

QUARTERLY REPORT OF RESEARCH

APRIL - JUNE 1971

NATIONAL MARINE WATER QUALITY LABORATORY

WEST KINGSTON, RHODE ISLAND

Dr. C.M. TARZWELL, DIRECTOR

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Environmental Protection Agency  
Water Quality Office  
Office of Research and Monitoring

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

## 1.00 GENERAL

As a member of the National Temperature Committee, the Director has attended several meetings with state representatives to discuss temperature requirements and the setting of temperature criteria. On April 5-6, he was in Montgomery, Alabama, to participate in the hearings on temperature and oxygen criteria for both marine and fresh waters in the state of Alabama. He presented a paper on water quality requirements for the record of the hearings. On April 18-20, he was in San Francisco for a meeting in the Regional Office and with state representatives for the discussion of water quality criteria for fresh, estuarine and marine waters of the State of California. Recommendations were made for criteria for each type of water as to allowable temperature increases and allowable maximum temperatures.

On April 21-23, he was in Atlanta, Georgia, to attend the meeting on Pesticides which was called for reaching some decision on pesticides to be used in research studies and the coordination of work on pesticides.

On April 29, the Director attended a meeting in New York City for the discussion of water quality criteria for the State of New York. Members of the Regional Office, Washington Office, and the National Temperature Committee and representatives of the State of New York participated in this meeting. All but a few items were resolved.

On June 16 he participated in a meeting of Regional Corp of Engineers and EPA representatives for a discussion of thermal problems in Narragansett Bay. On June 18 the Director participated in a meeting of Regional representatives of Sport Fisheries and Wildlife, Corp of Engineers and EPA with representatives of the New England Power Company to discuss temperature conditions and requirements for Mount Hope Bay.

On July 2, members of the Regional Office and the New England Power Company met at the National Marine Water Quality Laboratory to discuss methods of determining natural temperatures in Mt. Hope Bay. No agreement was reached due to the fact that very little data is available to indicate temperatures in Mt. Hope Bay before the Brayton Point plant was put into operation.

An agreement for the use of the continuous flow seawater "wet Laboratory" at the Northeast Water Hygiene Laboratory, EPA, Narragansett, R.I. has been made. This expands the continuous flow aquaria facilities from 1500 to 3000 square feet for both ecological requirements and toxicological studies carried on in the Rhode Island area. Extensive work has been accomplished in re-vitalizing the area including pump repair, replacement of storage tanks and addition of a new glass lined aquaria to increase the capacity of the system. This expansion has been identified primarily for studies of safe levels of pesticides in estuarine and coastal waters, the program for which was established during this quarter.

Additional laboratory space has also been made available, in NEWHL, for the newly purchased Finnigan 1015 Mass Spectrometer-gas Chromatograph with data reduction system. The unit is a low resolution mass spectrometer capable of identifying many of the potential organic chemical pollutants. It's primary program will be that of pesticide identity, both in the seawater aquaria system as well as in biological tissue for body burden. This capability has been sorely needed for some time.

The research barge houseboat was launched in the Pettaquamscutt River on June 16th. Marine electrical cable has been installed; the dissolved oxygen and temperature control and monitoring system is under contract and is expected to be completed in early July. Full scale studies will commence at that time.

A contract has been awarded for hull inspection, painting and necessary repairs to the Research Barge. This work is scheduled for October or November 1971 during a period of minimum disruption of the research program.

Research programs at the Bears Bluff Field Station have been continuing on a regular basis. Following the resignation of Dr. Richard Wade, Chief of the Station, Mr. Alston Badger has been named as Acting Chief until a permanent Chief can be appointed. Dr. James O'Hara has resigned and Dr. Don C. Miller, Assistant Professor Biological Sciences, Union College, Schenectady, N. Y. has been appointed as his replacement.

The demand for library services for both the principal laboratory and the field station have necessitated the addition of a library assistant. Current awareness searches in Biological Abstracts and Bio-research Index on toxicity of heavy metals to marine and estuarine organisms will be continued by the University of Georgia Computer Center. A extensive literature search for information on the toxicity of "water" soluble fractions of crude oils and petrochemicals by-products to marine organisms is in process.

At the request of the University of Rhode Island's Chapter of HEED (Humans to End Environmental Deterioration), Dr. Prager organized an April 21 panel discussion on Ecology for URI'S Earth Week activities. Panelists were Barbara Reynolds, ecologist, of Brown University; Jay Cronin, Biologist, of R. I. Department of Fish and Game; Milt Anderson, ecologist, of New England Electric Company, and Dr. Prager as a panel moderator. Students participated actively in the discussion which ran an hour over schedule. We received a very gratifying letter of thanks from HEED.

Personnel assisted in a NEBO investigation of a fish kill in the Lees River, a tidal arm of Mt. Hope Bay during the week of April 19. According to later newspaper accounts, the fish kill was traced to overchlorination of industrial cooling waters pumped into the Lees River and the responsible company was fined by Massachusetts courts for violating water quality standards.

At the request of Mr. John Rademacher, Director of Region VII, Dr. Prager evaluated Union Electric Company's Thermal discharge reports and their proposal to site a generating plant at Rush Island. Numerous deficiencies in the reports such as a lack of summer data, incomplete knowledge of thermal requirements of resident species, and environmentally undesirable features of plant design were pointed out, and recommendations were to insist upon "Green Book" thermal standards if closed system cooling towers could not be negotiated as a first choice.

## 2.00 RESEARCH

### 2.10 Ecological Requirements for Plankton

#### 2.11 Need 18080 OUE, Plan GJZ, "Dissolved Oxygen and Temperature Requirements of Marine Plankton."

The Brayton Point project has continued and intensified during the last part of spring. Many developments have been recorded on colored slides. Racks of asbestos plates were located at different points at the intake, effluent, and in Lees River to assess the effect of heated water on settling of organisms. Plates are removed every two weeks to measure the extent of settling of organisms. The settling has followed a typical succession pattern. First there was a slimy filamentous diatom that formed a "jungle" for harpacticoid copepods. Barnacles settled at a high rate with larger numbers in the effluent canal, possibly an effect of current rather than temperature. The settling was similar in both intake and effluent canals at the very beginning. However, the effluent plates became covered with hydroids at a faster rate, so that it is now nearly impossible to separate the plates. The intake rack is still mildly covered.

The growth rate of Mytilus edulis, a mussel, that set in the effluent canal during the winter months (perhaps late fall) has been followed biweekly. Growth was fast (over 2 mm/week in May). However, it has virtually stopped as temperatures in the effluent canal have approached their tolerance limit. Microscopic examination (fixing in situ, sectioning and staining) reveals that they have reached spawning stage (state). A new set (spat) of this mussel occurred in May, probably as the result of the spring spawn in other parts of the bay. Growth of this set has been followed also. The purpose of this study is to verify the effect of the heated effluent on the fluctuations in populations of this animal. Callinectes sapidus (blue crab) is invading the effluent canal, preying on Mytilus.

Temperature tolerance experiments have been run on Acartia clausi, the dominant copepod in Narragansett Bay at this time of the year. Critical thermal maxima and upper lethal temperature have been determined for animals held at various temperatures. There appears to be some correlation between the temperature at which they are held and their CTM and ULT.

Salinity-temperature interactions are also being studied with this animal. Long exposure to different temperatures indicates that A. clausi cannot live long at temperatures above 25°C. High mortality (50%) was observed in the field when the temperature of the effluent canal was 30°C.

Plans, drawings, and specifications have been prepared for a mobile laboratory facility which would greatly facilitate field work of this sort and also support regional and enforcement activities.

Most emphasis in the area of marine macrophyte ecology was placed on constructing facilities at the Water Hygiene Laboratory for the culture of marine algae in continuously running seawater. Progress to date has been very slow because of delays in receiving and assembling equipment. We have about 15 species of algae in one tank awaiting cleaning and beginning of culturing.

Some laboratory studies have been carried out on the green alga, Enteromorpha clathrata. E. clathrata was collected at Charlestown beach during March and was isolated free of other organisms. We have tested it against a series of salinities ranging from 0 - 60‰. Growth occurs most rapidly at 5-20‰ and drops off very rapidly above 40‰. The ability of E. clathrata to sporulate was examined as a function of salinity. Sporulation occurs at the same salinities that growth is best, between 5 - 20‰. To date, this organism has gone through two complete asexual cycles in the laboratory.

Thermal exposures to Skeletonema costatum and Cyclotella nana acclimated to 20°C have been completed. After 180 minutes exposure to 30°C, C. nana showed only a 15% reduction in growth rate during the subsequent 24 hr. At 35°C for 90 minutes there was a 77% reduction in growth rate during the subsequent 24 hr. with a resumption of normal growth rate after 48 hr. At 35°C for 180 minutes a 72 hr. lag occurred before resumption of normal growth rate resulted, and after 168 hr. Skeletonema was considerably more sensitive to thermal shock. At 30°C for 60 minutes there was a 33% reduction in growth rate during the subsequent 24 hr.; at 40°C a 5 minute exposure was completely inhibitory to growth. Future studies will involve evaluating thermal stress to Thalassiosira, Detonula, and chaetoceros.

In addition thermal stress to C. nana and Skeletonema will be re-evaluated at 25°C acclimation temperatures.

- 2.12 Need 18080 OUA, Contract 14-12-856, "Collection and Establishment in Culture of Species and Strains of Marine Phytoplanktonic Organisms, "University of Rhode Island, Graduate School of Oceanography. Contract Officer - Dr. R. L. Steele.

An unusual organism has been isolated and placed in the culture collection. We have isolated a cryptomonad, Cyanomonis, which is interesting because it contains the pigment phycocyanin. Normally this pigment is found only in the red algae or the blue green algae.

As of the last quarterly report, the culture collection contained 250 clones. The contractor is also continuously supplying Isochrysis galbana for raising clam larvae.

## 2.20 Plankton Toxicological Studies

- 2.21 Need 18080 OWA, Plan ETR, "Toxicity of Oils and Petrochemicals to Plankton."

Examination of cellular ATP (adenosine triphosphate) as a measure of biomass or of stress response has continued. An indirect measure of this sort is needed for studies of the effects of oil or other adsorbed materials on microorganisms in sediments. ATP/cell and ATP per unit cell volume were determined for Cyclotella nana, Skeletonema costatum, Rhodomonas baltica, Detonula confervacea, and Thalassiosira nordenskioldii. The value for ATP per unit cell volume are very similar for all species. These values vary slightly with stage of growth and nutritional history but are independent of stage of light-dark cycle. We are presently evaluating the relationship of ATP/ in stress situations (i.e., Cu, Cl, etc.). The ATP content of a variety of neritic copepods is similarly being determined in relation to total carbon and dry weight measurements. Investigations are also being made to determine the ATP content of these zooplankters under various stress conditions, such as exposure to copper or chlorine.



The measurement of grazing rates may prove to be a useful parameter in assessing the sublethal effects of toxic substances such as oil. Accordingly, preliminary experiments were undertaken to attempt to determine the filtration rates of various zooplankton taken both from natural environmental and laboratory culture. Results were somewhat inconclusive indicating need for further study and improvement of techniques.

2.22 Need 18080 OWB, Plan ETM, "A Measure of the Toxicity of Heavy Metals to Estuarine Plankton".

Laboratory screening studies have been initiated with chromium and mercury. Copper toxicity was investigated in a variety of unenriched natural seawater and in a minimally enriched waters. The test species were Cyclotella nana and Skeletonema costatum.

The effect of initial cell density was also investigated. There was a distinct decrease in degree of growth inhibition when cell density exceeded  $2 \times 10^4/\text{ml}$ .

A field study has been planned to validate laboratory results and to determine the extent of the natural variability in copper toxicity. The field study will include a bioassay procedure (now undergoing laboratory testing) for determining the copper complexation capacity of natural waters. Water masses sampled will be characterized as to biological and chemical content.

Chitosan was prepared from lobster carapaces according to the method of Muzzarelli (1970) and tested as a possible alternative to Chelex-100 for the removal of trace metals from marine culture media. It was confirmed that chitosan could be used to remove trace metals from seawater but chitosan had only 10 to 20% of the capacity to take up  $\text{Zn}^{65}$  as Chelex-100. Other trace metals such as silver, a metal not effectively taken up by Chelex-100, have yet to be tested. Copper-chitosan was also tested and found not to take up amino acids from seawater as efficiently as copper-chelex.

Grant 18050 DUI, "Toxicity of Metals to Marine Plankton," Texas A & M University, Dr. W.B. Wilson, Principal investi-

gator, Dr. C. S. Hegre, Project Officer.

Progress on this grant has been satisfactory. A continuation proposal has been received and reviewed favorably.

2.23 Need 18080 OSK, Preliminary investigations, "Toxicity to Marine Organisms of Free Chlorine and Chlorinated Compounds in Seawater."

The Chlorine demand of Brayton Point natural seawater and of synthetic TM-6 was investigated. After 24 hr. the titratable chlorine had decreased from 4.9 ppm to 1.1 ppm in natural seawater and 4.9 to 4.25 in synthetic. In a second series the values went from 10.21 mg/l to 5.75 mg/l in 24 hr. in Brayton Point water. It is probable that the chlorine is interacting with organic compounds. We found that Brayton Point water which was treated with 10 mg Cl/l had no detectable chlorine after 500 hr. Bioassays (enriched and unenriched) of this water indicated the presence of some inhibitory compounds not present in untreated water. Further studies of this nature are in progress and it is hoped that chemical analysis of treated water will help in characterizing possible toxic chlorinated organics.

Acute chlorine toxicities have been established for several algae. 24 hr. IL50's are: Skeletonema 95 ug/l, Rhodomonas 120 ug/l, Detonula 250 ug/l, Cyclotella 130 ug/l. Another series of experiments with Cyclotella is in progress which relates exposure time and chlorine concentration. Chlorine is removed at specific time intervals by thiosulfate titration. At 1.0 and 0.5 ppm, chlorine exposure for 30 seconds resulted in partial kill with growth resurgence after 48 hr., at 60 seconds exposure complete kill was observed. At 0.3 ppm an exposure of 60 seconds produced partial kill and subsequent regrowth after 48 hr. At 5 minute exposure inhibition was total and permanent. At 0.150 ppm slight inhibition was evident after 5 minutes exposure and only partial inhibition after 24 hr. exposure. Research is continuing on this study using different combinations of time and concentration.

Bioassays were conducted to determine the toxic effects of chlorine (as Hypochlorite) to select zooplankton species (Acartia clausi, Tigriopus japonicus, Pseudodiaptimus coronatus). Results show resistance of species tested to

levels of 7.5 ppm chlorine for 5 minutes time exposure. Tests have not yet been conducted to determine the effect of chlorine exposure in combination with temperature increase as is encountered in electric power plants.

#### 2.24 Special Project, "Toxicity of Waste from Antibiotic Production."

At the request of the New England Basins Office, sludge from antibiotic production was tested for toxicity to phytoplankton, zooplankton, and an amphipod. Acute toxicity of sludge to C. nana and R. Baltica was not evident at concentrations up to 1000 mg dry weight/liter. Extracts of sludge (72 hr. at 15°C in synthetic seawater) were found inhibitory to algal growth at 10% (v/v) concentration. The initial samples when added to enriched and unenriched seawater produced definite fungal growth; autoclaved samples did not. Subsequent "aseptic" samples didn't manifest any fungal growth. ATP analyses of initial sludge samples revealed definite presence of ATP indicating the presence of living organisms. In tests with a number of zooplankton species no mortality was observed in any of the bioassays using either the method of undiluted extract or of suspended sludge at levels as high as 500 mg dry weight/liter. Tests were also made with the amphipod Corophium sp. using the suspension only. No mortality was observed after 72 hr. exposure at 500 mg dry weight/liter. Amphipods have yet to be tested with the extract. Our determinations have shown the sludge to be 20% carbon on a dry weight basis.

#### 2.30 Ecological Requirements for Fish and Invertebrates

##### 2.31 Need 18080 OQA, Plan ETI, "Dissolved Oxygen, temperature and salinity requirements for selected marine fish and invertebrates."

Studies of temperature increments above ambient on adult and larvae and eggs of winter flounder, Pseudopleuronectes americanus were essentially completed for the season during this quarter. Results indicated that eggs are more sensitive to thermal stress than are larvae or adults. Egg mortality

became significant at 13°C while larvae as well as adult flounder started to succumb at 17°C.

A breakdown of thermal tolerance at 17°C for both adult and larval flounder is suspect however. In the case of the larvae, difficulty was encountered in identifying and supplying an adequate food supply. Some success was realized by providing larvae of the hard shell clam, Mercenaria mercenaria, however, limited supplies of clam larvae dictated the continued use of wild plankton collections as the primary food source.

Adult flounder died from an infection that was an indirect effect of thermal load. The upper thermal limit for adult flounder is 26.5°C for individuals acclimated at 20°C (McCracken, 1963, Seasonal movements of the winter flounder, P. americanus, on the Atlantic coast. Fish Res. Bd. Can. 20(2):557-586). Barge flounder acclimated at 12, 9 and 5°C died consistently at 5 or 6°C below values extrapolated from McCracken's data. The difference is most likely due to the bacterial disease which is of itself related to temperature level.

Drs. Cabelli and Levine of the Water Hygiene Laboratory and Dr. Wolke of the Sea Grant experimental station, University of Rhode Island, isolated the bacteria and carried out pathological examinations of diseased flounder. They reported on their work as follows:

Piscine Vibriosis is best described as an acute bacterial disease of marine fish characterized by dermal ulceration. Descriptions of histopathological examinations have been made. The vast majority of such descriptions have been associated with Vibria auquillarum infections.

The following brief report describes lesions associated with an outbreak of Vibrio ichthyodermis among Pseudopleuromectes americanus under aquaculture conditions, and subsequently observed and verified in natural populations.

#### Pathology:

Gross lesions: Gross lesions were confined to the skin and musculature of affected fish. These lesions in their acute phase were characterized by petechiae and ecchymoses which in their most chronic manifestations became frank ulcerations. They occurred with equal frequency on both pigmented and non-pigmented surfaces and often involved the fins, especially the anal fin.

Microscopic lesions: Histological changes were present in the skin, musculature and kidney.

Dermal lesions included acute necrosis of the epidermis, dermal edema and a lymphocytic infiltration. Brown-Bien and Giemsa stains revealed numerous gram-negative rods scattered throughout the dermis and about the edges of ulcers.

The lesion observed in the skeletal muscle was one of classical ZENKER's necrosis with little or no inflammatory response. Congestion of vessels was common and perivascular accumulations of bacteria were also present.

Kidney lesions were of two types. Firstly, a relatively consistent increase in the numbers of primitive HEMATOPOIETIC CELLS (Hemocytoblasts) both in cut section and in WRIGHT's stained impression smears were observed in affected fish. Secondly, focal interstitial necrosis and some tubular necrosis were noted.

In addition, histological examination revealed a gram-negative organism in the tissue. An organism biochemically identified as Vibrio ichthyodermis was consistently isolated from the lesions from fish which had been exposed to increased temperature. Lesions in fish cultures of the organisms when injected into the fish produced identical lesions. Vibrio ichthyodermis was reisolated from the lesions. This is the first report of mortality in Pseudopleuronectes americanus caused by Vibrio ichthyodermis.

Fish with lesions were observed in relatively high numbers by an experienced collector for the Graduate School of Oceanography, URI. His observation was that this year there were many more fish with lesions than he had ever previously noted.

The development of pigment changes for protective coloration characteristic of the winter flounder was assessed as a measure of stress. While pigment change to a uniform background took 10-12 minutes at ambient temperature, intensity failed to develop after half an hour in fish exposed to plus 5 and 10°C. This work was carried out by two high school students from a National Science Foundation special studies group.

The black fish, Tautogus onitus, a summer migrant into local waters, has been introduced into our temperature control system. Work this summer will be limited to temperature effects on adult fish. The scup, Stenotomus chrysops, another summer migrant, is currently being acclimated to our holding system for future experimentation.

A study of the effects of depressed levels of dissolved oxygen on eggs of the winter flounder indicated that 1.0 ppm D.O. is lethal and low oxygen concentrations generally retard egg development. Limited test facilities prevented extensive investigations in this area.

Studies have been initiated to evaluate use of static vs. fluctuating systems in assessing relationships between temperature and growth for developing larvae of the quahog, Mercenaria mercenaria. Experiments continue on the determination of effects from depressed levels of dissolved oxygen on the growth of larval Mercenaria mercenaria. Larvae of the oyster, Crassostrea virginica, are being introduced into the dissolved oxygen experimental system.

The first run of a series of comparative studies to check for biological artifacts resulting from the mechanical removal of dissolved oxygen by sparging with nitrogen has been completed. Preliminary results indicate that similar levels of D.O. have identical effects on survival of the shrimp, Crangon septim-spinosa, whether or not the levels of D.O. are depressed by nitrogen sparging, biological reduction through the green algae, Codium fragile, or by respiring blue mussels, Mytilus edulis. A future series will be run to verify these results. The data base generated through this study will then be used to compare similar studies which will be run on a houseboat located on the Pettaquamscutt River. The latter facility will utilize water of low oxygen content from a reservoir of oxygen deficient water created through natural processes.

Studies to determine preference of settling M. mercenaria larvae along a gradient of dissolved oxygen have been temporarily suspended pending re-design of the experimental apparatus.

Comparative studies of dissolved oxygen requirements between larvae of M. mercenaria and the coot clam, Mulinia lateralis, continue but are limited by the availability of sexually ripe coot clams.

A "live car" design is being tested for in situ bioassays using oyster larvae. Larvae will be distributed in cars located at various sites in the upper portions of Narragansett Bay. Growth and survival to the straight-hinge stage will be observed.

Ecological requirements for dominant members of the ubiquitously distributed soft-bottom community, which are important food items in the diet of the winter flounder, Pseudopleuronectes americanus, continue to be studied. The natural cycle of gametogenesis for the polychaete, Nephtys incisa is being documented. Studies of effects of environmental stress on reproduction require knowledge of "normal" gamete formation in the natural environment. Monthly histological examinations continue.

Preliminary factorial studies leading toward a determination of the long term effects of combining temperature and dissolved oxygen stress have been completed for the polychaete, N. incisa and the clams Yoldia limatula and Nucula proxima. A nitrogen bubble problem required modification of system design.

A factorial study of short - term effects of temperature and dissolved oxygen stress on the shrimp, Crangon septimspinosus, has been completed. The D. O. level at which 100% mortality occurred in spite of temperature was 0.72 ppm. The upper temperature level at which 100% mortality occurred, regardless of D. O. level was 29.5°C. Fifty per cent mortality occurred at 16°, 19.8°, 23.1°, 24.5°, 25.5°, and 27°C for dissolved oxygen levels of 1.52, 2.29, 3.37, 3.82, 4.2, and 4.8 ppm respectively. One hundred per cent mortality occurred at 1.52, 2.29, 3.37, 3.82, 4.2, and 4.8 ppm dissolved oxygen at 18.5°, 25°, 26.5°, 28.5°, 29°, and 29.5°C respectively. Survival was complete at 14.2°, 17.8°, 21.2°, 22.6°, and 23.8°C when dissolved oxygen levels were 2.29, 3.37, 3.82, 4.2, and 4.8 respectively.

A report of the total body burden of Fe, Zn, Co, and Sc in a marine worm, Nephtys incisa, and a clam, Yoldia limatula, has been developed. Both are organisms which are important as food for the winter flounder, Pseudopleuronectes americanus. Levels of the metals were found to be different between points on a relatively short transect of stations during the summer in Narragansett Bay. However, such spatial differences essentially disappeared during the winter months primarily due to a great increase in variability of metal levels.

The greatest differences in total body burden were related to seasonal change. All species at all locations reflected increased metal levels during the winter. An explanation for enhanced winter levels postulates a biological control system during summer changing to a physical chemical control system during winter. Metabolic and physical activities peak in the organisms during summer and are greatly retarded at temperatures below 10°C during the winter.

If further analyses substantiate the postulated dual seasonal control systems for heavy metals in the body of these organisms several pertinent questions arise:

1. How do levels of these metals in interstitial water compare to levels in associated sediments?
2. May there not be a "biological pump" that operates on a seasonal basis indicated? (such recycling from a reservoir of concentration should be taken into consideration in the establishment of water quality standards for heavy metals.)

3. Many not these organisms be most susceptible to heavy metal intoxication during winter (below 10°C) when biological regulation is least efficient, and body burdens of metals are highest?

These results were presented in a paper at the Third National Symposium on Radioecology held at Oak Ridge, Tennessee, May 13, 1971.

Lobster metabolism research continued during this quarter using salinity as the stress factor. High rates of mortality were observed at salinities of 15 and 40‰. Metabolic rates at 20, 25, 30, and 35‰ are being computed.

Temperature tolerance studies are continuing at the Bears Bluff Field Station on juvenile Mugil cephalus and on Uca pugnator. Conclusions cannot be reached at this time.

#### 2.40 Invertebrate and Fish Toxicological Studies

- 2.41 Need 18080 OSF, Plan GJY - "Effects of Sodium Nitrilotriacetate on Marine and Estuarine Organisms."

Flounder, scallop, oyster, and lobster chronically exposed to 10 ppm of NTA were examined for any changes in aminolevulinic dehydrase activity,  $^{14}\text{C}$  leucine uptake and RNA:DNA ratios. The only apparent difference was in the flounder muscle and liver nucleic acid ratio. Five NTA exposed and 4 control fish produced an average ratio of 8.0 and 5.6, respectively. The sample size was too small to test the difference for statistical significance. More NTA exposed animals will be tested to determine the reproducibility of the changes.

The study of the effects of NTA on the respiration rates of lobsters was resumed.

Histopathological studies were conducted on lobsters, scallops, and oysters exposed to NTA (10ppm) for 9 months. Sand shrimp were exposed for 6 months at the same concentration. Microscopic examination of the animals revealed no observable changes.

Animals were taken from the chronic exposure study on the barge after 9 months exposure to 10 ppm NTA. The water temperature during winter months just prior to sampling was -1 to 3°C. At these temperatures, electrophoresis of tissue homogenates showed that the metabolic activity is low, reflecting by lower enzyme levels. The water temperature now is 15-16°C and the animals are feeding better and are more active. This will be reflected in higher enzyme levels. Differences were obtained in the zymograms obtained from tissues of control and treated oysters when the enzymes malate dehydrogenase and alcohol dehydrogenase were detected. These differences occurred in the mantle, gill



and muscle tissue in case of malate dehydrogenase and in the mantle and gill in the case of alcohol dehydrogenase. These differences definitely suggest the NTA is being metabolized in at least oyster tissue.

Acute toxicity of 29 household synthetic detergents (purchased at local supermarkets) to the marine fish *Fundulus heteroclitus* was determined at 20‰ salinity and 20°C. A summary table is presented below:

TL-50, in mg/l total product	Number of detergents of 29 in each of the TL-50 toxicity range at 3 time intervals		
	24hrs	96hrs	168hrs
1.0	0	0	0
1.0-5.0	2	7	7
5.1-10.0	9	6	6
10.1-20.0	7	7	7
20.1-40.0	7	5	5
40.1-100.0	2	2	2
100.1	2	2	2

Of the 29 syndets, 13 produced 168 h TL-50 values in the range 1.0-10.0 mg/l total product. It is probable that the toxicant in each case is surface active component, linear alkylate sulfonate (=LAS). On the basis of estimated LAS content the 168 hour TL-50 values would be in the range of 0.2-2.0 mg/l. We consider these levels hazardous to marine fishes. Acute toxicity screening of the 29 syndets to the other species of marine organisms will be evaluated during the next quarter.

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

Preliminary studies are being carried out with an oil emulsification system designed to determine the toxicity of petroleum products to fishes. Earlier problems in design have been corrected, and toxicity assays are expected to proceed during the coming quarter.

Acute exposure studies were conducted on oysters, scallops, quahaugs and lobsters exposed to various concentrations of crude oil. Preliminary histopathologic studies of these animals, ranging in concentration from 30 - 550ppm, showed changes in the circulatory system of the oysters.

Acute exposure studies have been started on lobsters, oysters, scallops and quahaugs which are being exposed to water soluble oil fractions.

2.43 Need 18080 OSC, Plan ETO - "Toxicity of Heavy Metals to Marine Fish and Invertebrates."

168 hr. TL-50 values for chloride salts of mercury, zinc, cadmium, nickel, and chromium (as  $K_2CrO_4$ ) were determined for Fundulus heteroclitus. Additional assays are in progress with these compounds with sandworm, Nereis virens; muc snail, Nassa obsoletus, hermit crab, Pagurus longicarpus; and sand shrimp Crangon septemspinosa. Greater availability of animals during the summer months is anticipated.

Radiocadmium<sup>115m</sup> uptake studies with Fundulus heteroclitus under varying conditions of stable  $Cd^{2+}$  in the medium contained during this quarter. Preliminary results indicate that fish will continue to accumulate  $Cd^{115m}$  from the medium over a three week period, that radiocadmium uptake rate is inhibited by increasing stable  $Cd^{2+}$  in the medium, that gill is the initial site of cadmium concentration with eventual translocation to viscera and that loss rate of radiocadmium is slow. These studies will continue during the next quarter.

Progress was accomplished on the annotated bibliography of heavy metal effects on aquatic organisms. These abstracts (n=567) have been indexed by metal and cross - indexed by organisms. Final typing is in progress.

Studies on the effects of copper on the lateral-line system of Fundulus heteroclitus continued during the quarter. Intraperitoneal injections of copper did not induce pathological changes in the lateral-line system of Fundulus comparable to those observed following exposure to the metal solution. Experiments are being designed and implemented to determine the effects of copper on larval forms of Fundulus.

A second study was begun to elucidate the relative sensitivity of selected life stages of the common mummichog, Fundulus heteroclitus, to heavy metals. Study of the toxicity of cadmium to selected embryological stages has begun. Preparations are being made to culture embryos in order that the fry stage may also be studied. A histological examination of specimens is planned in conjunction with the survival phase of the study.

Fishes exposed to copper in the wild have been examined for histopathology. Preliminary investigation indicated that copper may be inducing a lesion under these conditions. The study will continue during the next quarter.

Amino-levulinic dehydrase inhibition was determined as a function of lead and other heavy metal exposure in killifish and winter flounder. Lead in sublethal concentrations inhibited this enzyme more than any other heavy metal tested. It was also observed that silver, zinc and to a lesser extent cadmium increased activity of this enzyme. A manuscript describing this work has been completed and will soon be submitted for publication. This enzyme will be studied in fish from lead contaminated waters and compared to that from uncontaminated areas. Ribonuclease inhibition will also be looked at using new simplified assay techniques.

Studies of heavy metal toxicity have continued at the Bears Bluff Field Station from the previous quarter.

The uptake, retention and TL-50 values were determined using Phenyl mercuric nitrate (PMN) and mercuric chloride (MC) in studies on Fundulus heteroclitus; Mugil cephalus, a common pompano; and Uca pugnator, the fiddler crab. All acute bioassays were carried out in one gallon glass jars containing 3 liters of seawater. A temperature of 20°C, ph of 7.7 - 8.0 and dissolved oxygen of 5.0 - 6.0 ppm were maintained except at 10% salinity where the ph value was 6.6.

Preliminary estimates of the 96 hour TL - 50 at 10‰, 20‰ and 30‰ salinity are shown in Table 1. Further replication of the experiments are in process for confirmation of the results reported.

Juvenile mullet and pompano had TL - 50 values which approximated each other for each toxicant. However, the Phenyl mercuric nitrate proved to be approximately ten times more toxic than mercuric chloride. In general the toxicity with respect to fiddler crabs was comparable at the three salinities for each of the toxicants with PMN being approximately twice as toxic as MC.

Tissue mercury concentrations were determined for Fundulus heteroclitis at 24, 48, 72 and 96 hours exposure for the several dosages of both PMN and MC as used in the acute bioassay tests. Although the data are incomplete, it appears that mercuric chloride exposed fish died with whole fish mercury loads of 150 - 170 ppb. Those having a body burden of .70 ppb or less survived the 96 hour test. Those fish exposed to phenyl mercuric nitrate died with a whole fish body burden ranging from 20 to 70 ppb. Survivors, after mercury levels lower than fish dying from mercuric chloride exposure.

Table 1. Estimated TL-50 values for Phenyl Mercuric Nitrate (PMN) and Mercuric Chloride (MC).

<u>Species</u>	<u>% Salinity</u>	<u>Toxicant</u>	<u>Ave. wt (gm)</u>	<u>Estimated 96hr. TL-50 (ppm)</u>
<u>F. heteroclitis</u>	10	MC	3.5	0.90
<u>F. heteroclitis</u>	10	PMN	3.5	0.21
<u>F. heteroclitis</u>	20	MC	3.5	1.2
<u>F. heteroclitis</u>	20	PMN	1.2	0.06
<u>F. heteroclitis</u>	20	MC	1.5	0.49
<u>F. heteroclitis</u>	30	PMN	3.5	0.14
<u>F. heteroclitis</u>	30	MC	3.5	0.87
<u>U. pugilater</u>	10	MC	3.5	0.39
<u>U. pugilater</u>	10	PMN	3.5	0.87
<u>U. pugilater</u>	20	MC	3.5	0.28
<u>U. pugilater</u>	20	PMN	3.5	0.52
<u>U. pugilater</u>	30	MC	3.5	0.31
<u>U. pugilater</u>	30	PMN	3.5	0.88
<u>M. aphelus</u>	20	MC	3.5	0.30
<u>M. aphelus</u>	20	PMN	3.5	0.02
<u>T. carolinas</u>	30	MC	3.5	0.45
<u>T. carolinas</u>	30	PMN	3.5	0.03

### 3.00 Project Support

#### 3.10 Fundamental Studies

Mya arenaria were collected and sent live to Roland Walker at the Polytechnic Institute in Troy, New York, for electron microscopy examination in an effort to substantiate our findings on morphological changes associated with a typical hyperplasia in the soft shell clam.

Futher studies have continued, where time permits, on the incidence of gonadal cancer in quahaugs from Rose Island.

Methods for tissue homogenization and gel electrophoresis for scallop gill, mantle and muscle; oyster gill, mantle and muscle; lobster gill and muscle; and sand shrimp (whole animal) have been devised and tested successfully. A few slight modifications are necessary to increase the resolution of certain enzyme bands.

Zymograms for the following specific enzymes were produced with success from the electrophoregrams of the above tissue homogenates: monoamine oxidase, DOPAoxidase, alcohol dehydrogenase, alkaline phosphatase, clucose-6-phosphatase, cytochrome oxidase, lactic dehydrogenase, esterase, malate dehydrogenase, succinic dehydrogenase, ATPase and glutamic dehydrogenase.

A study of bay scallops, *Aquiptecten irradians*, was begun to: (1) determine the respiration response of scallops to various DO-temperature regimes; (2) establish respiratory baseline values for this species for use in future toxicity studies. Data have been collected which indicate the respiration rates of scallops of various sizes exposed to three DO ranges at 10°C. Similar experiments are now being conducted at 20°C. Others are planned for 30°C.

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

Four populations of *M. mercenaria* were sampled: one each from the Bideford River, Prince Edward Island, Canada; Boothbay Harbor, Maine; Narragansett Bay, Rhode Island; and Wadmalaw Island, South Carolina. For comparison, Two populations of *M. campechiensis*, a southern congener, were sampled: one from Shackelford Banks, Beaufort, North Carolina, and one from Tampa Bay on Florida's Gulf Coast.

Disc-gel electrophoresis and histochemical staining techniques are being used to survey the isozyme patterns of various enzymes in Mercenaria. Since protein structure is closely controlled by DNA structure, genetic comparisons are inferred from comparisons of protein differences found within and among the six populations studied.

Work continued in two areas: one, the surveying of isozyme patterns in the six populations under study, and two, expanding the repertoire of analytical methods used in this sort of research.

The genetics of two enzymes, malate dehydrogenase and an esterase, were surveyed in 350 animals representing the six populations being considered. These results, along with previous work on the enzyme lactate dehydrogenase and tetrazolium oxidase, complete this phase of the Mercenaria population genetic study.

Histochemical and electrophoretic methods were developed, checked or modified for the following enzymes:

Lactate dehydrogenase: Methods currently used in this laboratory were checked by assaying a variety of tissues from rat and horseshoe crab. The isozyme patterns produced by our method agreed well with those reported in the literature for these two species.

Esterase: Four different electrophoretic systems were tried. A modification of a method reported by Ornstein and Davis works well and was used to survey the isozymes of this enzyme in 350 quahaugs.

Malate dehydrogenase: Two electrophoretic and two histochemical staining techniques were tried. Modifications of Ornstein and Davis's electrophoretic system and of Latmer's histochemical technique worked well and were used to survey the isozymes of this enzyme in 350 quahaugs.

Alkaline phosphatase, acid phosphatase, alcohol dehydrogenase, cytochrome reductase, succinate dehydrogenase and dopamine oxidase were all considered during this quarter. Attempts to develop analytical techniques for these enzymes are continuing.

### 3.20 Procurement of test Organisms (NMWQL)

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>Number</u>
<u>Homarus americanus</u>	96
<u>Homarus americanus larvae</u>	1000
<u>Crassostrea virginicus</u>	5 1/2 bushel
<u>Pseudopleuronectes americanus</u>	50
<u>Artica islandica</u>	150
<u>Palaemonetes pugio</u>	400
<u>Crangon septimspinosa</u>	1275
<u>Fundulus heteroclitus</u>	1850
<u>Tautogus onitus</u>	50
<u>Mya arenaria</u>	5 bushel
<u>Mercenaria mercenaria</u>	5 bushel
<u>Aquiptecten irradians</u>	3/4 bushel

<u>Species Collected</u>	<u>Number</u>
<u>Spisula solidissima</u>	1 1/2 bushel
<u>Nassarius</u>	1000
<u>Paguiris sp.</u>	1000
<u>Stenotomas chrysops</u>	57
<u>Paralichthys denatus</u>	3

Special collection trips were made to New York for scallops, to Maryland and Virginia for quahaugs, oysters, and scallops and Massachusetts for lobster larvae.

### Procurement of Test Organisms (BBFS)

Fifty two field trips were made during this quarter in order to collect various animals for pond stocking and for the Toxicology section. Methods of capture included otter trawls, hand seines, cast nets and minnow traps. These fifty two trips resulted in the capture of the following organisms:

<u>Species</u>	<u>No. Captured</u>	<u>Size</u>
<u>Penaeus setiferus</u>	311	5" to 7"
<u>Penaeus aztecus</u>	155	2" to 3"
<u>Leistomus xanthurus</u>	856	2" to 4"
<u>Micropogon undulatus</u>	2,479	2" to 4"
<u>Paralichthys dentatus</u>	62	5" to 7"
<u>Symphurus plagiura</u>	228	4" to 5"
<u>Callinectes sapidus</u>	756	4" to 5"
<u>Mugil cephalus</u>	7,500 (Imm)	1" to 2"
<u>Mugil cephalus</u>	175 (Mat)	6" to 7"
<u>Bairdella chrysura</u>	68	3" to 5"
<u>Menidia menidia</u>	5,000	1" to 2 1/2"
<u>Uca pugilator</u>	1,225	3/4" to 1 1/2"
<u>Trachinotus carolinus</u>	415	1" to 2 1/2"
<u>Menticirrhus americanus</u>	31	5" to 6"
<u>Menticirrhus littoralis</u>	49	1 1/2" to 3"
<u>Palemonetes vulgaris</u>	8,500	3/4" to 1 1/4"
<u>Brevoortia tyrannus</u>	97	4" to 5"
<u>Cyprinodon variegatus</u>	390	1 1/2" to 2"
<u>Trinectes maculatus</u>	51	3" to 4"

### 3.30 Technical Advances

Five groups of weighed flounder were held at the barge on different diets for two weeks. At the end of this period

fish weight changes were correlated with  $^{14}\text{C}$  leucine uptake and nucleic acid ratios. Since all fish lost weight for unexplained reasons, the experiment will have to be repeated. A start was made in collecting background information on nucleic acid ratios of freshly caught fish from local waters. Leucine uptake and nucleic acid ratios with respect to growth will continue to be investigated.

### 3.50 Facilities Improvements - Research Houseboat and Barge

An emergency warning radio communication system similar to that installed on the barge, is to be provided for the research houseboat and tied into the existing network. Water and electrical service will be monitored. Equipment has been ordered and installation started.

A new deck coating system for the research barge interior deck is being evaluated. The coating is 1/4 inch thick and composed of a natural aggregate bonded with epoxy resin. It provides a seamless impervious surface tightly bonded to the steel deck. If the system proves effective it could virtually eliminate deck maintenance on the barge.

### Facilities Improvements - Northeastern Water Hygiene Laboratory

The water system and troughs at the Northeastern Water Hygiene Laboratory have been completely cleaned and reconstructed so as to give a constant water flow. Animals have been added to the troughs and are awaiting additional numbers. To date winter flounder, sand shrimp, lobster, oyster, and bay scallop are being acclimated.

### 3.60 Grants and Contracts

#### 3.61 Grants

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

A renewal application and expansion of this grant has been reviewed.



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.

Studies on the concentration and storage of mercury in the rainbow trout were continued. Tissue concentrations of radio-mercury were made following injections.

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.

Fatty acid distributions in a marsh ecosystem has been studied at various times of the year. Distinct seasonal variations were observed.

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

Renewal application has been made for the third and final year.

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.

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 EFV "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.

Grant 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.

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

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

Grant 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.

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

### 3.62 Contracts

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

Final report has been submitted for publication.

14-12-872 "Remote Water Quality Monitoring System." Ocean Data Equipment Corporation; Mr. A. Petteruti, 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.

68-01-0122 "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.

#### 4.00 PRESENTATIONS

Dr. D.K. Phelps attended the Third National Symposium on Radioecology May 10-12, 1971 at Oak Ridge, Tenn., where he presented a paper co-authored by Gregory Telek entitled "Distributions of zinc, iron, cobalt and scandium in Nephtys incisa and Yoldia limatula."

Dr. D.K. Phelps presented a senimat on "Monitoring Water Pollution" at the Northeastern University Center for Continuing Education on May 5, 1971.

Dr. Ronald Eisler attended the Northeast Division meeting of the American Fisheries Society in Portland, Maine, on May 23-26, 1971, at which time he spoke on "Cadmium poisoning in marine organisms."

Allan Beck spoke to a group of 50 technical personnel on June 16<sup>th</sup> at Owens-Corning Fiberglass in Ashton, Rhode Island. Subject was "Effects of Pollution on the use and Development of Narragansett Bay."

#### 5.00 Papers Submitted for Clearance

Eisler, R., G. R. Gardner, R. J. Hennekey, G. J. LaRoche, D. F. Walsh and P. P. Yevich. "Acute toxicology of sodium Nitrilotriacetic acid (=NTA) and NTA-containing detergents to marine organisms." For publication in Water Research (The International Journal of the Association on Water Pollution Research).

Eisler, R. and G. J. LaRoche. "Elemental composition of the mummichog, Fundulus heteroclitus (Pisces: Cyprinodontidae) (Linnaeus)". For publication in Copeia Journal of the American Society of Ichthyologist and Herpetologists.

Barry, M.M. and P.P. Yevich. "Incidence of Cancer in the quahaug Mercenaria mercenaria." For publication in ONCOLOGY.

Voyer, R.A. and G.E. Morrison. "Factors affecting respiration rate of winter flounder, Pseudopleuronectes americanus (Walbaum)." For publication in the Journal of the Fisheries Research Board of Canada.

#### 6.00 Publications

George Morrison, NMWQL, "Dissolved Oxygen Requirements for Embryonic and Larval Development of the Hardshell Clam, Mercenaria mercenaria". Journal Fisheries Research Board of Canada, Vol. 28, No. 3, 1971

## 7.00 VISITORS

### 7.10 Narragansett

During this quarter 326 visits were recorded at the West Kingston Laboratory. Of these the distribution was as follows: Sales representative 95, employment interviews 51, technical discussions with professional staff 128, two group tours 46, and miscellaneous.

#### APRIL

Dr. Eugene Coan, The Sierra Club, San Francisco visited Dr. Hegre and was given a tour of the facilities.

Dr. W. Percy, Professor of Oceanography, Oregon State College visited the laboratory.

Dr. John W. Steenis, Patuxent Wildlife Center, USDI Fish and Wildlife Service met with the scientific personnel and presented a seminar discussion on pesticides.

Mr. David Mills, National Library of Medicine, Bethesda visited the laboratory to discuss the toxicology program and availability of reference materials.

Mr. Roy Levine, Lamont - Doherty Geological Observatory of Columbia University visited Mr. Gangaley to discuss copepod culture.

Dr. Walter Whitworth, University of Connecticut and sixteen of his students visited the laboratory and were given a discussion on the research aims of the laboratory by Dr. Hodgkiss and a tour of the principal laboratory. Dr. Phelps described the programs at the Research Barge during a tour at that facility.

Mr. Charles Wallker, Chief, Branch of Pesticide Control, Bureau of Sport Fisheries and Wildlife visited the laboratory, presented a seminar and discussed herbicide research activities and related problems of mutual interest.

Mr. Milton Anderson, New England Power Co. and Mr. G. C. Matthierson, Marine Research, Inc. visited Dr. Tarzwell to discuss thermal electric plants.

Mr. John Roper, Fish Research Biologist, NOAA, National Marine Fisheries Service, Oxford, Md. visited the library on sources of literature and Dr. Phelps on research approaches by this laboratory.

Dr. Philip Howard, Syracuse University Research Corp., visited Dr. Tarzwell as a portion of a National Science Foundation study of environmental laboratories.

#### MAY

Dr. E. Zuraw, General Dynamics, Groton, Conn., visited Dr. Gentile for a discussion on copepods.

Dr. Tom Siccan, Yale University Forestry School, visited Dr. Tarzwell to discuss heavy metals in salt marshes.

Dr. Peter Franls, Plant Physiologist, Plant Science Division, USDA Beltsville, visited the laboratory, presented a seminar discussing pesticide research problems of mutual interest.

Dr. Tom Maloney, Pacific Northwest Water Laboratory visited with Dr. Prager and the staff in regard to plankton research programs.

Capt. Vining Sherman, Ass't Director, Batelle Laboratory Duxbury, Mass.; Mrs. Marilyn Necet, Marine Biologist and Dr. R. Engel, Marine Biologist visited the laboratory to see and discuss the continuous flow bioassay system.

Dr. Gunter Zweig, Director, Life Sciences Division, Syracuse University Research Corporative visited the laboratory to discuss possible contract funding.

Dr. Richard Jagels, Research Associate, University of Vermont visited Dr. Prager to discuss a research proposal to EPA.

#### JUNE

Mr. Robert Delguidice, Teacher of an Ecology Class in the Gorton Junior H. S., Warwick, R. I. and thirty students visited the research barge. The research program there was described by Dr. Phelps and staff.

Richard C. Hamston and Philip Richardson from the Providence Country Day School assisted the the functions of the Research Barge for part of their senior year science studies. This is the second year that they have sent students to participate in our program.

Mr. Daniel A. Mills, Chief, Toxicology Information Program, National Library of Medicine, visited the library and informed us of the specialized information services available from their center.

#### 7.20 Bears Bluff Field Station

Mr. Paul Ramsey, S. R. E. L., Aiken, South Carolina

Miss Virginia L. Shelton and Family, Columbia, South Carolina.

Mrs. A. G. DeLones, Key West, Florida.

Dr. J. Barrier and Biology Class from the Baptist College of Charleston to collect marine organisms.

Mr. Jim Henderson, Charleston, South Carolina

Mr. Henry Gaud, Charleston, South Carolina.

Mr. R. M. Halcombe and Mr. E. J. King, South Carolina National Bank, Charleston, South Carolina.