote location. The progress during November and December included emplacement of additional sensors for Temperature, Dissolved Oxygen, conductivity, pH, and an experimental sensor for turbidity.

Histology Group

The Histology Group accomplished a number of projects for various research teams:

16AAT-12	Chronic cadmium exposure oysters	Bioassay
21AKF 2F	Nutrition study-*Menidia* (In progress)	Culture
21BBG	Ocean dumping	Ocean Disposal
16AAT-11	Dissolved oxygen and cadmium effects	Synergisms
16AAV	Chronic oil exposure	Oils

Two major reports were (1) Report to State of Maine on Histopathological Findings in Soft Shell Clams From Searsport, Me. Oil Spill site, (2) Histological Findings in Ocean Quahaugs From Newport, R.I. Ocean Disposal Site. Results of these histological findings are included elsewhere in reports of projects by the lead research team involved.

Marine Culture Team, Non ROAP Activities

Allan Beck covered the subject area of impingement and entrainment of organisms in power plant cooling water systems at an EPA lawyers' seminar organized by Dr. Prager and held at NMWQL, Narragansett. Subject area treated was directed towards biological considerations in 316(a) & (b) guidelines to implement provisions of water law PL 92-500.

Several publications resulted from technical assistance to EPA Headquarters, recommending marine water temperature requirements and guidelines for enforcing 316(a) and (b) provisions of PL 92-500. These are listed in the following sections.

Manuscripts and Publications

MARINE CULTURE TEAM

Beck, A.D. and D.C. Miller. 1974. Analysis of inner plant passage of estuarine biota. Proc. ASCE Power Div. Spec. Conf. Boulder, Colo. August 12-14, 1974. pp 199-226.

Beck, A.D. and N.F. Lackie. 1974. Effects of passing marine animals through power plant cooling water systems. Presented as Symp. Effects of Nuclear Power Plants on the Ecosystem. Am. Fish Soc. Ann. mtg. Honolulu, Hawaii. Sept. 7-11, 1974.

Miller, D.C. and A.D. Beck. 1974. Development and application of criteria for marine cooling waters. Proc. IAEA/ECE Symp. Physical and Biological Effects on the Environment of Cooling Systems and Thermal Discharges at Nuclear Power Stations Oslo, Norway, August 26-30, 1974.

MacPhee, G.K. 1975. Synopsis of biological data on the summer flounder, Paralichthys dentatus. Fisheries Bulletin. NOAA, National Marine Fisheries Service. In press.

HISTOLOGICAL GROUP

Rinaldo, Ronald G. and Paul Yevich P. 1974. Black Spot Gill Syndrome of the Northern Shrimp Pandalus borealis. Journal of Invertebrate Pathology, Vol. 24, pp 224-233.

Gardner, George, Paul Yevich, Margaret James, J.C. Prager; 1974. The Microscopic Perils of Marine Pollution. Underwater Naturalist, Vol. 8, #4. pp 15-19.

Betzer, Susan B. and Paul Yevich. 1974. Copper Toxicity in Busycon Canaliculatum L. The Biological Bulletin.

Voyer, R.A., Paul Yevich, and Carolyn Barszcz. Alterations in the Response Pattern of Cadmium-exposed Mummichogs Fundulus heteroclitus L. (Submitted for publication to Water Research)

Publications and Workshops

Allan Beck - October 4. Presentation of talk Biological Effects of

Power Plants on the Marine Environment. Interstate Seafood Seminar Atlantic Beach, N.C.

Paul P. Yevich - July 10 Mr. Yevich went to Washington, D.C. to give a paper entitled Ovarian tumors in the quahog and mesenchymal tumors in the softshell clam at the Interagency Collaborative Group on Environmental Carcinogenesis.

Sept. 20 Mr. Yevich gave a Seminar on The histopathologic effects of water pollutants on marine life to the Scientists at the Edgewood Arsenal in Edgewood, Maryland.

Sept. 23 Mr. Yevich was in Tampa, Florida for a pre-hearing meeting in the Belcher Oil Co. proposed refinery and tanker terminal complex.

Dec. 3-4 Congerence/Workshop, Marine environmental implications of off-shore drilling in the Baltimore Canyon region of the Mid-Atlantic coast, at the Center for Adult Education, University of Maryland, College Park, M.D.

Dec. 5-7 Attended the Eighth Advanced Seminar in Clinical Ecology, Denver, Colorado to give a talk on The histological effects of oil pollutants on marine life.

Dec. 9 Gave a lecture at Woodward Hall, University of Rhode Island, to Dr. Tarzwell's class on pollution.

Dec. 17 Sat in on a grant proposal by Rita Colwell at EPA headquarters, Washington, D.C.

Dec. 17 Consulted with pathologists at the Armed Forces Institute of Pathology, Washington, D.C. about soft shell tumor slides.

Dec. 18 Attended the meeting of the Working Group on Aquatic Carcinogens, National Institutes of Health, Bethesda, M.D.

Aug. 26-30 Carolyn Barszcz attended the American Society of Clinical Pathologists workshop on Histological Topics in Chicago, ILL. Aug. 26-30.

The Metals Project is devoted to realistically assessing metals as a problem in the marine environments. Field methods for determining metal levels in water, sediments, pore water, and biota are being developed and standardized for investigation of problem areas as they are identified. A matrix of existing toxicity and body burden data using animal species (including various life stages) as one axis and metals (including various species, chemical states, and modes of application) as the other is being created as a secondary goal by the Metals Project. The matrix will define the data base currently available for criteria decision making; it will point out information gaps in animals, their life stages, and metals and their various states, thereby defining needed research goals; and it will provide a basis for comparing metal levels and their modes of application in laboratory toxicity studies with levels and pathways defined in metal-problem-areas in the natural environment.

Metal levels and their natural "modes of application" (chemical states and combinations in seawater and food chains) derived from field studies will be applied to laboratory bioassays through close coordination with the Bioassay Systems Project. The results of this approach will be to assess, broaden, and validate the data base needed for metal criteria decision making.

ROAP 16-AAT (Previously 21-AKF - Task 21, 23): Criteria for heavy metals to protect estuarine and marine life.

ROAP OUTPUT: To determine the sources, fate and effects of heavy metals discharged into estuarine and coastal areas in order to recommend maximum allowable concentrations of metals that are not hazardous to marine biota or man via marine food chains.

I. ROAP Approach 16 AAT, All Tasks except 018: Construct matrix of toxicity data for metals and aquatic biota available from literature, using in-house research grants and other sources.

A. Metals Matrix

A matrix of existing toxicity and body burden data using

marine species (including various life stages) as one axis and metals (including various forms, chemical states and modes of application) as the other has been formulated by Marcia Barry, George Hartson, and Earl Davey. The matrix to date consists of information from the Metals Bibliography, Water Quality Criteria 1972 prepared by the National Academy of Sciences, in-house bioassays and experiments and more recent literature. We are now in the process of attempting to computerize this data base and continuously update this information with the help and cooperation of Don Worley and Joe Wilson of Data Systems Division, Office of Administration, US-EPA, RTP, N.C.

The metals matrix indicates that there is information on only 36 elements out of a possible 104. Of these, only 18 have toxicity data listed and of the 18, only Hg is sufficiently documented to perhaps formulate good criteria. Consequently, the metals matrix is now being utilized to suggest metals and their respective forms to be applied to in-house testing by the Bioassay Team.

B. Metals Bibliography

Dr. Eisler and student aide Maryjane Wapner are preparing Volume 2 of "Annotated Bibliography on Biological Effects of Metals on Aquatic Environments". A total of 725 references have been collected on toxicological, physiological and metabolic influence of stable and radiolabeled chemical species of metals to marine, estuarine, and freshwater fauna and flora. References are annotated and each is indexed by metal, taxa, and by author in cumulative indices which encompass the present report and the initial report in this series (published as EPA Report R3-73-007). The manuscript is now complete and a contract has been let for final typing. A target date of April 1 has been set for submission of the completed work to the Director, NMWQL. Publication will be in the US EPA Ecological Report Series.

II. ROAP Approach 16 AAT - Task 018: Formulate and implement biogeochemical laboratory and field investigations for marine waters impacted by high metal use point sources.

A. Biogeochemical impact of Quonset Point Naval Air Rework Facility (NARF) on Narragansett Bay, Rhode Island.

All marine samples taken during the autumn of 1973 from NARF have finally been completely analyzed for selected heavy metal concentrations by atomic absorption and/or neutron activation analysis. Statistical and graphical analysis beyond the preliminary observations summarized in the January-June 1974 semi-annual report are currently being prepared for publication. Highlights of our results are as follows:

1. Concentrations of Ag, Cd, Cr, Cu, Ni, Pb and Zn in acid-soluble components of sediments were highest at stations nearest the NARF discharge outfalls and lowest at the more distant stations. Some metals are distributed homogenously throughout the 50 cm core (Cd, Co, Fe, Mn), but all others concentrated in the upper (0-5 cm) sediment component.

2. Highest values recorded for interstitial waters from the study are in µg/ℓ (ppb) 7048 for Mn, 2351 for Zn, 559 for Fe, 55 for Pb, 46 for Ni, 44 for Cu, and <1.0 for Cd. Content of Mn, Zn, and Cu in extracted pore waters were significantly higher in upper (0-5 cm) than lower fractions; Pb, Ni and Fe were distributed homogenously.

3. Concentrations of selected metals in µg/ℓ (ppb), from bottom waters ranged from 0.1 to 0.38 for Cd, 0.10 to 0.41 for Co, 2 to 5 for Pb, 0.13 to 1.94 for Cu, 0.82 to 3.88 for Zn, 0.14 to 5.81 for Fe, 0.75 to 7.96 for Mn, and 1.07 to 9.08 for Ni. These bottom water values were 1 to 3 orders of magnitude lower than similar data for interstitial sediment water and 3 to 7 orders of magnitude lower when compared to NARF sediment data.

4. Data on elemental composition of biota from the NARF study area are now complete. These data will be analyzed and interpreted in terms of the surrounding sediment and water data.

In connection with the NARF study, Mr. Greg Telek reports neutron activation analysis has been completely automated with the application of automatic sample changers coupled either to a tape deck or disc for data storage. Data reduction is done by a computer program which corrects for flux variations, decay and gives the final results in ppm for five elements. The program can be readily altered to determine any number of elements.

Two separate systems are available for the counting of two samples simultaneously on a continuous 24 hour basis. Therefore, as many as 96 sediment samples or 48 biota samples could be analyzed daily for at least 7 elements (assuming 1 hour count for biota and 1/2 hour for sediment).

The interferences involved in Zn determinations in sediment have been determined. Sc, Ta, and Europium have interfering peaks whose primary peaks can be used to subtract their respective additions to the Zn peak. An experiment design utilizing Eu, Sc, Ta spikes of orchard leaves has been formulated for evaluating the accuracy of eliminating the interference of these spikes in Zn determinations.

A manual for neutron activation analysis is being completed.

B. Mr. Bickford, a part-time employee of the NMWQL and a geochemistry Master of Science candidate at Brown University, is continuing on a project to establish whether chromium can be used as a flag for organic pollution from sewage treatment plants in Narragansett Bay. He has completed a series of analyses on seston (material which settles out of the water column) samples which were found to contain 5-12% organic matter and a carbon to nitrogen ratio of 6 to 8 irrespective of where the samples were collected in the

bay. Total chromium analyses of the seston indicated a clear gradient with values ranging from 60 ppm dry weight in the relatively clean lower bay to 350 ppm in the highly impacted upper bay. A peroxide (H_2O_2) digestion, which only oxidizes organic matter in the samples, was found to release 30% of the chromium from lower bay samples, 60% from mid-bay and 90% from upper bay samples. These results tentatively indicate that Cr could be used as a tracer of organic pollution from upper to lower bay stations.

In a related project, Mr. Bickford has developed a technique to partition between the plus-three and plus-six redox states of chromium in seawater. Plus-three chromium is taken up on the chelating exchange resin, Chelex-100; whereas, plus six chromium is taken up on tin ($SnCl_2$) impregnated Chelex 100. The efficiency of the resin uptake and elution was verified to be 99% by using the chromium radio-isotopes. $^{51}CrCl_3$ and $Na_2\ ^{51}CrO_4$.

Seawater samples were also taken at Mr. Bickford's stations by E. Davey and A. Soper for dissolved and particulate trace metal analysis of eight metals of both 0.5 m from the surface and 10 cm from the bottom. In general, all metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) particulate and/or dissolved forms, were found to decrease with distance from the highly impacted upper end of Narragansett Bay.

EPA Grant No. R804000-01-1

Title: History of heavy metal pollution in estuaries

Investigator: Dr. Edward D. Goldberg, Scripps Institute of Oceanography, LaJolla, California

Earl Davey, Richard Lapan, and Tom Bickford of the NMWQL participated with Eric Gamble and Kenneth Bruhland of Scripps Institute of Oceanography in the selection and collection of sediment box cores from Narragansett Bay during the last week in August 1974. Dr. Goldberg, in a recent quarterly report, stated that they have completed Pb^{210} geochronology on one Narragansett Bay core which showed a sedimentation

rate of about 1 cm/year and an accumulation of shells that coincides with the 1954 hurricane. Analysis with depth were completed for cobalt and silver which show that silver is accumulating atfaster rates now than 50 years ago while there were no enhanced cobalt fluxes to the sediment. They are now in the process of accumulating data on plutonium and other heavy metals.

Manuscripts

Eisler, R. 1973. Latent effects of Iranian crude oil and a chemical oil dispersant on Red Sea molluscs. Israel Journal of Zoology 22:97-105.

Eisler, R., G.W. Kissil, and Y. Cohen. 1974. Recent studies on biological effects of crude oils and oil dispersant mixtures to Red Sea macrofauna. In Proceedings of Seminar on Methodology for Monitoring the Marine Environment held in Seattle, Washington, Oct. 1973. U.S. EPA Report 600/4-74-004, pages 156-179.

Phelps, D.K., G. Telek and R.L. Lapan, Jr. 1973. Assessment of Heavy Metal Distribution within the food web. Proceedings of the second Int'l Symposium on Marine Pollution, San Remo, Italy.

Presentations

Davey, E.W. and A. Soper. Apparatus for the In-situ concentration of trace metals from seawater. Symposium in Analytical Methods in Oceanography sponsored by the Analytical Division of the American Chemical Society. Atlantic City, New Jersey, Sept. 8-13, 1974. Portions of the paper submitted will be published in the A.C.S. Advances in Chemistry series. Other parts have been submitted for publication to Limnology and Oceanography.

Miscellaneous

Dr. Ronald Eisler ahs been invited to present "Toxic, sublethal, and latent effects of petroleum to Red Sea macrofauna" at the 1975 International Conference on Prevention and Control of Oil Pollution, jointly sponsored by EPA, API, and the USCG. The proceedings of the conference will be published.

Dr. Eisler received a letter of commendation from A.C. Trakowski, Asst. Administrator for Research and Development, for his participation in the Section 307 (a) hearings of PL 92-500, involving water quality criteria for mercury, cadmium, and other metals.

Dr. Eisler participated in an EPA Technical Workshop held at Gulf Breeze, Florida, August 6-8, and assisted in the preparation of the report "Physical, Chemical and Biological Considerations with Respect to the Dupont Permit for Ocean Dumping of Industrial Wastes".

Dr. Davey participated in a pre-hearing on the possible ocean dumping of arsenical-wastes in EPA Region II by Whitmoyer Laboratories subsidary of Rohm and Haas at EPA Headquarters in Washington, D.C. on Sept. 5, 1974.

ROAP 21 BBG - Ecological Assessment of Ocean Dumping

This ROAP is designed to establish criteria for the survey and ecological assessment of existing ocean disposal sites as well as provide the information necessary for the appropriate selection of new dump sites. Emphasis is on establishing criteria for monitoring sites and adjacent areas for ecological compromise, investigating routes, rates and mechanisms for recycling of materials from disposal sites through biological uptake as well as by physical and chemical processes, establishing standard bioassay technology.

ROAP 21 BBG, Task 2 - The Movement of Trace Materials from Sewage Sludge--Rates, Routes, and Mechanisms into the Biota

This study is being implemented by an interagency agreement with Dr. Frank Lowman of the AEC Nuclear Science Center at Mayaquez, Puerto Rico. Contaminant fluxes from secondary sewage sludge including uptake and transfer by biota are being studied in flow-through seawater systems. This study is proceeding. Secondary sludge from New York City treatment plant is being used. Prime emphasis is on metals, but hydrocarbons and pesticides will be considered also.

ROAP 21 BBG, Task 3 - History of Metal Pollution in Estuaries

Dr. E. Goldberg of Scripps Institution of Oceanography is studying contaminant impacts in historical context. Dr. Earl Davey is project officer and he reports on this grant in the Metals Team section.

ROAP 21 BBG, Tasks 4, 8 - Bioassay Methods Development

These tasks are being implemented by ROAP 21 AKK (Techniques for Water Quality Criteria Development), Task 115 (Bioassay Methods for Ocean Dumping). Dr. Gentile's team cooperated with Dr. Tom Duke, Director of the EPA Gulf Breeze Environmental Research Laboratory, to compile a manual of standard bioassay techniques for the ocean dumping permit program. Dr. Gentile was involved directly with

several Regional permit actions, most notably Region III's permit to Dupont for dumping acid wastes.

ROAP 21 BBG, Task 5 - Standard Site Monitoring Methods

Three representative sites are being studied to develop site monitoring methods. These include a dredge spoil site, an industrial wastes site, and a sewage sludge site.

The industrial wastes (Dupont acid wastes) and sewage sludge (Philadelphia) sites offer a unique opportunity to test the concept of analytically "finger printing" a waste material (Fig. 1). Both wastes may be distinguished by their metallic content. In the last semi-annual report we reported data from a monitoring cruise made in the fall of 1973. We found that metals were accumulating in the mahogany clams, Arctica islandica, in and around both dump sites. The metals accumulating were those being dumped at one or the other sites. The distributional patterns of accumulation reflected site sources. The concept of using metal "tags" or "tracers" to follow the fate of dumped wastes was substantiated. In this report we include data from a monitoring cruise made in the spring of 1974. Metal levels were measured in mahogany clams, Arctica islandica (Table 1) and sea scallops, Placopecten magellanicus (Table 2). These data conclusively confirm our initial findings. Figures 2 thru 7 illustrate patterns of accumulation of vanadium, cadmium, and zinc in both species of shellfish found in the vicinity of these dump sites. These metals serve as useful tracers for the dumped materials. Four metals characteristic of the Dupont waste (Fe, Mn, V, and Ti) and five metals characteristic of the Philadelphia sludge (Cu, Ag, Ni, Cd and Zn) showed statistically significant distribution patterns in the spring 74 data (Tables 3 & 4). Some of these tracers displayed elevated concentrations in areas extending from the dumpsites to the limits of the area covered by the spring cruise. Vanadium levels in the tissues of the clam, Arctica islandica, and the scallop, Placopecten magellanicus, clearly show

this pattern (Figures 2 & 3). Presumably these animals were exposed to Dupont wastes. Prevailing currents sweep wastes southwest from the dumpsite. Significantly, vanadium tissue levels remain elevated at the farthest station southwest of the Dupont site. The spring 74 cruise was not extensive enough to permit delineation of the area influenced by Dupont's wastes. A future cruise will extend the sampling area in an effort to delineate the total area of effect.

ROAP 21 BBG, Task 6 - Dredge Spoils and Sewage Sludge in the Trace Metal Budget of Estuarine and Coastal Waters

Dr. James Simpson of Columbia University has a grant to develop field methods for assessing metal fluxes from natural sediments. Simpson's approach has been "to build up the components which are necessary to make trace metal budget calculations, based largely on field measurements in the Hudson Estuary and adjacent coastal waters." Initially he "employed natural tracers which have less complicated chemical pathways than trace metals, to establish some of the parameters needed to compute trace metal fluxes from sediments." These tracers include radon^{222}, methane, chloride, Ra^{226} and Ra^{228}. These measures are being correlated with water column and sediment metal concentrations in the estuary and bight areas.

ROAP 21 BBG, Task 14 - The Problems of Ocean Dumping -- Stability and Resiliency in Experimental Ecosystems Exposed to Constant and Time-Varying Stresses

This task is being implemented by a grant awarded to Dr. Scott Nixon of the Graduate School of Oceanography, University of Rhode Island. This project represents a cooperative effort between Dr. Kenneth Perez of this lab and Dr. Nixon. Dr. Perez reports details of this study in his section of this semi-annual report.

ROAP 21 BBG, Task 15 - Influence of Dredge Spoils and Sediment Pollution on Trace Metal Assimilation by Organisms

Dr. Michael Bender of the Graduate School of Oceanography, University of Rhode Island has a grant to develop methods to assess metal fluxes between sediments and overlying water in controlled laboratory simulations of ocean dumpsites. At this point emphasis is on analyzing pore waters, for metals content. As the expertise develops the complexity of the system studied will be increased by including macrobiota.

Technical Assistance

During the period July - December 1974 the Ocean Disposal Team responded to assistance requests from Regions I, II, III, IV, VI, and X. We would like to acknowledge particularly Dr. Jan Prager's assistance with our efforts to support Region III. He served on review panels for two important public hearings on ocean permit applications.

Publications

Lear, Donald W. and Gerald G. Pesch. Effects of Ocean Disposal Activities on Mid-Continental Shelf Environment off Delaware and Maryland. Environmental Protection Agency publication series number 903/9-75-015. January 1975.

FIGURE 1

AREA OF STUDY

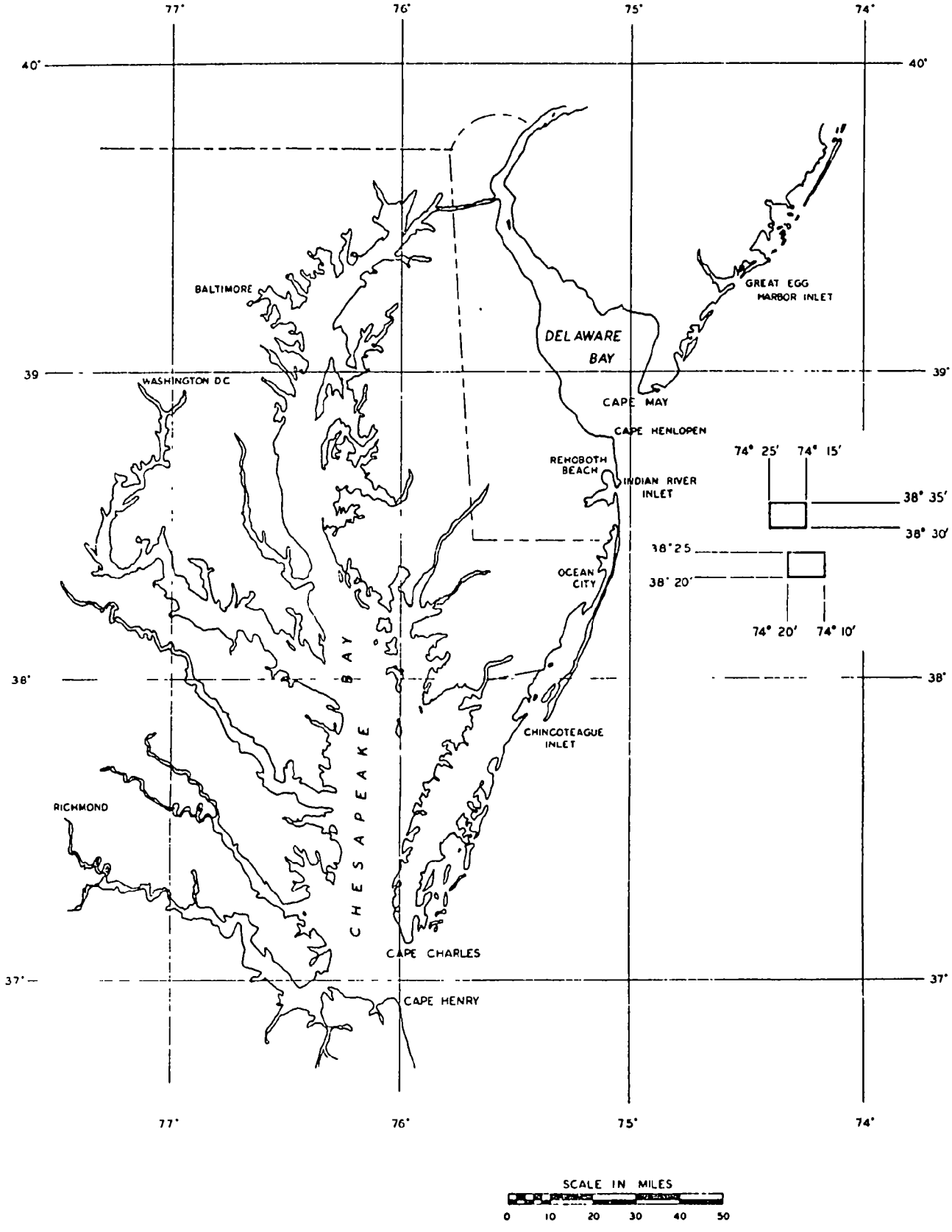


FIGURE 2

OPERATION "IDES"
ARCTICA
METAL CONCENTRATION
VANADIUM
ppm dry wt

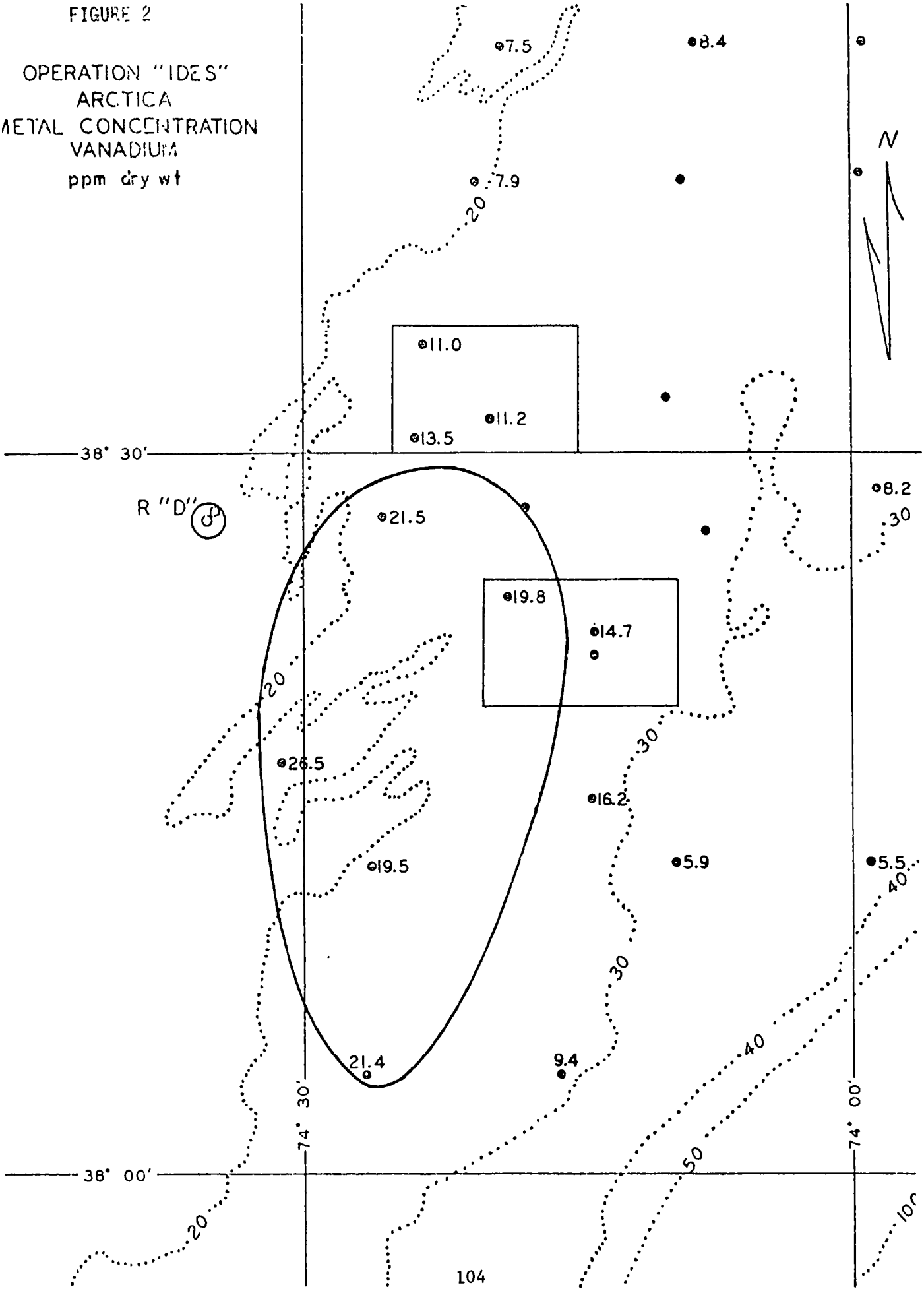


FIGURE 3

OPERATION "IDES"
SCALLOP
METAL CONCENTRATION
VANADIUM
ppm dry wt

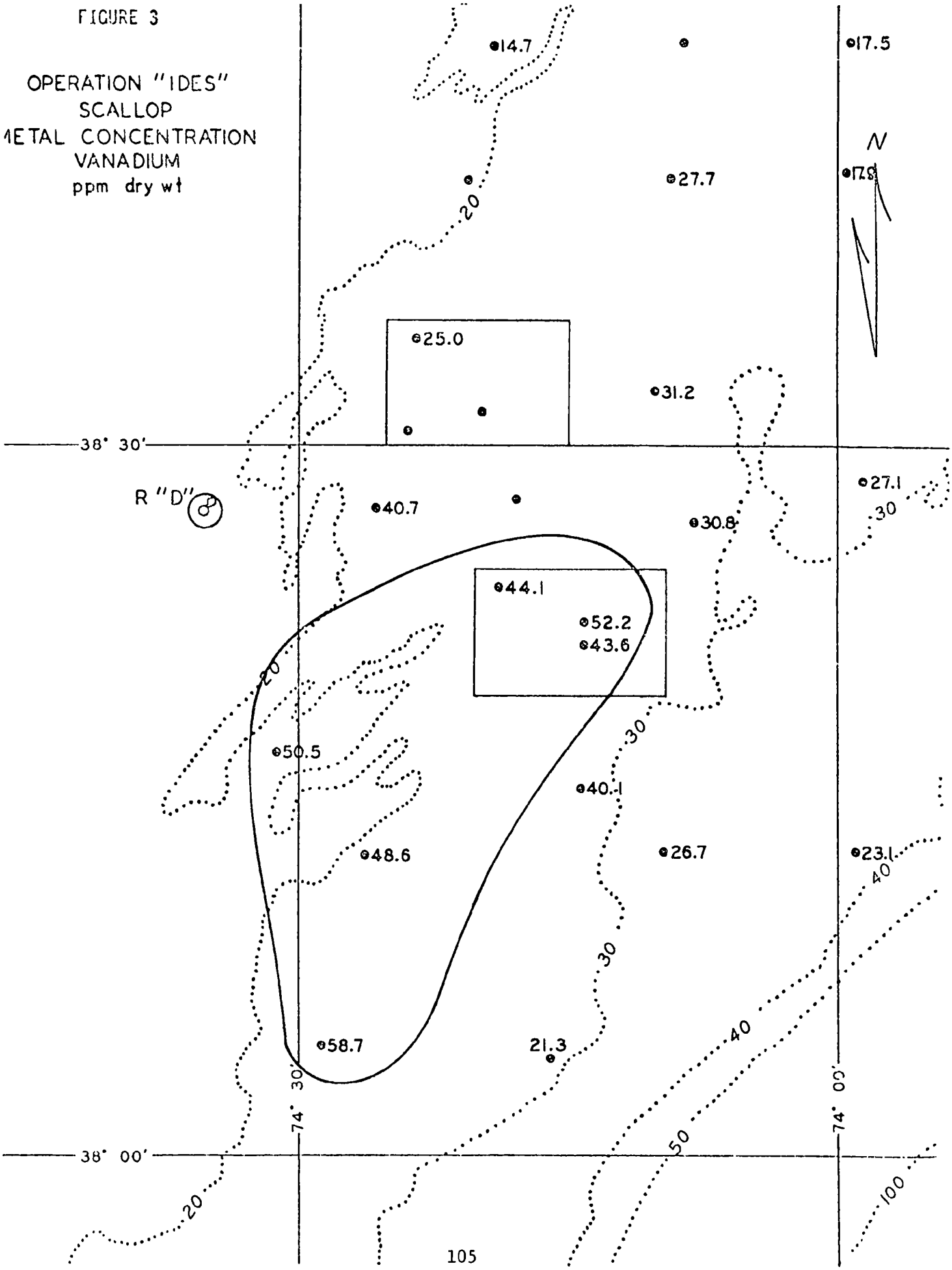


FIGURE 4

OPERATION "IDES"
ARCTICA
METAL CONCENTRATION
CADMIUM
ppm dry wt

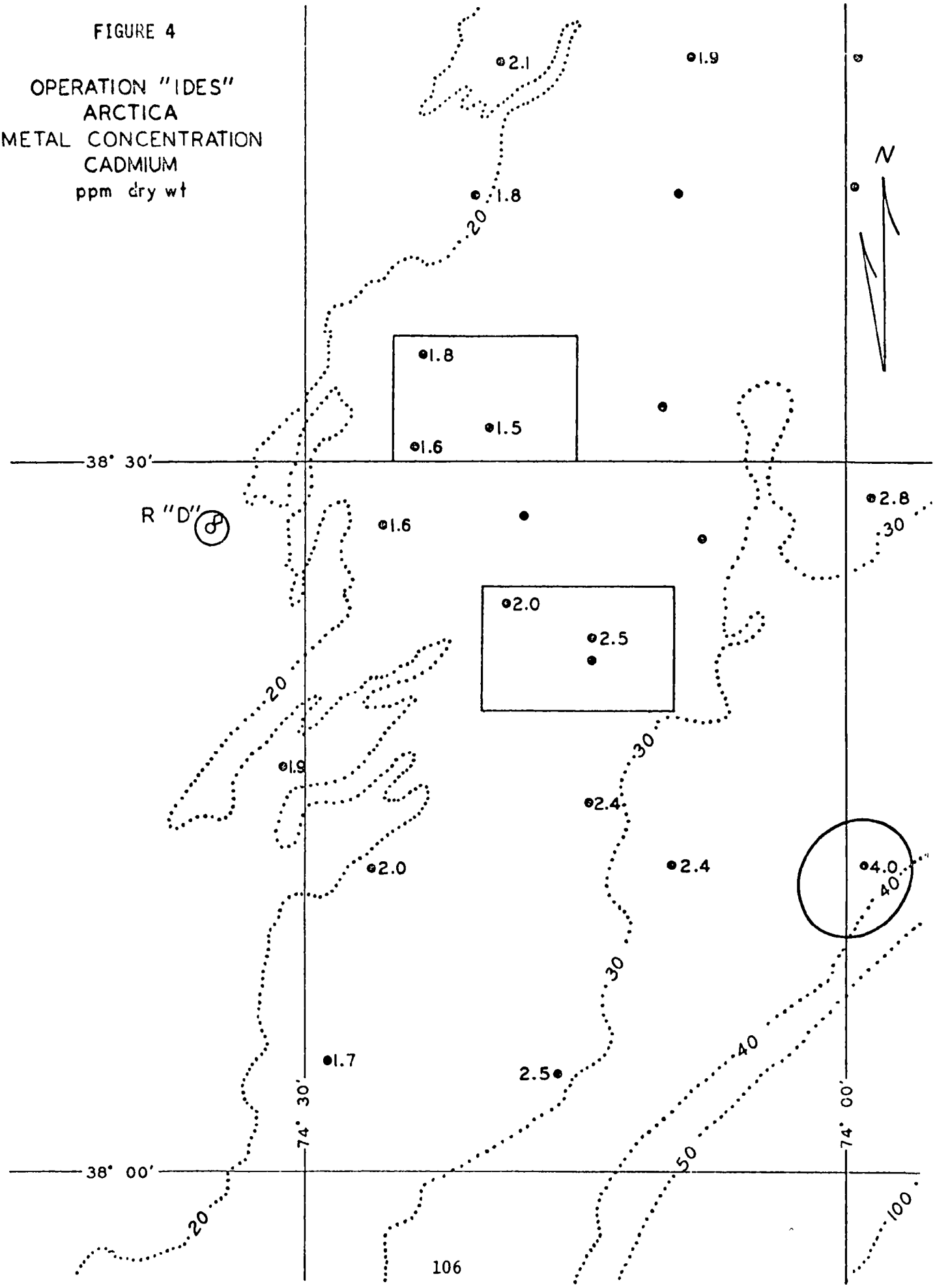


FIGURE 5

OPERATION "IDES"
SCALLOP
METAL CONCENTRATION
CADMIUM
ppm dry wt

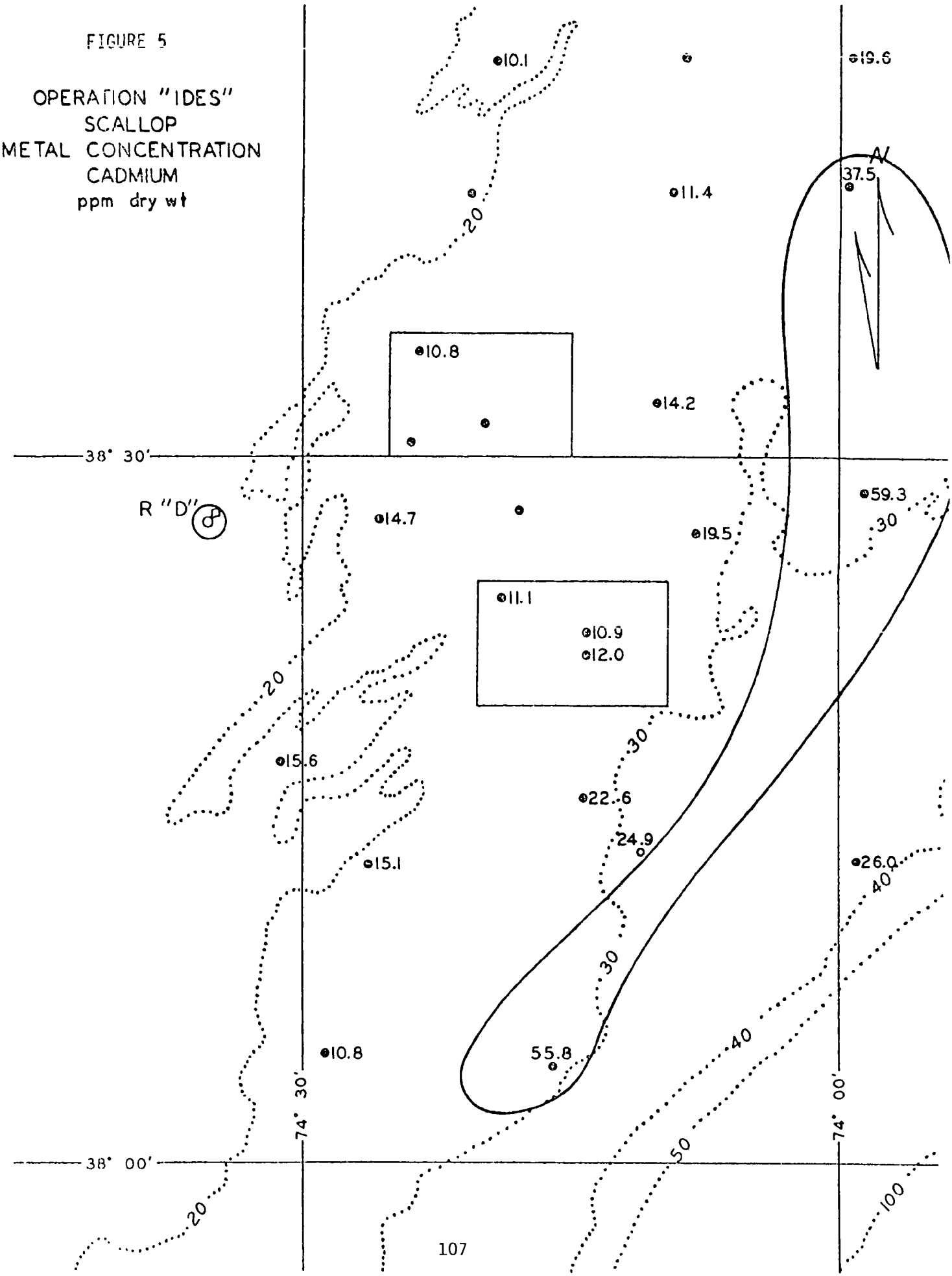


FIGURE 6

OPERATION "IDES"
ARCTICA
ETAL CONCENTRATION
ZINC
ppm dry wt

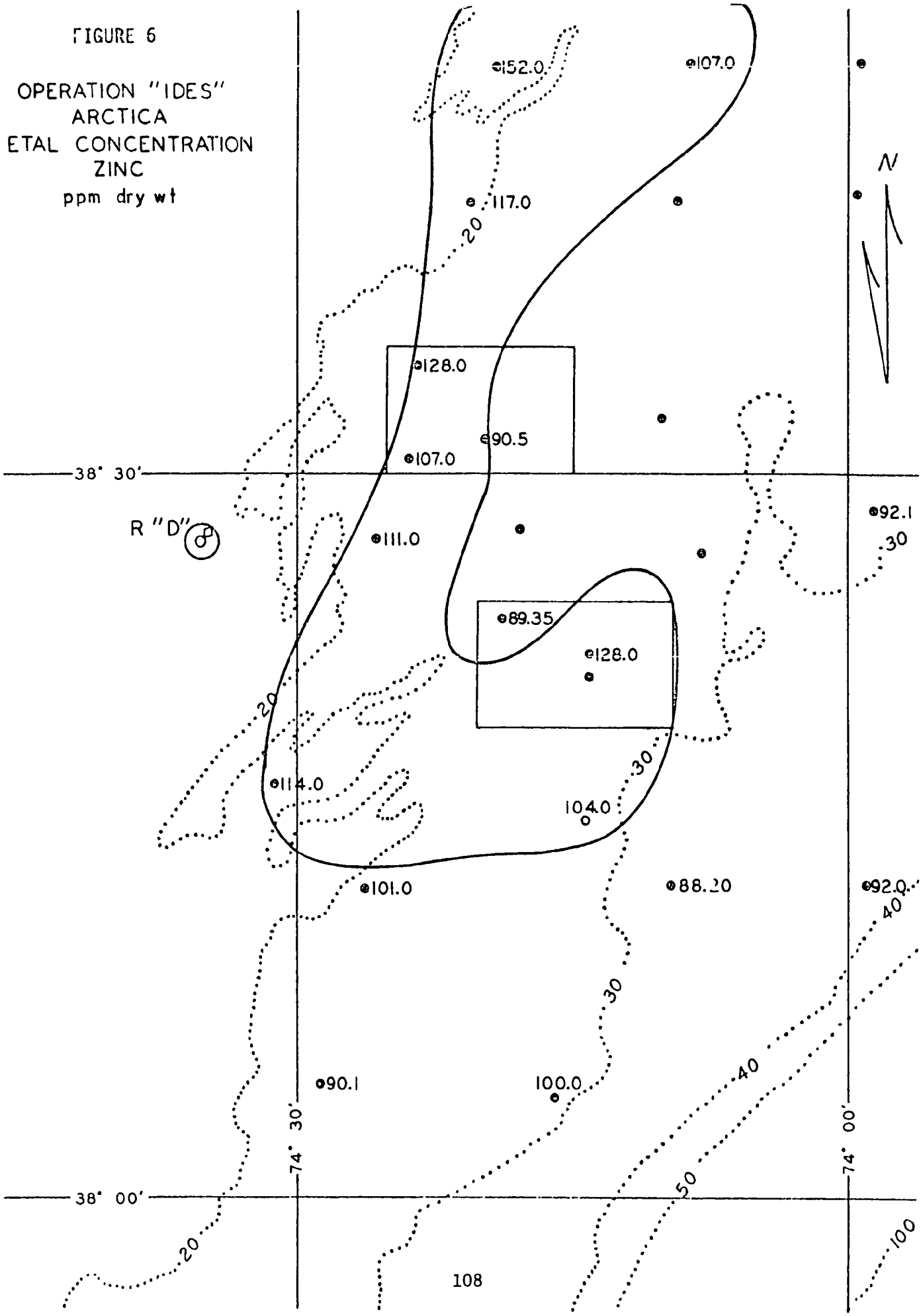


FIGURE 7

OPERATION "IDES"
SCALLOP
ETAL CONCENTRATION
ZINC
ppm dry wt

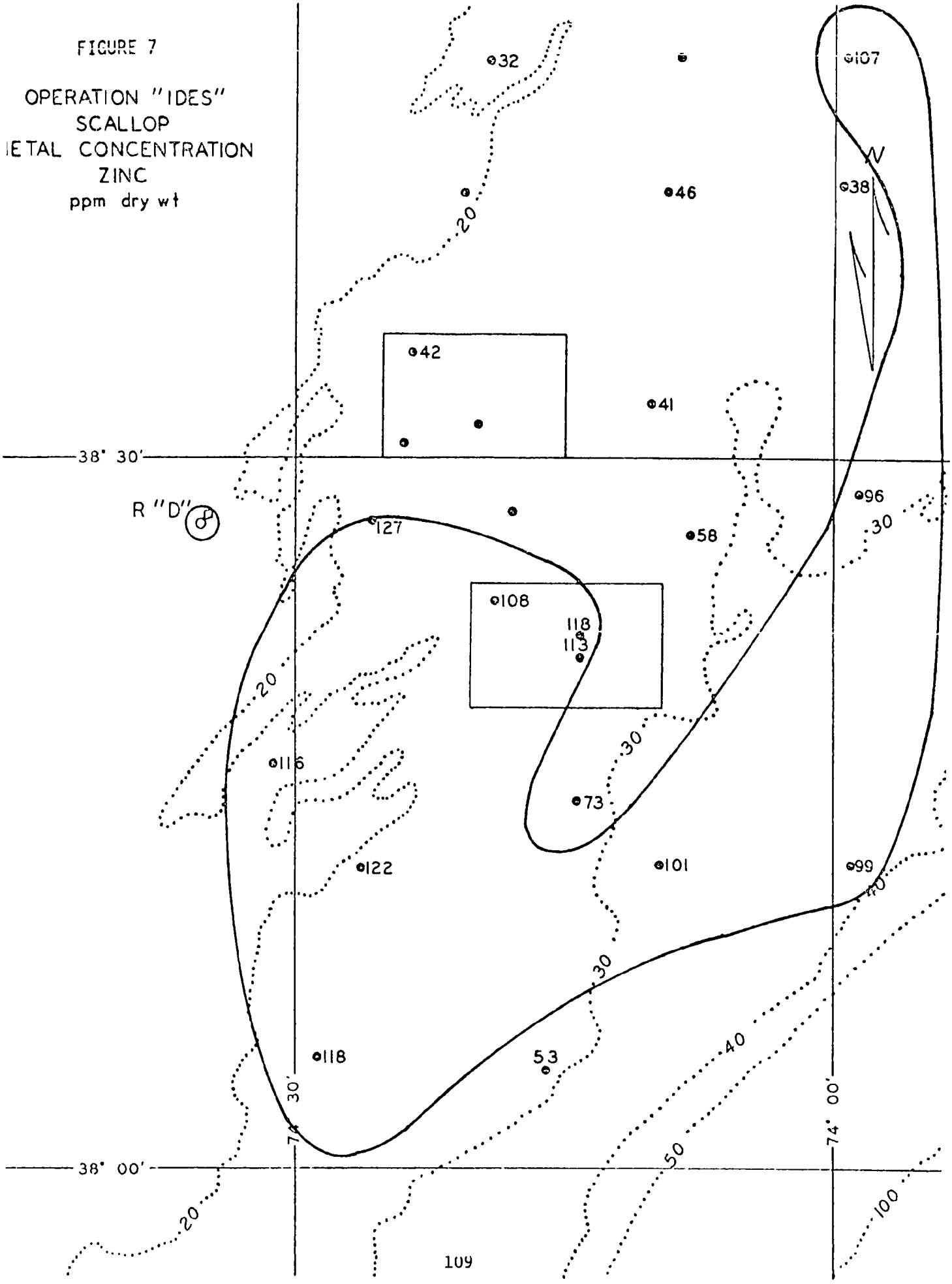


Table 1

OPERATION "IDES"

Metal Concentrations in Arctica
ppm/dry wt.

Sta/Reps.	V	Zn	Cd	Ag	Cu	Cr	Mn	Co	Ni	Ti	Al	Fe	Pb
2 (2)	14.66	128.5	2.52	1.53	9.55	4.13	9.63	1.09	11.59	6.55	103.4	438.6	6.11
9 (10)	26.46	114.4	1.73	2.33	9.73	3.99	19.89	1.07	6.63	5.20	125.4	354.7	3.61
14 (4)	8.18	92.1	2.78	1.11	7.04	2.95	9.39	1.17	13.36	2.54	40.6	264.3	6.08
17 (10)	19.49	100.7	1.98	0.85	7.89	3.65	14.03	0.83	6.77	3.53	38.0	320.0	4.92
20 (5)	10.95	127.7	1.77	0.76	9.67	4.92	26.09	1.32	9.56	3.49	60.1	562.1	6.47
22 (10)	21.48	111.0	1.62	0.76	10.36	5.04	13.83	1.12	6.47	6.35	162.5	469.6	3.09
24 (3)	16.20	104.5	2.36	0.69	8.02	4.09	5.20	0.87	9.11	1.62	36.3	142.9	2.41
25 (10)	5.46	92.0	4.01	1.41	8.34	5.26	7.98	0.68	10.80	2.40	103.5	281.0	5.33
26 (2)	21.44	90.0	1.73	0.58	8.85	5.17	10.96	0.57	4.21	4.79	87.6	290.6	3.44
27 (10)	9.41	100.1	2.46	1.09	6.59	4.80	10.07	1.01	10.61	2.96	85.0	317.5	5.34
28 (6)	7.47	152.4	2.08	1.77	11.31	5.67	16.95	1.56	11.73	3.01	48.6	261.3	3.57
29 (5)	8.38	107.5	1.91	0.66	7.63	4.03	17.98	1.23	8.85	3.36	65.0	288.5	4.14
A (4)	7.93	117.2	1.77	0.62	9.79	4.77	11.14	1.34	8.34	7.09	112.0	417.1	4.21
B (5)	13.46	107.3	1.61	0.73	9.55	6.07	22.37	1.06	7.79	20.38	341.1	562.4	4.51

OPERATION "IDES"

Metal Concentrationslin Arctica (continued)
ppm/dry wt.

Sta/Reps.	V	Zn	Cd	Ag	Cu	Cr	Mn	Co	Ni	Ti	Al	Fe	Pb
C (6)	11.23	90.5	1.48	0.83	8.86	5.18	12.85	0.72	7.35	7.20	121.6	480.4	5.89
E (7)	19.78	88.2	2.04	0.67	8.60	4.21	11.15	0.79	5.26	6.98	161.0	449.8	4.24
F (10)	5.86	89.4	2.41	0.65	6.64	4.02	10.61	0.95	10.53	8.77	183.5	492.3	3.54

Table 2

OPERATION "IDES"

Metal Concentrations in Scallops
ppm/dry wt.

Sta/Reps	V	Cd	Zn	Ni	Cu	Cr	Ag	Ti	Mn	Al	Fe	Pb	Co
28 (3)	14.66	10.06	31.59	1.10	4.79	1.28	0.46	7.35	17.11	144.7	575 7	1.22	0.33
18 (8)	27.69	11.44	45.52	1.91	5.16	2.76	0.71	17.27	41.76	505.9	1409.7	2.45	0.65
20 (2)	24.99	10.80	42.08	1.65	5.63	2.01	1.11	16.30	22.72	312.5	682.4	1.64	0.78
21 (4)	31.22	14.21	40.50	2.06	5.25	2.90	0.64	8.68	29.26	202.8	1180.8	2.06	0.43
23 (6)	30.75	19.55	57.77	4.34	8.30	2.08	1.24	9.26	11.95	139.1	250.9	11.15	0.66
27 (4)	21.29	55.88	53.43	1.35	6.28	3.26	0.43	9.19	22.83	204.1	1110.0	6.91	0.54
19 (3)	17.93	37.51	38.50	1.06	4.27	2.85	0.39	4.98	32.17	151.9	564.8	1.84	0.48
24 (10)	40.15	22.56	73.00	1.97	6.14	1.17	0.43	5.48	18.48	155.0	291.1	1.54	0.58
25 (2)	23.08	25.96	98.59	4.19	6.73	0.89	1.13	6.21	12.86	126.5	243.4	3.73	0.76
30 (3)	17.53	19.60	107.48	1.03	4.01	1.42	0.50	8.47	30.36	228.8	314.1	1.69	0.38
F (10)	26.66	24.87	100.70	2.99	6.81	4.56	0.81	26.22	63.21	903.9	852.9	3.96	1.02
14 (8)	27.13	59.28	95.90	1.89	6.69	3.12	0.48	12.00	32.76	617.1	299.3	1.89	0.65
2 (5)	52.16	10.95	118.03	14.67	12.66	2.66	9.08	5.02	16.11	92.4	190.5	2.45	0.52
8 (10)	43.60	11.98	113.32	7.14	8.83	2.49	3.76	6.34	27.29	183.8	380.1	6.48	0.35
17 (9)	48.56	15.12	121.71	6.00	8.81	4.19	2.15	10.97	38.77	774.9	342.1	3.99	0.41
26 (5)	58.65	10.79	117.95	13.65	9.50	6.88	4.93	1.06	13.01	78.2	285.9	5.78	0.69

OPERATION "IDES"

Metal Concentrations in Scallops (continued)
ppm/dry wt.

Sta/Reps.	V	Cd	Zn	Ni	Cu	Cr	Ag	Ti	Mn	Al	Fe	Pb	Co
9 (3)	50.55	15.60	116.33	2.08	7.54	2.64	0.45	4.53	24.26	91.9	260.7	2.52	0.29
E (10)	44.12	11.08	108.47	2.64	6.31	3.31	0.58	6.03	28.81	200.5	400.1	2.80	0.48
22 (10)	40.65	14.70	127.35	6.33	8.63	4.05	2.96	6.82	26.88	166.3	249.8	1.83	0.45

Table 3

Analysis of Variance Results for Metal Levels in *Arctica islandica* Collected in Spring 1974 in Vicinity of Delaware Dumpsites

Metal	F level	Significance
Vanadium	33.54	.01
Zinc	6.34	.01
Cadmium	5.12	.01
Silver	3.07	.01
Copper	3.02	.01
Chromium	2.80	.01
Manganese	2.74	.01
Cobalt	2.25	.01
Nickel	1.95	.05
Titanium	1.92	.05
Aluminum	1.62	--
Iron	1.36	--
Lead	1.29	--

Table 4

OPERATION "IDES"

Metals in Scallops
ppm/dry wt.

Metal	F ratio	Significance
V	11.885	.01
Cd	6.896	.01
Zn	5.376	.01
Ni	5.300	.01
Cu	4.194	.01
Cr	3.130	.01
Ag	3.096	.01
Ti	2.832	.01
Mn	2.347	.01
Al	2.088	.05
Fe	1.787	.05
Pb	1.434	--
Co	1.117	--

ROAP 16-AAV

The mission of the Oils Team is to determine the fate and effects of oils and petrochemicals in the marine environment. Our goals are to generate the data base from which defensible criteria can be written to protect the integrity of the marine ecosystem. To accomplish this, we have divided the problem into three somewhat arbitrary categories. These are the fate and effects of:

I. Spilled oil
II. The water-soluble fractions of oils
III. Oil-contaminated sediments

I. Spilled Oil

A. Field Work

Our program on the fate and effects of whole oil spilled in the marine environment continues to be primarily a grant and contract activity. A large contract with Mississippi State University on the fate and effects of crude oil added to a gulf coast estuarine environment is in its last year. A set of simulated ecosystem ponds has been constructed to study these effects in a field situation. Two ponds have been treated with Empire Mix crude oil and two have been maintained as controls. Over the previous two years, a series of laboratory exposures of oil to various organisms has been conducted. Various response parameters have been observed. The results of these laboratory observations are now being applied to the ponds in order to observe as many responses to the oil as possible. Some preliminary oil effects that have been noted are shifts in planktonic populations and increased incidence of disease in fish. These effects are being observed throughout the year to elucidate the chronic effects as well as the fate of the oil in these ponds.

We are also studying the effects of #2 fuel oil on clam (Mercenaria mercenaria) and oyster (Crassostrea virginica) thru a contract with the Virginia Institute of Marine Science. The animals

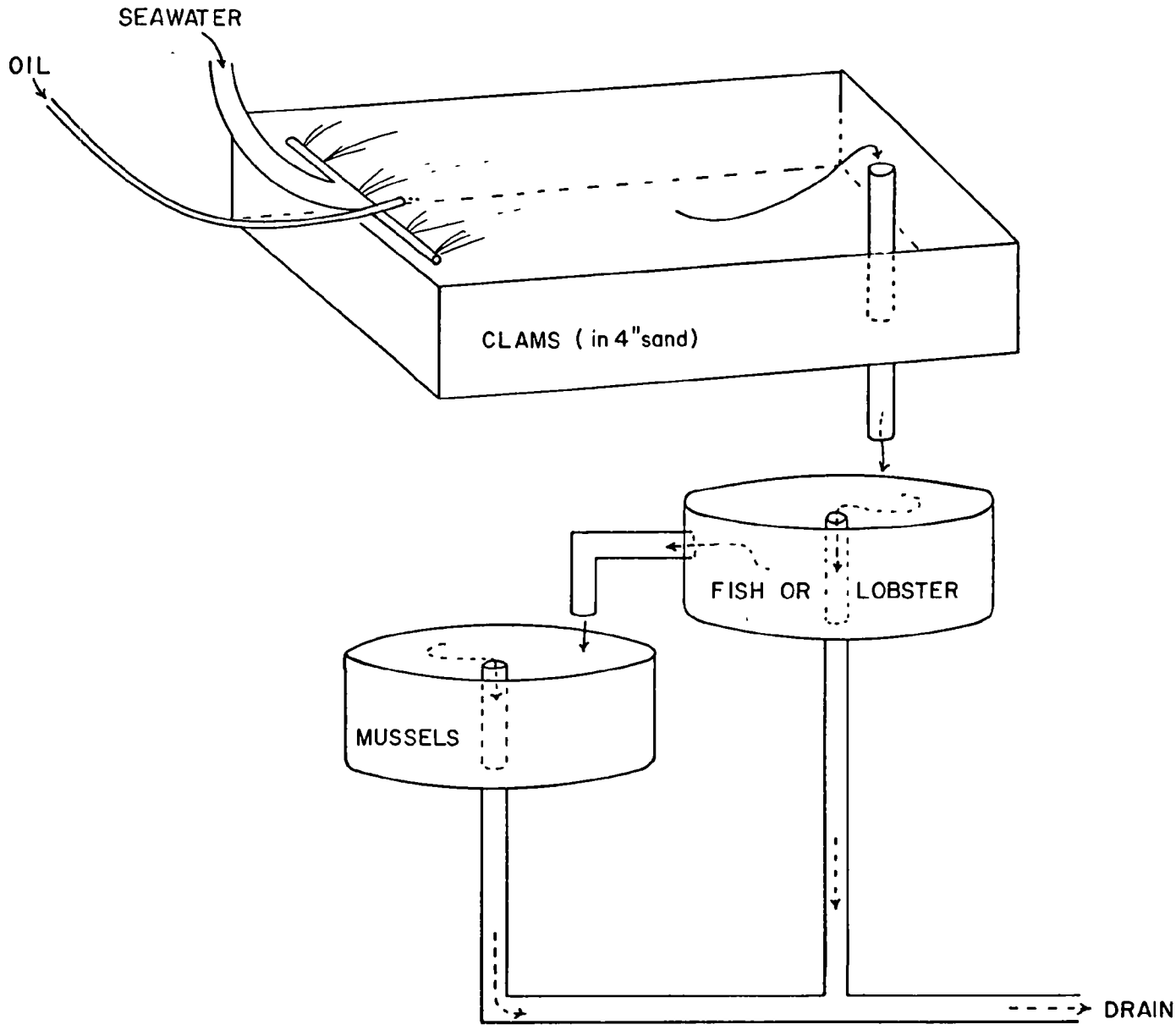
will be exposed to #2 fuel oil as in a spill situation in the field for a period of six months, with the effects on the animals being observed by histological examination. In addition, chemical analysis of the animals, as well as sediments and water, will be performed by gas chromatography and gas chromatography/mass spectrometry. This will give us some information on the fate of #2 fuel oil in the environment along with telling us how much #2 oil the animals were actually exposed to and how much they incorporated into their tissues. We had planned to conduct these studies this fall, but a number of problems delayed our spilling the oil until nearly December, which was too late in the season to initiate the study. The exposure is now set to start in the spring as soon as the water warms up.

B. Laboratory Bioassays

Our recent in-house bioassay work also has involved No. 2 fuel oil as the toxicant. We exposed the soft-shelled clam, Mya arenaria, for five months in the system shown in Figure 1. The exposure was on a continuous flow basis at ambient temperatures. An amount of whole oil equivalent to 10 ppm was metered into the top tank. This resulted in accommodated oil levels at mid-depth of between 0.1 and 0.2 ppm as measured by infrared absorbance. While the detailed histological examination of these animals is still underway, preliminary analysis shows the development of some lesions in the digestive diverticula.

During this same time we also exposed several other species to the No. 2 fuel oil effluent from the primary exposure chamber of the clam studies. Secondary exposures were conducted in two cylindrical fiberglass containers connected in series. Mid-water fuel oil concentrations were fairly constant at 1.8 and 1.5 ppm in the two tanks. Lobster (Homarus americanus) and Scup (Stenotomus chrysops) were exposed in the first tank, while mussels (Mytilus

FIGURE 1



edulis) and scallop (Argopecten irradians) were exposed in the second tank. Animals from these exposures are currently under-going histological evaluation. Dramatic behavioral responses were evoked by these exposures, including the absolute inability of the lobster to feed. Further studies with the system are planned.

A series of acute static bioassays of 96 hours duration were performed using No. 2 fuel oil as the toxicant and Atlantic silverside (Menidia menidia), shrimp (Palemonates pugio), and scallop (Argopecten irradians) as the test species. These tests were performed using standard methods in one gallon glass jars for comparison with the results obtained in continuous-flow systems. The results are shown in Table I.

TABLE I

Species	Temp, °C	48 Hr LC-50, ppm	96 Hr LC-50, ppm
Adult Atlantic Silversides	13	1000	370
4 cm " "	18	>5000	5000
Shrimp (Palaemonates)	13	180	70
" "	18	>340	340
Scallop	18		>500

II. The Water-Soluble Fractions of Oils

A. Field Effects

Our program to determine the fate and effects of the water-soluble (or accomodated) fractions of oil is also progressing with both in-house and out-house efforts. Discharges of these fractions have been largely ignored in the past since the major regulatory emphasis has been on the prevention of visible sheens. Although aesthetic considerations associated with sheens are important, it is also very important to control the potentially more toxic water soluble fractions. Our in-house effort is directed towards

the biological effects of the water-soluble fractions, while the fate of these fractions in the environment is being addressed thru an interagency agreement with the Navy. Our study with the Navy addresses the problems of the fate of dissolved and dispersed hydrocarbons in the effluent from ballast water processing plants. Such plants function primarily as oil-water separation facilities with the discharged water still containing all of the water accommodated fractions. The Navy is sampling water and sediments in the vicinity of several different ballast treatment plants to determine the extent of the plume in the water column and the rates of accumulation in the sediments. These data can then be correlated with the biological effects data that we are generating in the laboratory to delineate the magnitude of the impact of such a plant on an environment.

B. Biological Effects of Water-Soluble Fractions of Oils

The purpose of this phase of the oils program is to determine the biological effects of the water-soluble-fractions of oils. An in-house continuous-flow bioassay has been designed to expose a variety of organisms and their life stages to various concentrations of the water-soluble-fractions on No. 2 fuel oil on a long-term basis.

The apparatus consists of an oil-water separation chamber (Figure 2) wherein a constant inflow of oil and unfiltered sea water (~32% salinity and ambient temperature) is circulated over a series of baffles and mixed. The insoluble oil slick is skimmed from the surface and collected in a waste chamber, while the water-soluble-fraction of the oil is distributed to the various dosing tanks (Figure 3). The 55 gallon dosing tanks include a control and three experimentals, one containing 0.1 ppm fuel oil, another containing 1.0 ppm fuel oil, and a third containing 10 ppm fuel oil. The expected concentrations of oil within each of the

FIGURE 2

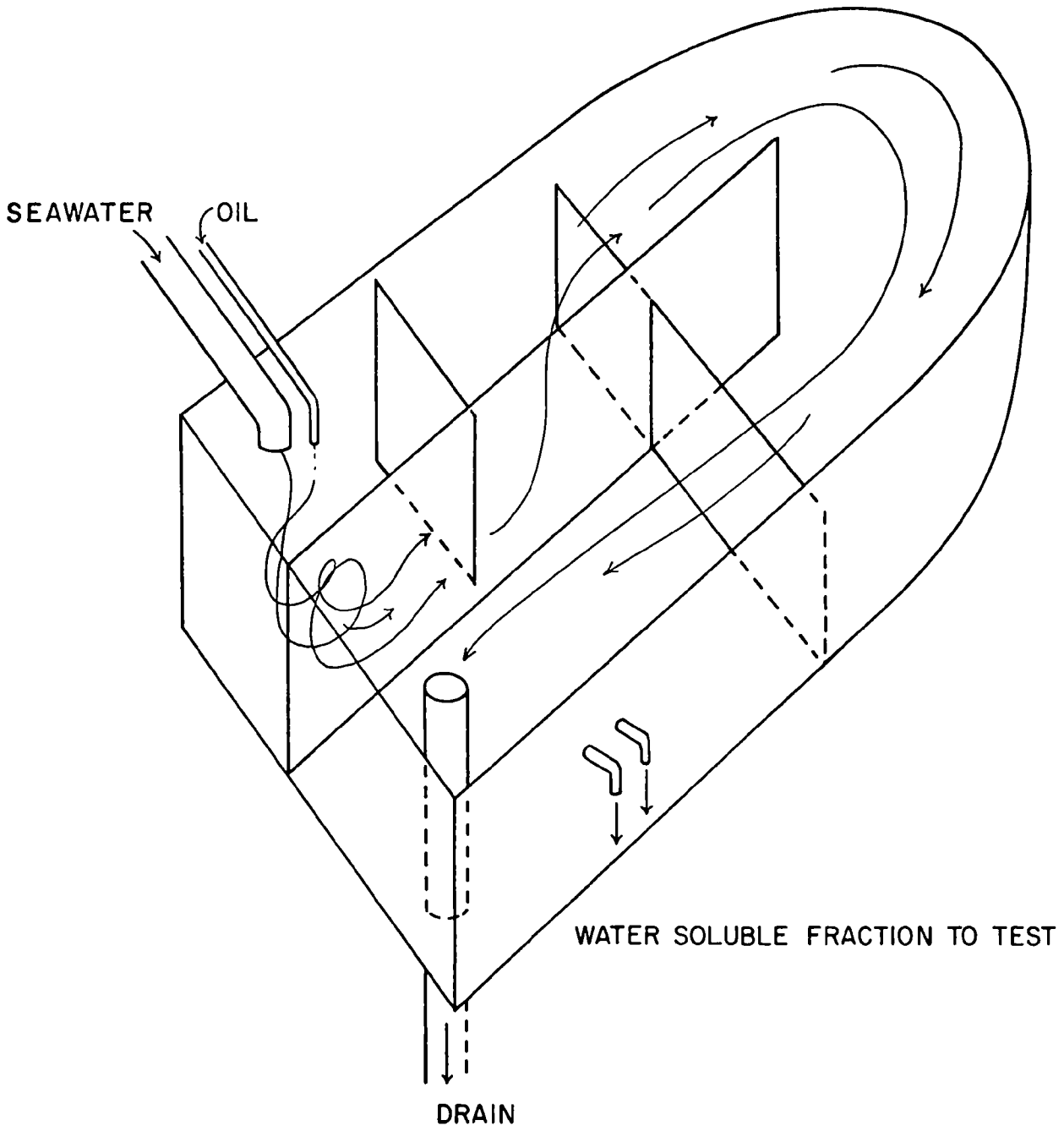
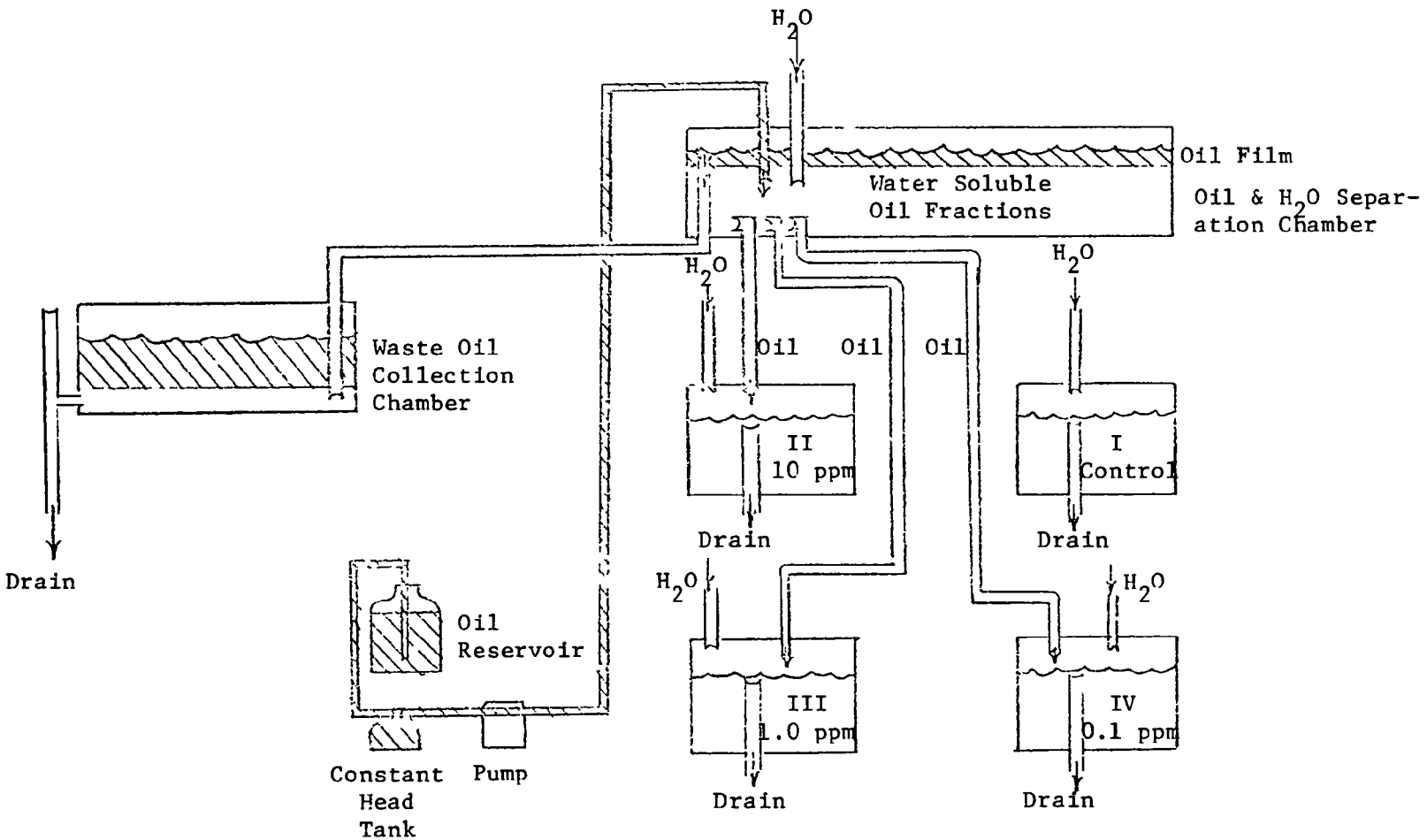


Figure 3

Continuous Flow Dosing Apparatus



experimental tanks are controlled by a metered flow of oil and water (total rate of flow within each tank=3ℓ/min), and are verified on a daily basis by infrared spectrophotometry. The characteristics of the water with respect to temperature, dissolved oxygen, pH, and salinity are also determined on a routine basis.

The test organisms exposed thus far have included adult grass shrimp, Palaemonetes pugio, juvenile quahogs, Mercenaria mercenaria, adult bay scallops, Argopecten irradians irradians, adult mud snails, Nassarius obsoletus, embryonic winter flounder, Pseudopleuronectes americanus, and adult stages of the marine polychaete, Capitella capitata.

Several parameters of response are being employed to evaluate the chronic and sublethal effects of the toxicant to the above organisms; for example, with the Mercenaria, Palaemonetes. Argopecten, and Capitella we are evaluating the animals histological condition. Furthermore, an attempt is being made to determine effects of fuel oil on the hatchability of winter flounder (Pseudopleuronectes americanus) eggs, and larval development. In addition, an attempt is being made to evaluate the effects of fuel oil on the growth rate of Palaemonetes by comparing the ratio of RNA to DNA in muscle tissue.

Behavioral studies are also being conducted on several of the test species. For example, daily observations are being noted on respiratory ventilation and feeding rate in the molluscs, Mercenaria and Argopecten, and on feeding activity, orientation, and general stamina of Palaemonetes. Further, the chemotactic response of Nassarius in the presence of water-soluble #2 fuel oil is being evaluated. We are also attempting to see whether or not fuel oil inhibits an escape response of the scallop, Argopecten, to the predatory starfish, Asterias forbesi.

Although our primary objectives are to evaluate sublethal and chronic effects of the oil, we have also kept a daily record of mortality throughout the exposure period (see Table II and Figures 4 and 5). The data reveal that the highest concentration, 10.6 ppm, is extremely toxic to the test organisms, causing 100% mortality of Palaemonetes within 6 days, Argopecten within 5 days, and Mercenaria within 20 days. The next lower concentration, 0.56 ppm, has caused a gradual increase in mortality of Palaemonetes, which has reached 32% thus far (i.e. after 72 exposure days).

Finally, we are analyzing the exposed test organisms for uptake of petroleum hydrocarbons. We are using gas chromatography to measure the amount of hydrocarbons taken up by the organisms, and using our gas chromatograph-mass spectrometer to verify the identity of the individual hydrocarbons. The sample preparation techniques for these analyses were described in the last semi-annual report (Jan-June, 1974; ROAP 16-AAV). These residue values will then be compared with the biological response data from the bio-assays to determine at what body-burden level of hydrocarbons the biological effects manifest themselves. We can then compare these body-burden levels with those of various field populations exposed to oil contamination to predict possible biological effects in the field populations.

TABLE II

Summary of Percentage Mortality in Response to the Water-Soluble Fraction of No. 2 Fuel Oil to Date (1/16/75) (72 Exposure Days Unless Other Wise Noted)

Expected Concentration	Actual Conc. (by I.R.)	Palaemonetes	Argopecten	Mercenaria
Control	0.006 ± 0.006 ppm	0.0	3.0(37 Days)	0.0
0.1 ppm	0.069 ± 0.029 ppm	1.0	0.0(37 Days)	0.0
1.0 ppm	0.56 ± 0.13 ppm	32.0*	10.0(37 Days)	0.0
10.0 ppm	10.6 ± 1.3 ppm	100.0* (6 days)	100.0(5 Days)*	100.0 (20 days)*

*STARTED VALUES INDICATE SIGNIFICANT MORTALITIES FOR WHICH CURVES HAVE BEEN GENERATED

Figure 4

Exposure Time Vs Mortality
for
Several Species in 10.6 ppm Water Soluble Fraction of No. 2 Fuel Oil

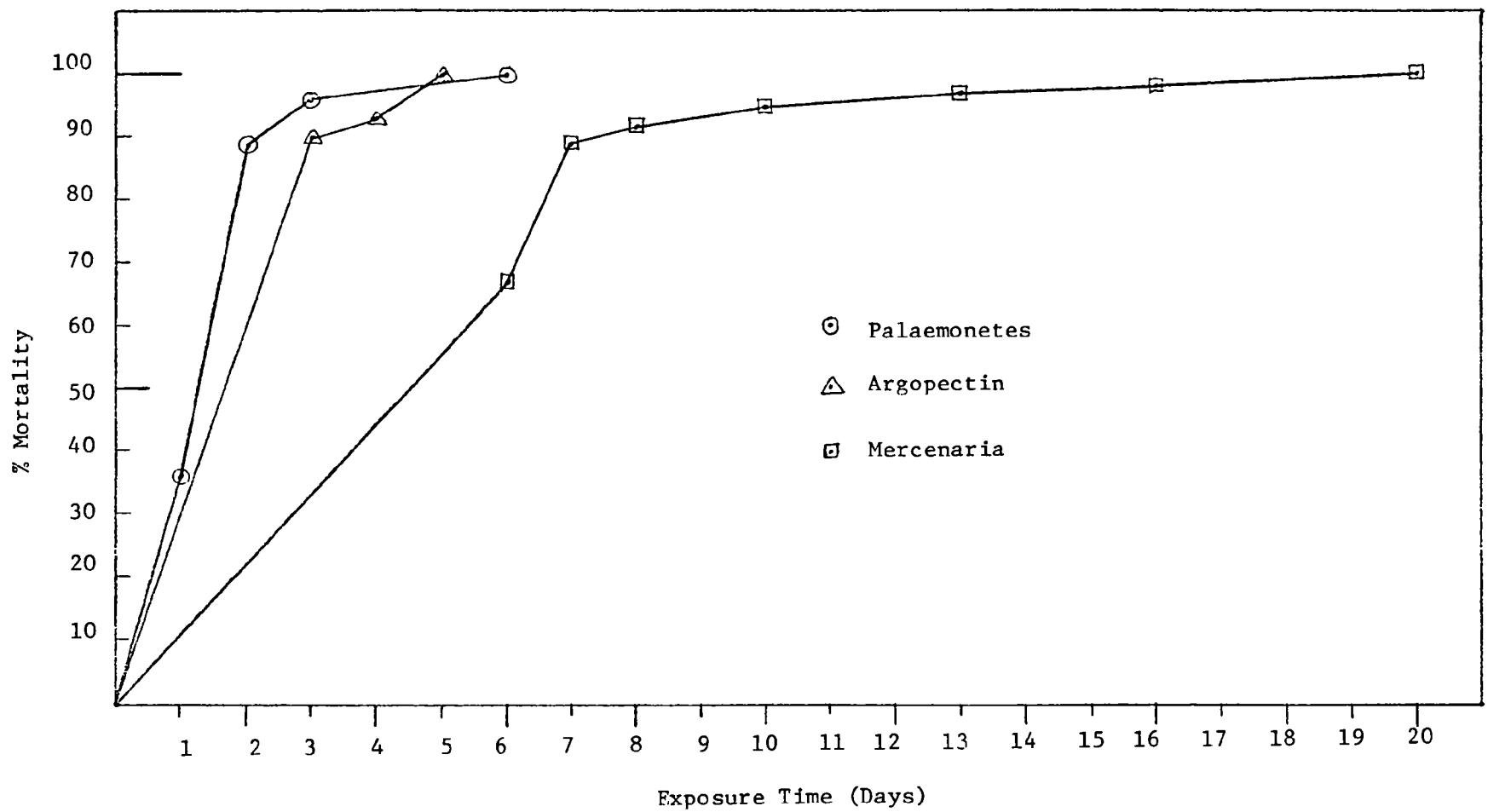
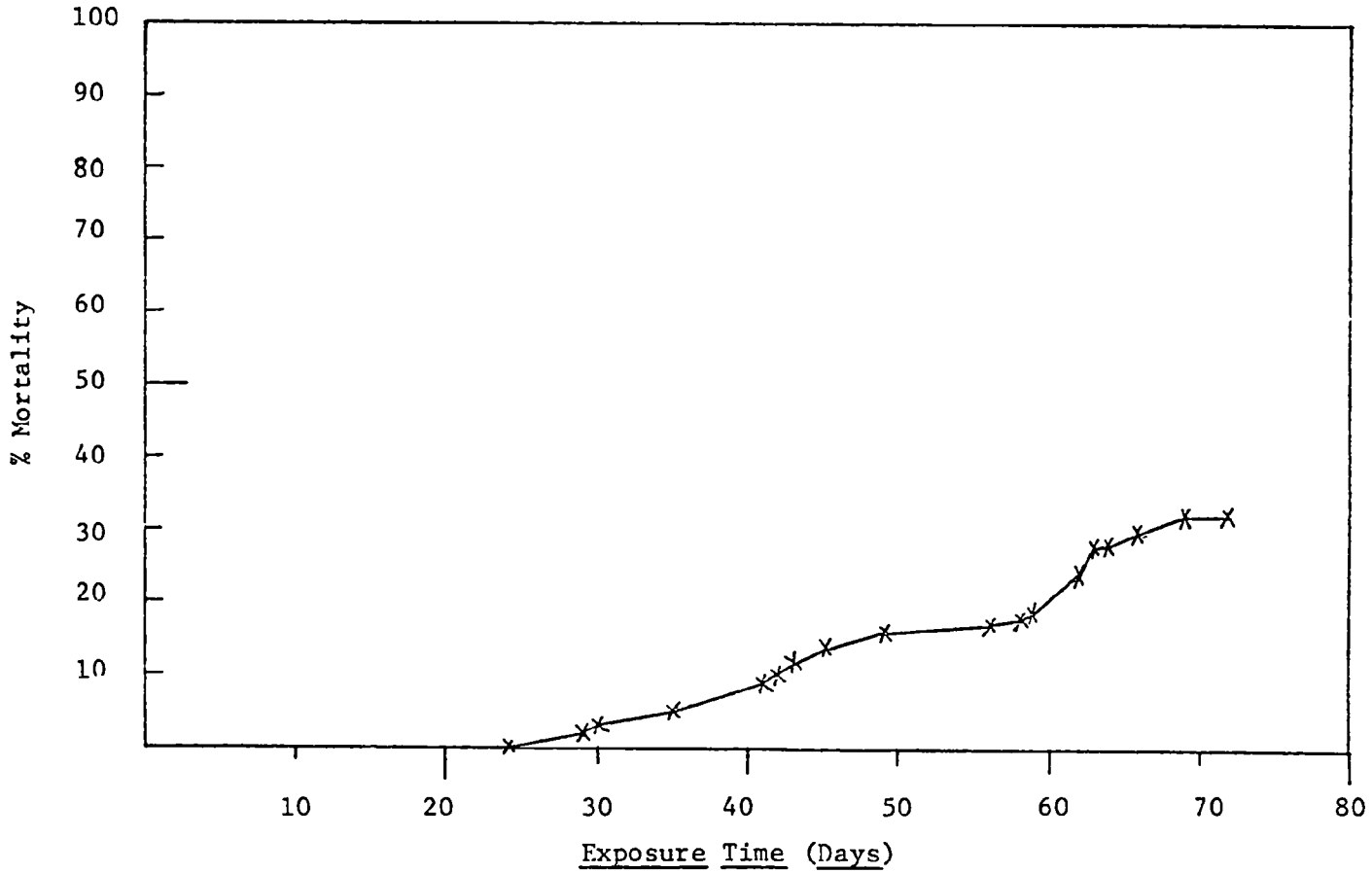


Figure 5

Exposure Time Vs Mortality

for

Palaemonates Pugio in 0.69 ppm Water Soluble Fraction of No. 2 Fuel Oil



It is also interesting to compare the mortality figures generated in the acute static bioassays to those from the continuous flow bioassay. It took less than two days for over 50% of both the grass shrimp and the scallops to die when exposed to 10 ppm of the water-soluble fraction of No. 2 fuel oil on a continuous-flow basis at 14°C. On the other hand, the static exposures were much less lethal. As can be seen from Table 1, the shrimp were roughly 20 times less sensitive and the scallops more than 50 times less sensitive in the static exposure system.

Some of the difference is of course due to the differences in the toxicants-we are comparing the water-soluble fraction of No. 2 fuel oil to the whole oil. In addition, one might expect some differences between continuous flow and static exposure for any toxicant. However, the magnitude of these differences requires further consideration because of the possibilities that criteria could be written and standards set on the basis of static exposures. One possible explanation of these large differences the typical use of aeration in static exposures. Petroleum hydrocarbons can readily partition between air and seawater, with the partition coefficient being heavily weighted towards the air. Thus, the aeration may effectively strip out the toxicant during the test period. In addition, the exposure concentrations reported in the two kinds of bioassays are not based on equivalent measurements. The exposure concentrations reported for most flow-thru bioassays are values that have actually been measured in the water column. This is not the case for most static bioassays, where the reported values are usually based only on the amount of toxicant added to the jar, not the amount that the test organisms are actually exposed to in the water column. The use of static bioassays is justifiable when only relative toxicity values are desired either for a variety of oils or a variety of organisms. However, if one is attempting to establish water

quality standards for the protection of an ecosystem then it is necessary to use flow-thru bioassays to establish the permissible levels.

III. Oil Contaminated Sediments

The third phase of our oils program deals with the chronic long-term effects of oil-contaminated sediments. The biological effects of this form of pollution have yet to be studied in detail, partly because the analytical methodology to measure low levels of petroleum hydrocarbons in sediments is still under development. We have a grant with Woods Hole to fund Farrington's continuing efforts to improve this methodology. In addition, we have a grant with URI to study the distribution of hydrocarbons in sediments and organisms in the vicinity of the Newport R.I. dredge spoil disposal site. This site received the dredge material from the Providence river about five years ago, and the material is known to have been contaminated with hydrocarbons. We are investigating whether the hydrocarbons have been mobilized and whether they have become incorporated into the surrounding biota. We suspect that hydrocarbons have become incorporated into the surrounding biota because of histological findings of masses of black particulate matter in the kidneys of Artica islandica taken from around the dumpsite.

Our final project in this area is a project funded jointly with NOAA to investigate the behavior of oil placed on the bottom in a tropical environment. This is being conducted by Dames and Moore, using saturation dives from an underwater habitat in the Bahamas. Oil is being mixed with several sinking agents and placed on the bottom. The oil can then be observed extensively during the dive, and its fate and gross effects within the area noted.

Analytical Services

During the past few months it has become obvious that the various people conducting routine analyses in our lab should be coordinated into a single unit. A major benefit from such a move is to generate a

viable quality control program to enhance defensibility of data generated by the lab. An additional benefit is that each team in the lab will not need a person trained in each technique or kind of analysis that the team requires to accomplish goals. We are currently offering heavy metals analysis by atomic absorption or neutron activation analysis, ATP analysis, and hydrocarbon analyses by infrared or gas chromatography and mass spectrometry. In addition, we plant to offer CHN and total organic carbon analyses on solid samples as well as micronutrients by Technicon auto-analyzer as soon as possible.

We have progressed the farthest with the metals analysis. We currently run all of the routine determinations of metals in sediments and tissues for the laboratory. We have been using nitric acid digestion techniques and feel that we have control over the tissue level determinations, but still have some unanswered questions with the sediment digestions. Our tissue determination methods have been checked using the National Bureau of Standards bovine liver as well as the Food and Drug Administration round robin oyster sample. Our results are summarized in Table III and graphed in Figures 6 and 7. We feel the agreement shown is quite satisfactory, although we seem to be a bit low on the iron in the liver sample. The NBS values are their provisionally certified values, but the FDA values are simply the averages from a variety of different labs. All results are in a μg per gram (μg/g) dry weight basis, with the range, standard error, and standard deviation shown on the graphs where applicable.

These are just our first attempts at ensuring the quality of our analyses. The problems are many, especially with the sediment samples because there are no available NBS-type reference materials. In addition, one has to consider that an added spike may not act in the same manner as the metal that may be tied up in complexes within the sample. Also, the natural variation within replicate

samples especially for organisms is very high, so that a large number of replications must be compared to find statistically valid differences. We are currently formulating plans to deal with these problems.

TABLE III

Metals Analysis of NBS Bovine Liver and FDA Oysters

	NBS Bovine, µg/g		FDA Oysters µg/g	
METAL	NBS	NMWQL	FDA*	NMWQL
Cd	0.27 ± 0.04	0.33 ± 0.03	2.06 ± 0.33	1.64 ± 0.03
Cu	193 ± 10	188 ± 2	24.8 ± 2.6	20.7 ± 0.2
Fe	270 ± 20	239 ± 4	-	-
Mn	10.3 ± 1.0	10.8 + 0.3	-	-
Pb	0.34 ± 0.08	0.43 ± 0.12	0.54 ± 0.34	0.61 ± 0.01
Zn	130 ± 10	134 ± 2	583 ± 86	523 ± 1

* Value given is average of 15 laboratories

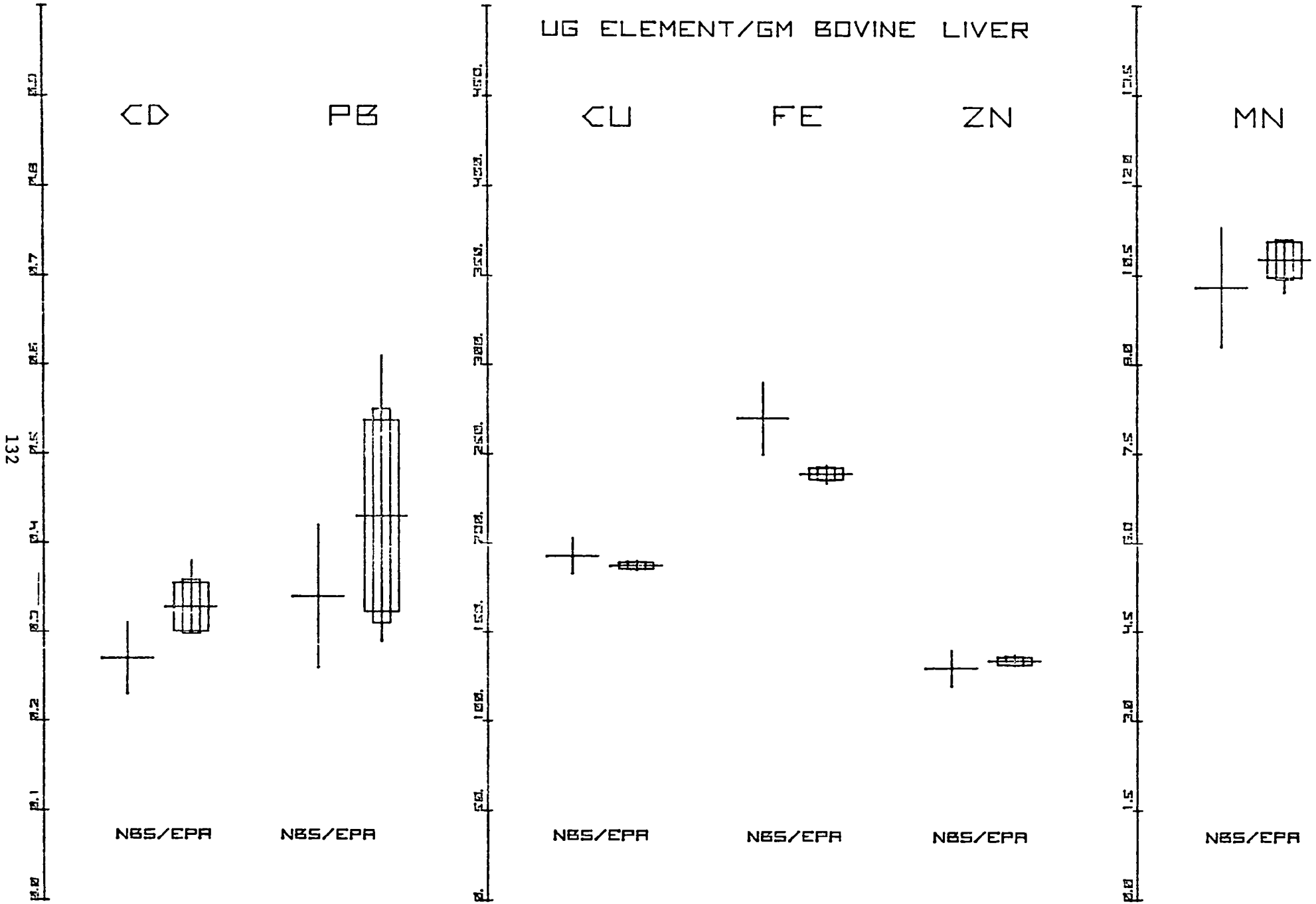

UG ELEMENT/GM BOVINE LIVER
CD
PB
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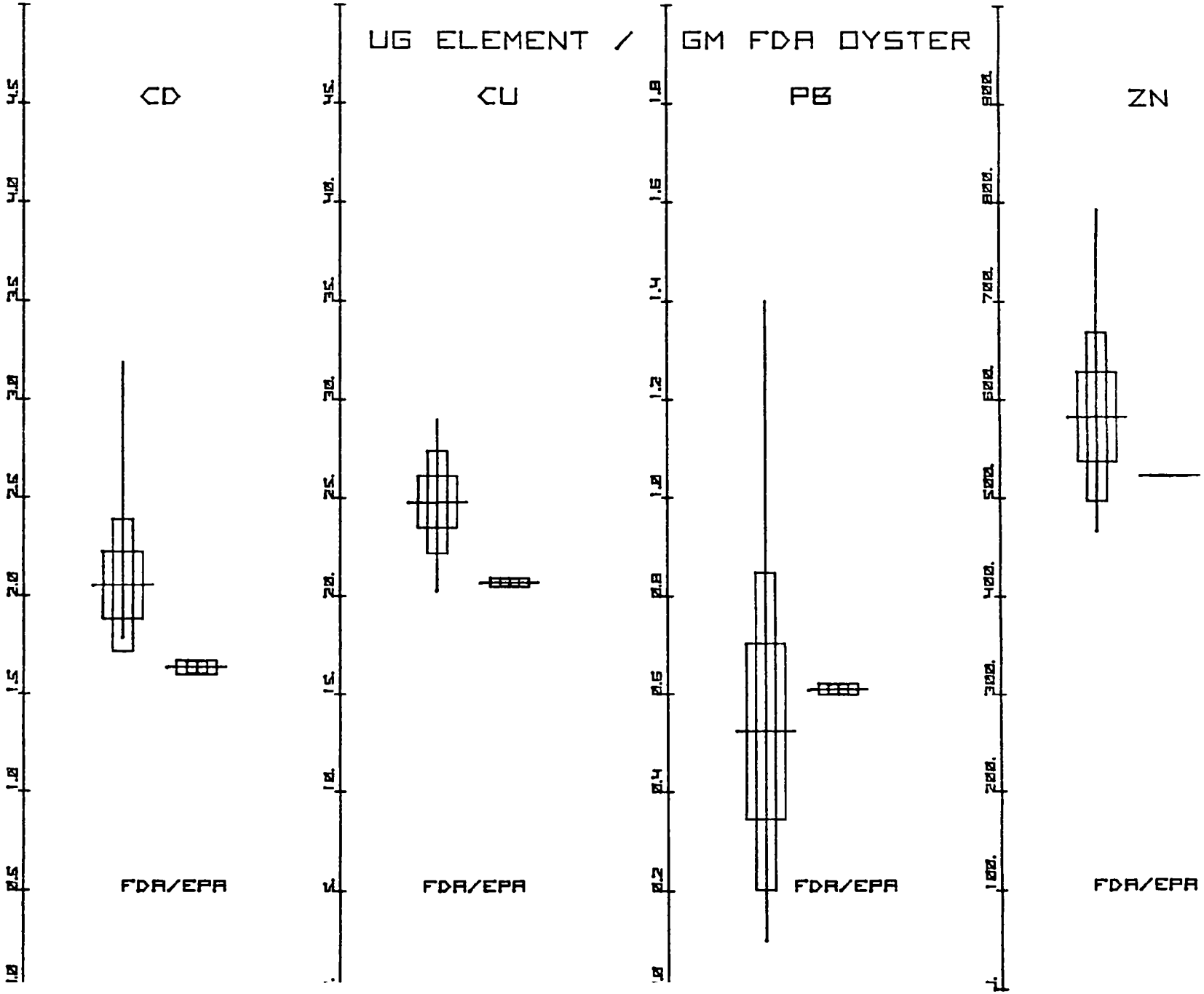
FIGURE 7


UG ELEMENT / GM FDA OYSTER
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ROAP 21 BEZ - Development of Criteria for Marine Recreational Waters

A statistically significant increase in the incidence of gastrointestinal symptoms among swimmers relative to non-swimming beachgoers was observed at the Coney Island ("barely acceptable") but not at the Rockaway beach ("relatively unpolluted"). Such was not the case with respiratory or "other" symptoms nor with the symptom severity index. However, the severity index (stayed home, stayed in bed, visited a physician) was higher among swimmers than among non-swimmers at the Coney Island beach. These findings were derived from about 8000 useable responses to illness inquiries obtained in the course of eight, Phase II, epidemiological-microbiological trials (in only 6 of the trials were enough responses obtained for individual trial analysis) conducted at the two beaches in the vicinity of New York City. Although the differential (swimmers less non-swimmers) gastrointestinal symptom rate at Coney Island was less than half that observed during the previous summer (pretest), because of the larger number of individuals queried (N), this essential observation from the previous summer's study was confirmed[1].

The decreased rate of gastrointestinal symptoms relative to that obtained the previous summer appears to coincide with decreases in the mean densities of *E. coli*, *Klebsiella*, fecal streptococci and *Aeromonas hydrophila*. The mean densities of the various potential indicators in the water are shown in Table 1. Coprostanol assays

[1] Cabelli, V.J., M.A. Levin, A.P. Dufour and L.J. McCabe. The Development of Criteria for Recreational Waters. *International Symposium on Discharge of Sewage from Sea Outfalls*, London, 28 August 1974.

are still being performed. Although the analysis of the data from the individual trials has not been completed, the rates of gastrointestinal symptoms appears to correlate best with E. coli densities in the water. This was observed from the analysis of the data from individual trials during Phase II and of the overall rates for the two series of trials (pretest and Phase II) at the barely acceptable and relatively unpolluted beaches.

Further analyses of the data are in progress, including interactions such as severity versus type of symptoms and analysis by trial. Confirmation of significant differences in gastrointestinal symptomatology among swimmers relative to non-swimmers at the Coney Island beach does attest to the applicability of experimental design. However, it would appear that these rather low attack rates fall into the lower portion of the indicator-illness curve described by Cabelli and McCabe (1974)[2] and that, in the absence of any significant differences in "severe" symptoms as defined herein, more polluted beaches must be sought in order to describe the full nature of said curve. The analysis of the data by trial should provide some insight whether temporal variability in indicator rates, which occur as a consequence of rainfall with combined sewage systems and of other factors, is associated with changes in the rate of gastrointestinal disturbances among swimmers. Thereby, changes at a given beach as well as beaches on a pollution gradient could be used in defining the entire indicator-illness response curve.

An analysis of the confirmation frequencies for Clostridium perfringens as obtained from the mCP method indicated that additional in situ biochemical tests would be required if the picking of colonies for confirmation is to be avoided. An additional in situ test incorporating three biochemical characteristics was developed,

[2] Cabelli, V.J. and L.J. McCabe. Recreational Water Quality Criteria News of Environmental Research in Cincinnati. November 11, 1974.

and the procedure thusly modified is under evaluation. Similarly, two additional in situ biochemical tests have been incorporated into the mVP method for the quantification of Vibrio parahemolyticus. The evaluation of the modified procedure is in progress; and the precision and specificity of the modified procedure appears to be within acceptable limits.

The search for additional specific inhibitors which will increase the selectivity (an increase in the quantity of water which can be examined without overgrowth due to background microorganisms) of the mC and mA procedures for differential coliforms and Aeromonas hydrophila respectively is continuing.

The capability to quantify f-2 coliphage has been obtained. These RNA, "tailless" phage are reported to be more resistant that T phage and, at least, of comparable resistance to polio I as regards chlorine and other environmental effects. A number of workers have suggested that such phage may be an appropriate indicator for enteroviruses. Said procedure will be incorporated into the examination of bathing water during next summer's trials.

A lab study comparing the survival in seawater of coliform biotypes, Aeromonas hydrophila, Pseudomonas aeruginosa and fecal streptococci, as influenced by the source of the organisms (sewage versus pure cultures), temperature (4 versus 20 C) and the concentration of sewage (0-50%), has been completed. A manuscript describing the findings has been sent forth for review.

Table 1. Comparison of log mean indicator and pathogen densities (per 100 ml) at the "barely acceptable" and "relatively unpolluted" beaches

Organisms	Method	Recovery per 100 ml at Coney Isl.	Rock.	C.I./Rock.
Total coliforms	MPN	1213	43.2	28.1
Total coliforms	mC	549	14.6	37.6
Fecal coliforms	MPN	545	28.4	20.0
E. coli	mC	15.3	2.4	6.4
Klebsiella	mC	59.2	3.5	16.9
Enterobacter-Citrobacter	mC	434	6.6	65.8
Fecal streptococci	mSD	16.4	3.5	4.7
C. perfringens	mCP	18.2	12.6	1.5
P. aeruginosa	mPA	45.8	3.1	14.8
A. hydrophila	mA	9.6	4.9	2.0
Total staphlococci	mSA	243	178	1.4
S. aureus	mSA	104	69.2	1.5

Papers Accepted for Publication

1. Dufour, A.P., and Cabelli, V.J. A Membrane Filter Procedure for Enumerating the Coliform Group and its Component Genera in Sea-Water. Appl. Microbiol.

2. A.P. Dufour and V.J. Cabelli. Comparison of membrane filter brands for recovery of the coliform group. Presented at the ASTM Symposium on Recovery of Indicator Organisms Employing Membrane Filters. January 1975. Ft. Lauderdale, Fla.

Manuscripts submitted for review

1. Dufour, A.P., and V.J. Cabelli. Survival of Indicator Microorganisms in Sewage and Seawater.

Non-research efforts from June through December in support of Regional office activities and Headquarters needs required nearly 16% of our resources. Major categories of activities supported continue to be industrial cooling water use and ocean disposal of wastes. These is an emerging trend toward greater participation of individual research scientists in Regional and Headquarters responsibilities. At present, this has not exceeded 15% of our efforts, but projections indicate that the coming press of Public and Adjudicatory Permit Hearings among seacoast regions, most of which concern PL 92-500 Section 316(a) applications, can consume up to 25% of our total resources and involve over 60% of our senior scientific staff. We view this as a short range expediency which can have long range consequences damaging EPA's marine research capability.

We have initiated a program to systematize our technical assistance efforts, expending some additional resources on efficient dissemination of our knowledge to Regional permits program personnel. In addition, we have requested and received coordinating functions from Headquarters staff in setting priorities and negotiating assignments among Regional Offices requesting our help. Our hope is to continue to meet all technical assistance requests from Regions, States, private citizens groups, other agencies and Headquarters without diverting more than 15% of our research resources from their primary mission. Meanwhile, our series of "Bio-legal workshops" is well underway and training individual Regional personnel through involvement in research and technical assistance is continuing, along with close coordination with Headquarters OR&D's Office of Program Integration and OEGC's office of Water Enforcement.

First Bio-legal Workshop:

Section 316(a) of the 1972 Water Bill Amendments has been the source of considerable controversy - and litigation is now in

progress over its proper administration by EPA. Deputy Assistant Administrator for Water Enforcement, Richard Johnson requested that NMWQL initiate a program to inform Regional attorneys who would be administering Section 316(a) permits about common biological phenomena associated with industrial cooling water use. Together with personnel from our sister institution, the National Water Quality Laboratory (Duluth, Minnisota) we planned and presented a workshop on the Biology of Power Plants.

One attorney from each Regional Office attended, along with two Headquarters OEGC attorneys who would be coordinating all Section 316 hearings for EPA. Workshop staff consisted of biologists from Duluth, Headquarters, and Narragansett. Curriculum included a day of illustrated lectures, a tour of an operating power plant and discussions with company personnel, a mock 316 hearing including direct testimony and cross examinations, and a water tour of proposed power plant sites. Biological issues of legal importance were emphasized (e.g. the kinds, firmness, and limitations of biological evidence) along with legal techniques in dealing with biological evidence. The workshop staff benefited from the exposure to these issues as much (or more) than did the participants, who requested that a second such workshop be conducted after the mass of Section 316 Hearings began in the spring.

Headquarters Planning Functions:

Several senior staff scientists have been involved in a research planning effort aimed at administration of special Presidential programs for evaluating environmental impact of energy exploration and exploitation. NMWQL has worked closely with the staffs of the Office of Planning and Evaluation, and OR&D's Office of Program Integration as well as "Sub-sector groups from NERC-Corvallis. Major tasks included identification, prioritization, and cost analysis of energy-relates environmental research. In addition to numerous meetings held in Washington, Denver, Dallas and Corvallis,

NMWQL staff consulted together in seminar fashion to be certain that all marine impacts were included and given appropriate priority rating by field personnel.

Surprisingly, staff felt that for certain categories of energy exploration and exploitation activity, research funds might be spent most wisely on impact-preventative technology rather than ecological damage prediction or assessment. These specific recommendations were passed on to appropriate sub-sector groups for technology development research.

Office of Planning and Evaluation personnel met at Narragansett with staff members to discuss specific issues of general industrial siting and thermal impact on estuarine and open coastal environments. Matters of project priority, levels of funding, and appropriate governmental and non-governmental sources of research expertise formed the nucleus of discussion.

Dr. Schneider formed and consulted with an ad hoc panel of University experts on energy-related impact on the marine environment. This panel has actively developed research priorities and identified sources of expertise as well - paralleling intramural efforts and adding to the conceptual basis of Project Independence's environmental efforts.

Headquarters Regulatory Functions:

1. U.W.A.G. Hearings on PL92-500, Section 316

Drs. Schneider, Miller, Perez and Prager participated in a Public Hearing held in Washington which had been requested by Edison Electrical Institutes Utility Water Action Group (UWAG). Hearings concerned evaluation of Section 316 decisions based upon ecological and economic factors. NMWQL participants were involved in testimony explaining the technical basis of EPA's Draft Technical Guidance Manual on Section 316(a)-providing anecdotal evidence for the soundness and necessity of several suggested methods of environmental

evaluation. We also participated in discussions of UWAG's proposed environmental and economic modeling systems.

2. Draft Technical Guidance Manual on Section 316(a)

Considerable NMWQL resources were expended on the EPA effort to complete this technical guidance manual. In addition to refining and re-writing several sections of earlier drafts, a new section concerning criteria for decision at each level of proof was drafted and submitted to headquarters for approval. The entire manual was reviewed by several NMWQL senior staff members, and editorial comments communicated to Office of Planning and Evaluation personnel were discussed in a series of meetings held at Headquarters and at Narragansett.

This effort demonstrated that a high level of communications among field-personnel and headquarters personnel representing OEGC, OPE, and OR&D was not only possible, but highly useful in developing regulatory documents. NMWQL staff was very pleased by the degree to which their technical knowledge industrial cooling water effects on marine biota could be translated into regulatory guidelines, and by the close cooperation of OEGC and OP&E personnel.

3. Standards and Regulations Information System

NMWQL staff members participated in a two day seminar at R.T.P. held to outline"Scientific and Technical Assessment Report" document development. Office of Program Integration Deputy Assistant Administrator John Buckley described the system of STAR report preparation and uses to which such documents may be put. No STAR assignments have been received by NMWQL to date.

4. Toxic Effluents Standards:

Dr. Donald K. Phelps met with headquarters staff to assist in the development of toxic effluents standards (mandated by PL92-500, Sect. 307(a)) for the estuarine and marine environments.

Assistance to Regional Offices

Region X: Drs. Pesch and Prager reviewed an ocean dumping

permit application concerning the disposal of large quantities of salt off Prudhoe Bay, Alaska. An advisory was prepared for the Region X Permits Office and submitted in favor of the one-time disposal.

Region IX: An advisory was submitted to Region IX on Hawaiian Electric Co.'s request for a Section 316(a) exemption for the Kahe Power Plant. Drs. Gentile and Prager had visited the plant and conducted an underwater spot check of effluent effects in September at the request of Region IX and OEGC's Counsel for Administrative Litigation. NMWQL staff advised granting the 316(a) exemption under certain specified effluent limitations.

Region IV: Mr Paul P. Yevich traveled to Tampa, Florida to attend pre-hearing meetings concerning construction of an offshore oil terminal in the Gulf of Mexico and a refinery complex proposed for St. Petersburg, Florida.

The Interagency Research Advisory Board for Crystal River Nuclear Unit 3 of which Dr. Prager is a member met with Florida Power Corp. and their consultants to discuss research performed in support of Florida Power Corp. NPDS Permit Application. The Board decided that data accumulation for that activity was complete but that additional analysis would be required.

Drs. Prager and Eisler provided Region IV and South Carolina Department of Industrial Water Control with further information on Manoa Metals effluent permit questions for adjudicatory hearings.

Drs. Prager and Eisler assisted Region IV by providing additional information on Manoa Metals Permit Application for Public Hearing Testimony.

Mr. Reynolds, Dr. Eisler, and Dr. Prager provided information on the toxicity of antimony of marine life to permits personnel in Region IV. Little information exists in the scientific literature, but indications are that antimony is not among the highly dangerous metals. Biological assays were recommended to be performed by the

applicant before permit issuance.

Region III: Dr. G. Pesch and Bruce Reynolds acted as members of the Region III Staff Panel and Dr. J.C. Prager acted as member of the EPA Region III Hearing Board for a Public Hearing concerning the City of Philadelphia's Ocean Dumping Permit for its sewage sludge.

Region III's Permit personnel and staff and consulting scientists representing E.I. DuPont de Nemours Co. met with NMWQL's Ocean Dumping Team to discuss research strategy for the Edge Moor iron acid waste ocean dumping permit. Field monitoring and laboratory bioassays were designed.

At the request of Region III, Dr. G. Pesch testified on the effects of E.I. DuPont de Nemours ocean dumping off the coast of Delaware at Public Hearings held in Ocean City, Maryland. Mr. Reynolds, who also attended the hearings, and Dr. Pesch had conducted research cruises in the dumpsite area. Dr. Prager served on the Region III hearing review panel.

Dr. G. Pesch and Messrs. G. Morrison and B. Reynolds traveled to the U.S. Naval Academy, Annapolis, Maryland., for a meeting which was jointly sponsored by NOAA and EPA concerning (1) the recent submarine survey of the Dupont and Philadelphia Ocean Dumping Sites located off Delaware; and (2) use of a submersible as a research tool for monitoring dump sites. Messrs. Morrison and Reynolds delivered short papers at the meeting.

Region II: Mr Eric Outwater, Dpty. Reg. Admin., Region II, visited NMWQL to discuss testimony and strategy for Public Hearings to be held in New York concerning the dumping at sea New York sewage sludge.

Personnel from Region II's Permits Branch met with NMWQL staff to discuss power plant permits on the Hudson River. The Oyster Creek Plant on Barnegat Bay, New Jersey, was also a subject of

discussion. Personnel from NMFS and DI attended the meeting to add their knowledge of these specific power plants to EPA's permit decisions.

Dr. G. Pesch, B. Reynolds and Paul Dix sailed on the sludge ship Newtown Creek out of NYC observing a normal dump at the dumpsite in the New York Bight. After returning from the dumpsite, these same people toured the Newton Creek Sewage Treatment Plant where the sludge is processed. They observed the normal treatment process and an experimental treatment process being developed.

Dr. G. Pesch, Bruce Reynolds and Paul Dix conducted an aerial survey on dumping activities in lower New York Harbor. Sludge dumping and iron acid waste dumping were observed and photographed.

Ms. Barbara Pastalove, biologist in the Thermal Regulatory Branch of Region II's Permits Office, spent one week at NMWQL for training in biological aspects of PL92-500, Section 316(a).

Region I: Dr. D.K. Phelps accompanied Dr. Warren Oldaker of Region I to Boothbay Harbor, Maine to discuss an EPA Grant to the State of Maine's Department of Marine Resources with Dr. John Hurst.

Mr. Gilbert Chase, Army Corps of Engineers visited NMWQL to take part in a joint Agency (cooperative) diving program. NOAA, Corps, EPA and University SCUBA divers studied transects across a dredge spoil dumpsite off Newport, R.I. during 3 cruises last week.

Drs. Eric Schneider and D.K. Phelps met with Region I and Army Corps of Engineers personnel at Regional Hdqtrs. regarding dredged spoil disposal at sea.

Dr. D.K. Phelps attended the hearings held at the Federal Court, Hartford, Connecticut, for an injunction against the Navy's dredging of the Thames River and the dumping of the spoils in the New London section of Long Island Sound.

Dr. Prager and Management Intern Sigmund A. Ustaszewski assisted Region I in a meeting with Boston Edison Company concerning its 316(a) demonstration for the Pilgrim Power Station.

Drs. Davey, Eisler and Prager provided advice to Region I Permits Branch on the disposition of a permit application from Kerramerican Mine Co.'s to discharge heavy metal wastes into an estuarine environment.

Dr. Prager and George Gardner reviewed comments by DOI & NOAA on Millstone 316(a) Demonstration for Region I.

Mr. Gardner and Dr. Prager accompanied Region I EPA Permit Program personnel to an interagency meeting held at Milford, Connecticut., NOAA laboratory to discuss NOAA and DOI objections to Connecticut's conduct of a 316(a) Public Hearing concerning the Millstone Nuclear Generating Plant.

Dr. D.K. Phelps, member of Interagency Research Advisory Board on Ocean Dumping, attended the Board's meeting to discuss Army Corps of Engineers problems with the New London Dump Site in Boston, Massachusetts.

International Technical Assistance:

Dr. W.D. Oliff, Durban, S. Africa, visited NMWQL to discuss recreational water methodology and other research programs.

Dr. Sotoaki Onishi, Mgr., Nuclear Power Survey Office, Electric Power Development Co., Ltd., Tokyo, Japan, visited NMWQL to discuss nuclear power plant information with staff members.

Assistance to the Private Sector:

Messrs. J. Fornes, J. Eckels and H. Palmer, representatives from Westinghouse Ocean Systems, Annapolis, Md., visited NMWQL to discuss applications of bioassay and possible EPA funding for their research.

Dr. Hugo Freudenthal, V.P. H2M Corp., Melville, N.Y., visited NMWQL to consult with staff members on EPA Water Quality criteria.

Members of the Manufacturing Chemists Association Water Resources Committee visited NMWQL, and after a briefing on the program were taken on a tour of the laboratory facilities.

Drs. Richard Toner and George Mathiessen of Marine Research, Inc., E. Wareham, Mass. visited NMWQL to discuss data requirements of a 316(a) demonstration for the Brayton Point Thermal Electric Plant with Drs. Miller, Beck, and Prager.

American Petroleum Institute personnel, Dr. N.K. Weaver, J.R. Gould and R.E. Eckardt visited NMWQL to discuss oil pollution studies with various staff members.

A Graduate Class in Community Planning from the University of Rhode Island met with Dr. Prager at NMWQL to discuss power plant siting problems and to observe NMWQL photographs of well and poorly sited facilities.

Miscellaneous Short-Term Assistance:

Drs. J. Gentile and S. Cheer held a Workshop at ATP Techniques in Denver, Colo. at the request of NFIC.

Capt. Willard M. Adams, FDA, visited NMWQL and consulted with Dr. M.A. Levin and A.P. Dufour on enumerative technology.

Drs. D. Phelps and P. Rogerson traveled to Washington, D.C. to discuss the Oil Section of the draft Water Quality Criteria document with Mr. K. Mackenthun.

Mr. Joel Fisher of R&D Program Integration, Hdqtrs, visited NMWQL to consult with staff members on the re-write of Chapter V of the CEQ Report.

Dr. D.K. Phelps traveled to Washington to meet with Headquarters staff on Toxic Effluent Standards, Section 307(a) of PL92-500.

Dr. Roy Irwin, John Christian, Jeff Goodman, and Diane Olsson and Michelle Zarubica of EPA Hdqtrs., Dr. Wm. Brungs of the Duluth Lab, and Bruce Tichenor, PNERL, visited NMWQL to draft a final 316(a) Guidance Manual with staff members.

Dr. Jan Prager attended a Hdqtrs OWP Meeting to assist R&D personnel in outlining Agency efforts to fulfill Sec. 104(n) of

PL92-500.

Miss Dreena Felton, Water Planning Div., Hdqtrs, visited NMWQL to gather information to be used as references in the upcoming suit against EPA by several power companies.

Dr. Schneider, Miller and Prager reviewed the Edison Electrical Institute "Utility Water Act Group" comments on EPA's draft guidence manual on Section 316(a), PL92-500. A public hearing on this subject was held at headquarters at which these persons participated in an EPA panel constituted to receive and answer comments from the public and concerned industrial interests.

Narragansett, RI

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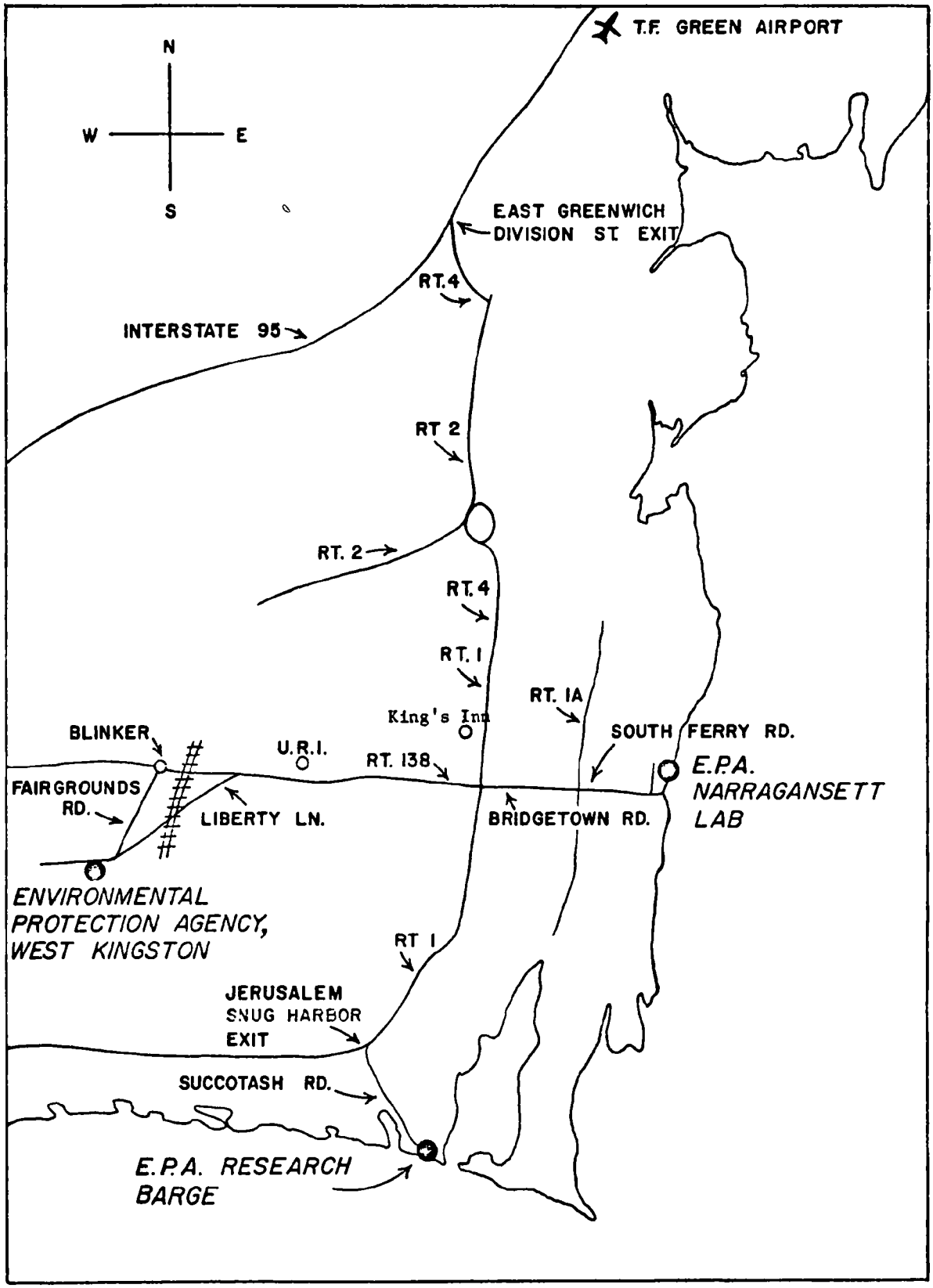
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