

QUARTERLY REPORT OF RESEARCH  
JULY - SEPTEMBER 1971  
NATIONAL MARINE WATER QUALITY LABORATORY  
WEST KINGSTON, RHODE ISLAND  
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Environmental Protection Agency  
Office for Research and Monitoring

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Quarterly Report of Research  
National Marine Water Quality Laboratory  
West Kingston, Rhode Island  
July, August, September, 1971

1.00 GENERAL

Dr. Tarzwell, Dr. Prager and Mr. Gonzalez are involved in the Region I evaluation of New England Electric Company's application to construct and operate a fourth fossil fuel generating unit at Brayton Point, Somerset, Massachusetts. A series of meetings with representatives of FPC, Army Corps of Engineers, other EPA offices, and various state of Massachusetts and Rhode Island agencies have been attended and will continue into next quarter.

A second Turkey Point study was initiated, performed, analyzed, and a report written, and submitted during the quarter at the request of the Deputy Assistant Administrator for Enforcement. This report served as an adjunct to last summer's Turkey Point report and was used in determining EPA's terms for the Florida Power and Light Company settlement. Prager, Steele, Gonzalez, and Johnson designed and executed the studies and authored the report, which analyzed benthic diatom and zooplankton populations in the southwestern quadrant of Biscayne Bay and northern Card Sound.

Dr. Prager is completing a biological evaluation of the environmental impact of a proposed 5000 MWE thermoelectric plant proposed by Houston Light and Power Company for Cedar Bayou, Texas, at the request of Region VI and the Office of Technical Analysis, Enforcement Branch, and personnel of the Dallas Office. Prager had represented EPA at a meeting of biologists representing Houston Light and Power Company, Florida Power and Light Company, Corps of Engineers, Texas Water Quality Board, Texas Parks and Wildlife Service, and NOAA in Baytown, Texas, at which resolution of the considerable biological problems posed by the plant's design was discussed. Resolution of the problem is still a matter of considerable controversy and our involvement may be expected to continue for a considerable time. The Office of Technical Analysis has requested our assistance in design of a study and evaluation of procedures for answering questions raised by the Cedar Bayou proposal.

Use of wet lab facilities in the Water Hygiene Laboratory, Narragansett, Rhode Island has been hampered by disrepair of the sea water system. This necessitated repair of all pumps, replacement of water storage tanks, cleaning and/or renewing most of the interior distribution system. Despite problems, progress has been made. The sea water system is now functional, several diluters have been fabricated and part of the continuous flow aquaria facilities are in operation.

The dissolved oxygen and temperature monitoring and control system for the research houseboat has been completed and is now operating.

Full scale research studies are planned and personnel from the research barge staff will accomplish this work during the interval when the barge is in dry dock for repairs and painting.

The research barge will be moved on October 26th to Newport Shipyard of Rhode Island for contract repairs and painting. The barge should return to mooring at Jerusalem, Rhode Island approximately in the middle of December. The barge marine species holding facilities will be established on-shore at the mooring site during the repair period.

An epoxy resin-natural aggregate deck coating up to 3/8" thick has been applied to the interior deck areas and evaluated for several months. Results have been excellent. The completed coating provides a seamless impervious protective layer tightly bonded to the steel decking. It is planned to coat the entire interior deck during the time interval of contract repair, approximately October 26th to Mid-December.

Studies on biological concentrations of conservative pollutants in fishes was initiated during the quarter through arrangements to procure off-shore samples from fishermen participating in local fishing tournaments. Either tissue samples or whole fish samples have been collected both at the Bears Bluff Field Station and in the Galilee, Rhode Island area. Analyses for heavy metal and pesticide content will be carried out as time permits.

## 2.00 RESEARCH - WATER QUALITY REQUIREMENTS

Subprogram - 310209

Marine Fishes, Other Marine Life and Wildlife

### 2.10 Ecological Requirements for Plankton

#### 2.11 Need 18080 OUE, Plan GJZ, DISSOLVED OXYGEN AND TEMPERATURE REQUIREMENTS OF MARINE PLANKTON

Experiments on the green alga, Enteromorpha clathrata, were repeated early in the quarter. Data obtained were much the same as acquired during last quarter in that growth is greatest at 5-20‰ and drops off at about 40‰ salinity. Samples collected at Brayton Point are being evaluated weekly and phytoplankton counts made from the influent and effluent of the generating plant. Much taxonomic literature was accumulated for this study and additional time has been spent on literature research.

Samples have been collected from two stations at Rome Point in Narragansett Bay and evaluating the algae collected there. We want to establish base line values for population biomass and diversity before the proposed electrical generating plant is built. This population will serve as a reference for the population collected at Brayton Point.

During this quarter, considerable attention was given to studying the temperature distribution in Mount Hope Bay at high and low tide. Some similar areas were studied in the upper region of Narragansett and Greenwich Bays. These studies were undertaken with two main objectives:

- a. Extent of movement of heated water resulting from the operation of New England Power Company at Somerset, Massachusetts, during the summer months.
- b. An attempt to find clues to what the summer ambient temperature was prior to the operation of the plant eight years ago.

A study of the temperature tolerance was conducted on the calanoid copepod, Acartia tonsa, from Bahia Fosforescente, Puerto Rico, from July 19 to July 25. It was found that the tropical form of this species, as expected, was more tolerant to high temperatures than the isolates from Biscayne Bay, Florida, or Narragansett Bay, Rhode Island. The critical thermal maxima (CTM) values were 1°C higher than for A. tonsa from Biscayne Bay or Narragansett Bay.

Live samples were brought to this laboratory and so far are in satisfactory condition. Live samples were obtained from Biscayne Bay and these, also, are in satisfactory condition.

Experiments on temperature tolerance in the laboratory and on interbreeding of the various forms have been run for the past four weeks on this ubiquitous copepod.

Twice weekly (at both high and low tide) we have been taking surface and bottom temperatures at close intervals around Brayton Point with the objective of following the movement of heated water as ambient temperature comes down during the season. There is considerable shrinking of the heated area based on last year's information. We will be able to make a proper assessment of the fate of the heated water mass as a continuous intensive survey is expected to follow its movement during fall, winter, and spring. Previous temperature information had been based on surface temperatures only.

Settling plates, two square feet in surface area, were placed at various stations including intake, the effluent canal, and in Lee River in April 1971. These plates were collected at two week intervals and the organisms preserved in formulin. At present the various species are being identified, dried and biomass is being determined. Further studies are being carried out on temperature, dissolved oxygen and zooplankton population

There is a dramatic difference between the organisms in the effluent canal and the intake. The effluent canal contains mainly Campanularia, a week-hydrind and barnacles while the intake shows a much wider species diversity such as Mya, Mulinia, Mytilus, starfish, several species of hydroids and harpacticod copepods.

## 2.20 Plankton Toxicological Studies

### 2.21 Need 18080 OWA, Plan ETR, TOXICITY OF OILS AND PETROCHEMICALS TO PLANKTON

Examination of cellular adenosine triphosphate (ATP) as a measure of biomass or of stress response has continued. ATP is being correlated with growth rates, chlorophyll "a", biomass particulate organic carbon, and C-14 fixation under conditions of heavy metal or chlorine stress. These studies are being conducted with both phytoplankton and zooplankton. The technique is also being applied to microorganisms in sediment samples where petrochemicals may be expected to concentrate.

### 2.22 Need 18080 OWB, Plan ETM, A MEASURE OF THE TOXICITY OF HEAVY METALS TO ESTUARINE PLANKTON

Acute toxicity bioassays have been conducted with a number of metals using the calanoid copepod Acartia clausi, Pseudodiaptimus coronatus and Acartia tonsa as well as the harpacticoid copepod Tigriopus japonicus. Median tolerance have been established at 24, 48, and 72 hr for  $\text{Cu}^{+2}$ ,  $\text{Cd}^{+2}$ ,  $\text{Pb}^{+2}$ ,  $\text{Hg}^{+2}$ ,  $\text{Zn}^{+2}$ ,  $\text{Ni}^{+2}$ , and  $\text{Cr}^{+3}$ . Exposed animals have been processed for future histopathological examination. Both cultured and field samples have been examined to determine the constancy of ATP to carbon ratio. As indicated in 2.21 above, ATP is also being tested as a possible index of sub-lethal stress in both zooplankton and phytoplankton. The

field study on variations in metal toxicity to phytoplankton which was planned next quarter has been postponed indefinitely. This was done in order to try using natural field populations in their own water as a better model of field conditions. We also wish to examine the influence of the particulate load of water on metal toxicity and indications that colloidal or precipitated forms of the metals may affect growth.

Cyclotella nana has been successfully used to obtain a quantitative measure of the copper complexation capacity of sea water. The method is sensitive enough to measure  $1 \times 10^{-7}M$  EDTA at a precision of  $\pm 5\%$ . These experiments indicate that C. nana is unable to successfully compete with EDTA for copper. Other copper chelates appear to be weaker or more avoidable to the test organism. The method is therefore a quantitative measure of the ability of a given water mass to render metals biologically inactive as far as phytoplankton in the water column are concerned.

Two methods were investigated for the quantitative determination of heavy and trace metals at seawater concentrations.

- a. Anodic stripping voltammetry (ASV) was found to be an extremely sensitive technique and could be used for the measurement of seawater levels of copper, lead, zinc and cadmium. This method is also being investigated for the possible differentiation and quantitation of complexed and noncomplexed metal ions in seawater.
- b. Neutron activation analysis of metals concentrated on Ammonium Chelex-100 from seawater was found to be sensitive for the determination of Mn, Cu, Zn, and Al. More work is proposed to quantitate the method and ascertain whether other seawater elements can also be analyzed.

2.23 Need 18080 OSK, Preliminary Investigations -- TOXICITY TO MARINE ORGANISMS OF FREE CHLORINE AND CHLORINATED COMPOUNDS IN SEAWATER

No activity this quarter.

2.30 Ecological Requirements for Fish and Invertebrates

2.31 Need 18080 OOA, Plan ETI, DISSOLVED OXYGEN, TEMPERATURE AND SALINITY REQUIREMENTS FOR SELECTED MARINE FISH AND INVERTEBRATES

Data reduction for temperature studies on winter flounder (Pseudopleuronectes americanus) eggs and larvae was completed.

Newly fertilized winter flounder eggs survive a thermal addition of 5°C above ambient temperature throughout their natural spawning season.

Rate of development is increased at elevated temperature but the sequence of developmental events is not altered. Eggs appear to be more temperature sensitive before gastrulation than after.

Results indicated that thermal shock as well as long-term exposure to elevated temperatures may produce high mortality figures at 10°C above ambient. Continued efforts will concentrate on study of eggs acclimated to elevated temperatures either by (1) gradual acclimation of newly fertilized eggs, or (2) obtaining eggs from gravid adults acclimated to elevated temperatures.

Newly hatched larvae seem to be less temperature sensitive than eggs, but because of temperature control problems in the 15° and 20° tanks, the results were not conclusive. All attempts to hold adults for extended periods at elevated temperatures failed due to disease. (See last quarterly report.)

The effects of elevated temperature on adult scup, Stenotomus versicolor, are continuing to be studied. To date, results indicate that they will survive 5°C above ambient during the summer when ambient temperature is highest. Mortality was very high in the 10°C above ambient tank. However, reduced flow in the experimental system during the course of this experiment may have compromised the results. The test will be repeated next summer.

A study was initiated to induce spawning of Fundulus heteroclitus, by hormone injection. Two methods are being used: (1) injecting 25 IU Chorionic gonadotropin every 24 hours, and (2) injecting a single dose of 150 IU of Chorionic gonadotropin. If spawning is successfully induced in Fundulus, similar experiments will be conducted on other species of fish so that a continuous supply of eggs will be available for temperature studies.

Oxygen requirements for eggs and larvae of the quahog, Mercenaria mercenaria, have been determined. Studies currently in progress are aimed at establishing effects due to artifacts (if any) from experimental systems on apparent biological results. The next step in determining oxygen and temperature requirements for the quahog has been initiated on the juvenile stage of its life history. Animals between



2 and 10 mm in length are being held at ambient temperature, plus 5 and 10°C at dissolved oxygen levels of 0.8, 1.7, 2.2 and 5.5 mg/l (ppm) respectively. Growth, growth rates, mortality and selection for particular phenotypes of lactate dehydrogenase and luciferase amino peptidase will be used to evaluate the degree of stress experienced by the juvenile quahogs. Results will be compared with the dissolved oxygen requirements which are being established for eggs and larvae.

The responses of adult clam species, Mercenaria mercenaria, and Spisula solidissima to increases in temperature and decreases in oxygen concentration have also been initiated. Two types of activity under consideration are burrowing behavior and heart rate. Burrowing activity is a gross response involving the integration of many facets of clam behavior and physiology. Observations show that clams of the two aforementioned species will attempt to bury themselves unceasingly within the limits of environmental tolerance. Unsuccessful attempts to bury usually precedes death or moribund condition. It was shown that negative burrowing activity could be reversed by returning the clams to known optimum conditions.

A series of burrowing experiments to determine oxygen requirements related to that activity were performed with Mercenaria mercenaria in August and September. Clams were exposed to dissolved oxygen concentrations of approximately 2, 3, 4 and 6 ppm at an average temperature of 26.5°C (range 25-30°C). Every 24 hours, the clams were displaced from sediment in which they had burrowed and placed on top of the sediment layer. Results obtained to date demonstrate that clams of this species can withstand exposures to 2 ppm dissolved oxygen for more than one month before succumbing (100% cessation of burrowing activity). After one month at the 3 ppm level only 33% of the clams had ceased burrowing activity altogether. At oxygen values higher than 3 ppm, burrowing continued. Evidently, oxygen requirements of this clam are relatively low (<3 ppm). High temperatures and conditions of repeated exertion are not apparently major factors in limiting its tolerance to environmental changes. The burrowing activity of the adult quahog, if accepted as a reasonable indication of the total response to environmental stress, suggests that the water quality criteria of 4 ppm recommended by the National Technical Advisory Committee is adequate to protect the adult stage of the quahog, Mercenaria mercenaria.

A procedure for monitoring heart rate has been developed whereby fine platinum wire is inserted through small holes drilled into the valves of the clam approximately above the

pericardial region. These wires are connected to leads from a polygraph instrument which records the surges with the beat of the heart. Experiments performed during July and August indicate that the heart of Mercenaria mercenaria (acclimated three weeks or more) beats slower in late summer than in spring or early summer at temperatures which are moderately high for this clam (23-27°C). Limits of survival decrease in late summer from about 36°C in July to 33°C in August (50% survival, three-week exposure).

Oxygen-temperature requirements of the squid, Loligo peali, (Series to be identified) were determined using nitrogen-sparging facilities at the barge. Egg sacs with about 90 embryos were maintained at dissolved oxygen concentrations of 0.8, 1.5, 2.2, 2.8, 3.3, 3.8 and 4.8 mg/l and temperature regimes of ambient (control), ambient plus 5 plus 10°C. Three experiments involved three age groups of developing larvae: 10, 14, and 21 days previous to hatching. The latter group was stressed as blastulae. Developing embryos were examined daily. Percentage hatching was used as criteria for success at each stress level. RESULTS: The embryos maintained in ambient plus 10°C died within 48 hours. At ambient plus 5°C development and hatching took place only at above 4.8 mg/l DO (approximately 25% hatching success). At ambient temperature no hatching occurred at 2.8, while there was 44% success at 3.3, thus establishing a breaking point over a range of .5 ppm dissolved oxygen. When stress was begun before gastrulation (21 days before hatching), no hatching was observed at 0.8 mg/l. (Blastulae stressed at 0.8 did not undergo gastrulation, did not hatch, but survived about 18-20 days.) However, 95% hatching occurred at 3.3 mg/l and 80% at 2.8 mg/l.

A series of nine comparative studies to check for possible biological artifacts resulting from the mode of removal of dissolved oxygen from experimental systems has been completed at the barge. Nitrogen stripping, a mussel bed (Mytilus edulus) and an algal column (Codium fragile) were used to reduce oxygen in three discreet systems. The test organism in this series was the sand shrimp, Crangon septemspinosa, and test criteria was percent mortality after 24 hours. Gross examination of the data from these pilot control systems indicates no measurable difference in shrimp survival between the three experimental systems of oxygen control.

A study of the dissolved oxygen requirements for Mulinia lateralis larvae has been completed. The data are currently being evaluated.

Heavy metal total body burden studies continue. Determination of seasonal and spatial levels in Nephtys incisa and Yoldia limatula (important food organisms for the winter flounder) continue on the basis of field collections made on a bimonthly basis.

A study to determine effects of a diet enriched in heavy metals, on winter flounder body burden of these metals, is underway. The first phase of the study, covering a feeding period of 30 days has been completed. Samples are being prepared for analysis of Phase II, covering a total of 60 days. Comparisons are being made on levels of the metals found in the eyes, stomach, intestines, skin, flesh and fins.

The neutron activation techniques used in obtaining trace element data on sediments and organisms was expanded to include Mn, Al and Cu. This is accomplished with one-minute irradiations. Due to the nondestructive nature of this method, samples previously analyzed may be reirradiated and the presence of these elements determined.

A study of cores to be taken in Narragansett Bay is being initiated to obtain the following trace element data (Ni, Pb, Cu, Zn, Sc, Co, Fe, Cr, Ag) in:

1. Interstitial water
2. Sediment
3. Water

The data is expected to show the distribution of these trace elements in the sediment, interstitial water and overlying water mass and attempt to correlate this information with existing data on organisms living in the interstitial water and at the sediment water interface. The analysis of replicate cores will yield data as to the variability of these elements at the same station. Sampling will coincide with the bimonthly collection of organisms and will eventually provide the necessary information needed to interpret the seasonal variations of the elemental content of Nephtys and Yoldia.

At the Bears Bluff Field Station, project planning and preliminary requirements have been initiated. Projected work will focus on temperature, salinity and oxygen requirements of selected animals, with particular emphasis on the effects of sublethal temperature and oxygen stress conditions. Experimental material will be drawn from available estuarine and coastal fishes, shrimp, and pelecypods. Requirements for all life history stages, including the egg, will be considered.

In addition to project planning, scientific activities have centered on literature reviews and preliminary work with shrimp, particularly Penaeus setiferus, the white shrimp. This species shows unusual tolerance to high temperature (33° and 34°) at salinities ranging from 6.2‰ to 27‰. Low temperature work was conducted at 10°. The rate of cooling and body size were found to be variables influencing tolerance, with small animals (50 mm total length) more sensitive than larger specimens (90 mm total length).

#### 2.40 Invertebrate and Fish Toxicological Studies

##### 2.41 Need 18080 OSF, Plan GJY - EFFECTS OF SODIUM NITRILOTRI-ACETATE ON MARINE AND ESTUARINE ORGANISMS

After one year of exposure to 10 ppm trisodiumnitrilotriacetate (Na<sub>3</sub>NTA), bay scallops, oyster, lobsters, and sand shrimp were harvested and tissue samples were prepared for electrophoresis. The gill, mantle, and adductor muscle tissue were sampled from the oyster and scallop; whereas for lobster, the gill, hepatopancreas and tail muscle tissues were used. Whole sand shrimp was used.

After electrophoresis, the following enzymes were detected: Lactic acid dehydrogenase, malic acid dehydrogenase, alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, alkaline phosphatase, ATPase, monoamine oxidase, cytochrome oxidase, cytochrome-c-reductase, acid phosphatase and dopamine oxidase.

No differences between control and exposed tissue of scallops were found in the zymograms.

Differences in the zymograms of tissues from lobsters exposed and nonexposed were found after 9 and 12 months exposure. The differences were noted between lactic dehydrogenase and cytochrome oxidase.

Zymograms of oyster tissue revealed differences between exposed and nonexposed tissue when the enzymes malate dehydrogenase and alcohol dehydrogenase were detected after 9 and 12 months exposure.

Differences were found in the zymograms of shrimp tissue exposed and nonexposed when alkaline phosphatase was fractionated after 9 and 12 months exposure.

Acute toxicity assays resumed this quarter with household heavy duty synthetic detergents and marine organisms. Preliminary tests indicate that teleosts are more sensitive than

molluscs, crustaceans, echinoderms, or annelids. Tests with linear alkylate sulfonate (LAS), the surfactant component in almost all commercial household syndets, and marine fishes indicate that teleosts are the most sensitive group of organisms tested with a computed TL-50 (168 h) value of 2.12 mg/l LAS to Fundulus heteroclitus at 20°C and 20 o/oo salinity.

- 2.42 Need 18080 OSB, Plan ETL - "Toxicity of Petroleum Oils, Dispersants and Petrochemical Wastes to Marine Fish and Invertebrates."

Histopathologic studies were started on soft shells which were collected from a No. 2 fuel oil spill in Searsport, Maine. Histopathologic examination of these animals, so far, has shown the presence of tumor in both the male and female gonadal tissues. Lesions of the gill and possibly the blood vessels were also noted.

Studies were started during this period on the histopathologic effects of the water-soluble portions of crude oil (Louisiana crude, Texas Languinillas crude, and fuel oil No. 2). The oil in proportions of one liter to 40 liters of seawater were mixed in an aerator for a period of a half hour. The oil and watersoluble portions were allowed to separate for various periods of time from ten minutes to four days. The water-soluble fractions were placed into tanks and scallops and oysters were exposed until death or moribund conditions were evident. In addition, water-soluble fractions were diluted with different volumes of seawater, and scallops were then exposed. Preliminary histopathologic examination of these animals show a histopathologic effect on the cardiovascular system.

- 2.43 Need 18080 OSC, Plan ETO - "TOXICITY OF HEAVY METALS TO MARINE FISH AND INVERTEBRATES."

Studies on the effects of copper ( $\text{CuCl}_2$ ) to the acoustico-lateraloclitus system of fishes continued. Larval forms of Fundulus heteroclitus were examined after acute exposures; pathology could not be noticed at the sensory center, however, the stage of development of the system did not permit a thorough examination. Fertilized eggs were exposed to various concentrations of copper to determine the effects on the hatching rate. All levels of copper studied substantially reduced hatching. Further, higher elevations of copper retarded emergence. Work on larval forms has been curtailed due to a lack of specimens.

Acute copper exposure studies are being conducted on the silver side, Menidia menidia, to determine if the lateralis system is affected. Chronic exposures will begin during the ensuing quarter to determine if copper does affect some of the schooling behavior of these fishes.

Fishes exposed to copper in the wild, as reported last quarter, have been undergoing continuous study. In some cases the earlier damage has been found to progress with time. Special techniques are being used to identify probable sites of copper deposition. Laboratory assays will be initiated next quarter to determine if the lesion can be duplicated under these conditions.

TL - 50 (168 h) values for chloride salts of mercury, nickel, chromium (as  $K_2CrO_4$ ), cadmium, and zinc were determined for adults of various species of marine organisms at 20°C and 20 o/oo salinity and these are summarized below. All values are in mg/l element.

Element	sand worm, <u>Nereis vivens</u>	hermit crab, <u>Pagurus longicarpus</u>	mud snail, <u>Nassa</u> <u>obsoleta</u>	killifish, <u>Fundulus</u> <u>heteroclitus</u>
Mercury	0.056	0.052	0.067	0.76
Chromium	0.7	0.7	10.0	44.0
Cadmium	0.7	0.7	16.0	40.0
Zinc	2.6	0.22	7.4	52.0
Nickel	25.0	30.0	16.0	150.0

Assays are in progress with the above compounds and starfish, Asterias forbesi; soft shell clam, Mya arenaria; and sand shrimp, Crangon septemspinosa.

Studies on uptake, retention, and translocation of radio-cadmium-115 m by Fundulus heteroclitus, begun in January will terminate next quarter. Results obtained during the past quarter indicate that tissues which accumulate Cd-115 m from the medium (on d/m/g basis) are, in order: GI tract, gill, liver, spleen, heads and carcasses, gonad, with no measurable activity in heart or gall bladder under the conditions studied.

A study was begun to evaluate the effects of heavy metals on the early stages of the mummichog, Fundulus heteroclitus. Due to a shortage of embryos, only preliminary experiments were conducted. The effect of reduced dissolved oxygen levels on the embryology of the mummichog was also studied. A comparison of the results of this experiment with those of an earlier one indicated that embryos of this species are more sensitive to low oxygen levels than are the adults.

The study of the effects of dissolved oxygen and temperature on the respiration metabolism of the bay scallop, Aquiptecten irradians, was continued through part of last quarter. This investigation was postponed when the supply of scallops was exhausted.

A study of the effects of low dissolved oxygen levels on the toxicity of heavy metals to marine animals was begun. Initial experiments using  $\text{Cd}^{2+}$  have been conducted.

Inhibition of the enzyme, aminolevulinic acid dehydrase in killifish and winter flounder, by lead, was completed. A manuscript describing this work is now under review. Chronic effects of heavy metals on RNase are now in progress.

The evaluation of factors which influence the rate of  $^{14}\text{C}$  leucine incorporation in fish was completed. A manuscript describing this work is now under review.

Growth studies using  $^{14}\text{C}$  leucine uptake as a response measure will be continued. In addition measurements of specific enzyme activity, the degree of polysome aggregation and an evaluation of free tissue amino acids will be evaluated as possible measurements for growth.

Heavy metal studies at the Bears Bluff Field Station emphasizing acute toxicity bio assays to determine the 96-hour TLm values for as many organisms as possible using heavy metals compounds.

Brown shrimp, Penaeus aztecus, exposed to phenyl mercuric nitrate and mercuric chloride had 96-hour TLm values of approximately 0.12 ppm and 0.28 ppm mercury respectively. Mortalities occurring during the bioassay as well as survivors were analyzed for mercury. Higher mercury levels were present in phenyl mercuric nitrate - exposed shrimp than in those exposed to mercuric chloride. Also, although the number of shrimp that molted during this bioassay was small, there appeared to be higher levels of mercury in the molted shrimp when compared to the non-molted shrimp.

Acute toxicity tests were conducted exposing juvenile white shrimp, Penaeus setiferus (average weight, 1.5 g) to phenyl mercuric nitrate and mercuric chloride at 20‰ salinity. The 96-hour TLm was approximately 0.05 ppm mercury as phenyl mercuric nitrate and less than 0.21 ppm mercury for mercuric chloride.

Additional studies were conducted to determine the acute toxicity of zinc, copper, nickel, and lead to juvenile white shrimp. The 96-hour TLm for these metals as the chloride salts were approximately 15.0 ppm zinc, 15.0 ppm copper, and 16.0 ppm nickel. No mortalities resulted during the 96 hours with lead at concentrations as high as 56.0 ppm due to precipitation of the metal. Environmental parameters for these static bioassays were 20‰ salinity, pH 7.8, 20°C, and dissolved oxygen 6.0 mg/L. All TLm values are reported as concentrations of metal added. Exposure concentrations will be reported later.

Juvenile striped mullet, Mugil cephalus exposed in acute bioassays to copper chloride, zinc chloride, lead chloride, and phenyl mercuric nitrate had the following 96-hour TLM values: 5.0 ppm copper, 12.0 ppm zinc, and 0.020 ppm mercury. Again, concentrations as high as 56.0 ppm lead caused no mortalities during 96 hours. Environmental parameters for the mullet bioassays approximated those for the white shrimp tests. Concentrations shown are for metal added to the bioassay.

Chronic studies exposing spot, pompano and mullet to phenyl mercuric nitrate were conducted to determine possible long-term exposure effects.

Spot, Leiostomus xanthurus, (average weight 10 g) and pompano, Trachinotus carolinus (average weight 2.8 g) were exposed continuously to 0.25 and 0.50 ppb mercury as phenyl mercuric nitrate in flowing seawater bioassays. Average salinity and temperature for the spot and pompano bioassays were 20‰, and 22°C and 19.8 ‰, 27°C, respectively. At the end of 50 days, muscle tissue of spot exposed to 0.25 and 0.50 ppb mercury contained a 3-fold and 6-fold increase, respectively, in mercury content when compared to actual exposure concentrations. After 25 days exposure, pompano, regardless of exposure concentrations, contained whole body mercury levels of ten times that of actual exposure dosages.

Juvenile mullet, Mugil cephalus, were exposed for 25 days to 10.0, 5.0, and 1.0 ppb mercury. As before, this bioassay was conducted in flowing seawater with continuous exposure of the fish to the toxicant. Rapid accumulation of mercury resulted in total mortality within 13 days and 22 days, respectively. Fish dosed at 1.0 ppb mercury all survived the 25-day exposure, with the exception of two fish dying on day 17 and 22. Total body mercury analyses indicated that on the average, fish dying within the 10.0 and 5.0 ppb mercury concentration contained the same amount of mercury regardless of time of death. On the 25th day, those mullet exposed to the lowest mercury level concentrated the metal 30 times that concentration which was available.

#### 2.43 Need 18080 OSE. "Safe levels of Pesticides in Estuaries and Coastal Waters."

Construction of the wet laboratory facilities at the Northeast Water Hygiene Laboratory has continued. One of two seawater storage tanks has been installed and connected to a trough form which seawater for experimental systems will be taken.

Two flowing seawater systems for use in exposing marine macroorganisms to different concentrations of pesticides have been assembled. Each system consists of a proportional



diluter with twelve 10-gallon glass aquaria. Construction of two additional systems is in process.

Preliminary work has been started on developing a clean-up system for pesticides in the effluent water from the experimental systems. Various filter and adsorbing materials have been ordered and a prototype filter has been constructed.

### 3.00 PROJECT SUPPORT

#### 3.10 FUNDAMENTAL STUDIES

Mr. Harold Mears, a University of Rhode Island graduate student is investigating changes in trace metals content of marine teleosts with increasing age. Tissues of tautog, Tautoga onitis; bluefish, Pomatomus saltatrix; and tilefish, Lopholatilus chamaeleonticeps, are being analyzed by atomic absorption spectroscopy, under the supervision of Dr. Eisler.

The toxicity of mixtures of salts of various metals to marine fishes is being investigated at the NMWQL by Mr. George Burgess, a graduate student at the University of Rhode Island, under the direction of Dr. Eisler.

#### Population genetics of the quahaug, *Mercenaria mercenaria*

Preliminary work on the molecular genetics of Mercenaria mercenaria and Mercenaria campechiensis was completed. Four populations of M. mercenaria were sampled; one each from the Biddeford River, Prince Edward Island, Canada; Boothbay Harbor, Maine; Narragansett Bay, Rhode Island; and Wadmalaw Island, South Carolina. Two populations of M. campechiensis were sampled: one from Shackelford Banks, Beaufort, North Carolina, and one from Tampa Bay on Florida's Gulf Coast. Four enzymes were examined: lactate dehydrogenase, esterase, tetrazolium oxidase, and malate dehydrogenase. All but malate dehydrogenase were found to be polymorphic. Phenotypic differences in these enzymes were inferred to represent genetic differences at the loci controlling their primary structure. Selection favoring heterozygotes was found at the lactate dehydrogenase locus, particularly in the Canadian population of M. mercenaria. Heterozygotes have two forms of lactate dehydrogenase and presumably this facilitates metabolism in an environment requiring alternating aerobic and anaerobic postures of this bivalve.

Both populations of M. campechiensis had a total of 14 alleles segregating at four loci for an average of 3.5 alleles per locus. The Canadian, Maine, Rhode Island, and South Carolina populations of M. mercenaria averaged 2.0, 2.75, 2.75, and 3.0 alleles per locus respectively. The variation contained in the genome of M. mercenaria potentially permits an almost infinite variety of

genotypes. The reproductive rate of the hard clam is geared to exploit this potential. An average adult female produces about 25 million eggs during a single spawning season. High fecundity permits reshuffling of genetic material within a particular population. Only a small fraction of the enormous number of fertilized eggs eventually survives. The survivors are thought best suited for their environment. Extending this concept of selection to laboratory situations it is thought that stresses controlled under laboratory conditions will cause differential mortality in groups of juveniles. Shifts in the genetics of these groups will provide a handle on the responsiveness of particular species to selected stresses. It is postulated that toxicant concentrations causing only subtle shifts in the metabolism of adults will cause differential mortality of juveniles clearly evidenced by shifts in the molecular genetics of survivors.

Genetic markers are needed to conduct the above research. The molecular forms of the enzymes; lactate dehydrogenase, malate dehydrogenase, tetrazolium oxidase, and an esterase were determined for the following species: Homarus americanus (lobster); Nephtys incisa (a polychaete), and the bivalves Aquiptecten irradians (scallop), Mulinia lateralis (coot clam), Mytilus edulis (mussel) and Yoldia limatula.

A temperature-oxygen stress study using juvenile quahogs is in progress. About 430 animals between 2 and 10 mm in length were divided into 12 groups of equal numbers and are being held at temperatures of ambient, ambient plus 5°C, and ambient plus 10°C with oxygen levels of 0.8, 1.7, 2.2, and 5.5 mg/l. These are being held in flowing sea water. Growth, mortality, and selection for particular phenotypes of lactate dehydrogenase and leucine amino peptidase will be used to evaluate the degree of stress experienced by the quahogs.

Pesticides will be bioassayed by in vitro testing of range of concentrations of each pesticide, starting with malathion. Esterase activity is affected by low concentrations of pesticides, particularly organophosphates. So the pesticide will be included with the medium for developing zymograms of esterase activity. Electropherograms of a variety of marine animals will be subject to combinations of esterase medium and pesticides. This approach should provide an easy but sensitive assay of pesticide effects on a large number of marine animals. The results may thus be used in two ways; first as a preliminary measure of pesticide toxicity and second as a screening method for later in vivo testing of these same pesticides. Again, for in vivo testing the method of plotting shifts in molecular genetics of large numbers of animals will provide a sensitive handle on the effects of pesticides in the marine environment.

### 3.20 PROCUREMENT OF TEST ORGANISMS. (NMWQL)

#### Larval Cultures:

Larvae cultured and held during past quarter:

1. Mercenaria mercenaria
2. Crassostrea virginica
3. Mulinia lateralis
4. Aquiptecten irradians

This was our first attempt to rear A. irradians. We found that spawning could be induced by physical agitation. The larvae were held using standard culturing methods. Only limited success was obtained, however, in rearing the organisms through metamorphosis.

RV/BILLIE II, operated by URI, made benthic collection trips each month to maintain a continuing supply of test organisms. During this quarter eight trips were made.

<u>Species Collected</u>	<u>Common Name</u>	<u>Number</u>
<u>Palaemonetes pugio</u>	grass shrimp	200
<u>Crangon septemspinosa</u>	sand shrimp	200
<u>Fundulus heteroclitus</u>	killifish	1000
<u>Mercenaria mercenaria</u>	quahog	4 bushel
<u>Aquiptecten irradians</u>	scallop	1/2 bushel
<u>Mulinia lateralis</u>	coot clam	1000
<u>Asterias</u>	starfish	200
<u>Pagurus sp.</u>	hermit crab	500
<u>Nassarius</u>	mud snail	1000

Special collection trips were made to New York for striped bass and white perch.

<u>Roccus saxatilis</u>	striped bass	500)
<u>Roccus americanus</u>	white perch	1000)

New York

Continuous field collections are being carried out at BBFS for support of their activities.

### 3.30 LIBRARY SERVICES

#### LITERATURE SEARCHES REQUESTED

Scientific Information Exchange of the Smithsonian Institution.

All current research dealing with benthic ecology and neptys

Toxicity of "Water Soluble" fractions of crude oil and petroleum products to marine organisms.

National Library of Medicine - Toxicity Information Program

Roster of experts in the following: Marine microbiologists experienced in studies of the effects of pesticides - particularly marine microbial geneticists and marine microbial ecologists.

Defense Documentation Center

Bibliography on oil pollution studies

Bibliography on ballast pumping hazards.

Bibliography on oil pollution in harbors

Bibliography on stability of emulsions

Bibliography on standards for water insolubles.

Work Summary - DDC

Acrylic plastic embedding.

Metering of toxicants into body of water through a membrane

### 3.40 GRANTS AND CONTRACTS

Grants and contracts previously assigned to Drs. Wade and O'Hara of the Bears Bluff Field Station as Project Officers have been assumed by the Washington Office.

#### 3 41 GRANTS

16020 EIB "Assays of Toxic Pollutants by Fish Blood Aberration". Sias Research Laboratories, Brookline, Mass. W.A. Curry, Principal investigator; Dr. W. S. Hodgkiss, Project Officer

The program has been funded for an additional year. Further studies will attempt to equate changes seen electrically on a Biological Multi-Channel Analyzer with microscopic and photomicrographic observations on fish blood samples taken simultaneously

16020 EGG "Development of Sample Preparation Methods for Analysis of Marine Organisms." Southwest Research Inst ; Dr H.C. McKee, Principal Investigator; Dr. W. S. Hodgkiss, Project Officer.

A renewal of this grant has been approved. Reviews of reports show satisfactory progress.

16020 DXT "Development of a Membrane Salinometer for Monitoring Estuaries." Woods Hole Oceanographic Inst.; Dr. T.R.S. Wilson, Principal Investigator; Dr. W. S. Hodgkiss, Project Officer

The grant has been continued The objective is to develop a membrane salinometer which will produce a continuous record of in situ salinity change with depth or time

16020 GCQ "Adsorption of Pesticides from seawater," Woods Hole Oceanographic Inst.; Dr. George R. Harvey, Principal Investigator; Dr. E. W. Davey, Project Officer.

18080 DOJ "Development of an in situ Marine Bioassay with Clams " State of Washington, Dr. C.E. Woelke, Principal Investigator; Dr. D. K. Phelps, Project Officer.

Contract in third and final year Additional Funding has been requested by the Pacific NW Region, and approved for additional field bioassays to support enforcement efforts.

18080 DHM "Estuarine Ecology Research." University of Washington; Dr E.O. Salo, Principal Investigator; Dr. D. K. Phelps, Project Officer.

Final Report in preparation.

18050 DHM "Effects of Oil Pollution on the Ecology of a Tropical Shore - Galeta Island, Panama Canal Zone." Smithsonian Institution; Peter W. Glynn, Principal Investigator; Dr. D. K. Phelps, Project Officer.

Currently functioning under a funded extension in order to evaluate data collected to date.

18050 DDN "Thermal Loading Effects on the Hawaiian Marine Biota." University of Hawaii, Sidney J. Townsley, Principal Investigator; Dr. D. K. Phelps, Project Officer.

18080 DFP "Environmental Biology of Tomales Bay." University of the Pacific; Dr. Edmund Smith, Principal Investigator; Dr. D. K. Phelps, Project Officer.

Final report being prepared.

18080 DNV "Marine Waste Disposal and Sea Urchin Ecology." California Institute of Technology; Dr. W. J. North, Principal Investigator; Dr. D. K. Phelps, Project Officer.

Final report submitted for publication.

18050 DUI "Toxicity of Metals to Marine Phytoplankton Cultures," Texas A&M University; Dr. W. B. Wilson, Principal Investigator; Dr. C. S. Hegre, Project Officer.

18050 DXI "Effects of Pollutants on Submarine Plant Synecology," Western Washington State College, Dr. Maurice A. Dube, Principal Investigator; Dr. J. C. Prager, Project Officer.

18050 EBN "Interaction Between Marine Organisms and Oil Pollution," Woods Hole Oceanographic Institution; Dr. Hunt, Principal Investigator; Dr. C. S. Hegre, Project Officer.

18050 FBW "Environmental Requirements of Selected Estuarine Ciliated Protozoa," University of New Hampshire; Dr. A. C. Borror, Principal Investigator; Juan Gonzalez, Project Officer.

18050 DRJ "Physiological Adaptation of Two Species of Fish, Fundulus heteroclitus and Salmo gairdneri, to Altered Water Quality Parameters"; University of New York at Buffalo; Dr. E. J. Massaro, Principal Investigator; Dr. G. LaRoche, Project Officer.

Mercury uptake studies were made on the rainbow trout. Concentration factors were developed for this fish. Mercury concentration factors have been studied over a period of 1 hour to 113 days. The author has also applied for a renewal of this grant.

18050 DTX "Chemical Responses by Marine Organisms to Stress II"; University of Rhode Island; Dr. H. P. Jeffries, Principal Investigator; Dr. G. LaRoche, Project Officer.

Work was conducted on the fatty acid distribution in a marsh ecosystem near Narragansett Bay. This grant is to be terminated shortly.

18050 DBT "Pesticide Residue Effects on Larval Marnie Fishes"; University of Massachusetts; Dr. C. F. Cole, Principal Investigator; Dr. G. LaRoche, Project Officer.

Final report is in preparation.

### 3.42 CONTRACTS

14-12-836 "Planning and Design of Narragansett Bay Synoptic Monitoring System." Nereus Corp.; Dr. Richard Hill was Principal Investigator; Dr. D. K. Phelps, Project Officer.

The final report has been completed, approved and forwarded for publication.

14-12-856 "Collection and Establishment in Culture of Species and Strains of Marine Phytoplanktonic Organisms," University of Rhode Island, GSO; Dr. T. Smayda, Principal Investigator; Dr. R. L. Steele, Project Officer.

The culture collection now contains more than 250 isolates constituting about 125 species most of these are now being grown bacteria free. During the last quarter we have requested and received 20 different species of plankton.

14-12-872 "Remote Water Quality Monitoring System" Ocean Data Equipment Corporation; Mr. A. Petteruty, Principal Investigator; Dr. D. K. Phelps, Project Officer.

The evaluation period for sensor reliability and accuracy over time has been extended to February 4, 1972, without additional funding.

68-01-0048 "Dissolved Oxygen and Temperature Monitoring System-Research Houseboat." Ocena Data Equipment Corporation; Mr. Allan P. Alarie, Principal Investigator; Allan D. Beck, Project Officer.

The monitoring and controls system has been designed and installed on the Research Houseboat and preliminary evaluation completed. A final report is in process.

68-01-0122 "Services for the Quantitative Analysis of Pesticides in Seawater and in Biological Samples." Analytical Biochemistry Laboratories. Mr. James Ussary, Principal Investigator; Dr. W. S. Hodgkiss, Project Officer.

68-01-0151 "Determination of the Biological Effects of Methyl Parathion, DDT, TDE, and Azodrin on the Marine Copepod *Acartia tonsa*," TRW/Hazleton Laboratories, Vienna, Virginia; Dr. F. H. Khattat, Principal Investigator; Dr. J. H. Gentile, Project Officer.

The first progress report indicates that the literature review is well underway and that all necessary facilities and biological materials have been assembled. Progress is satisfactory.

#### 4.00 PRESENTATIONS

Mr. Paul Yevich participated at the Offshore Drilling Conference held at Montauk, New York (New York Ocean Science Laboratory) on September 13, 1971. A paper was given on the impact of oil spills on Marine biota.

A paper was presented at the 6th Conference of the European Comparative Endocrinologists at Montpellier, France, by Mr. N. J. Blake and Mr. P. P. Yevich entitled, "The Neurosecretory Cycle of the Bay Scallop, Aquipten irradians."

#### 5.00 PAPERS SUBMITTED FOR PUBLICATION

Pesch, G. Isozymes of lactate dehydrogenase in the hard clam, Mercenaria mercenaria, submitted to Science. Pesch, G. Protein polymorphisms in the hard clam, Mercenaria mercenaria, to be submitted to Genetics.

The manuscript, "Annotated bibliography on biological effects of metals in the aquatic environment (No. 1-567)," by Ronald Eisler, has cleared in-house review preparatory to publication in the EPA research report series.