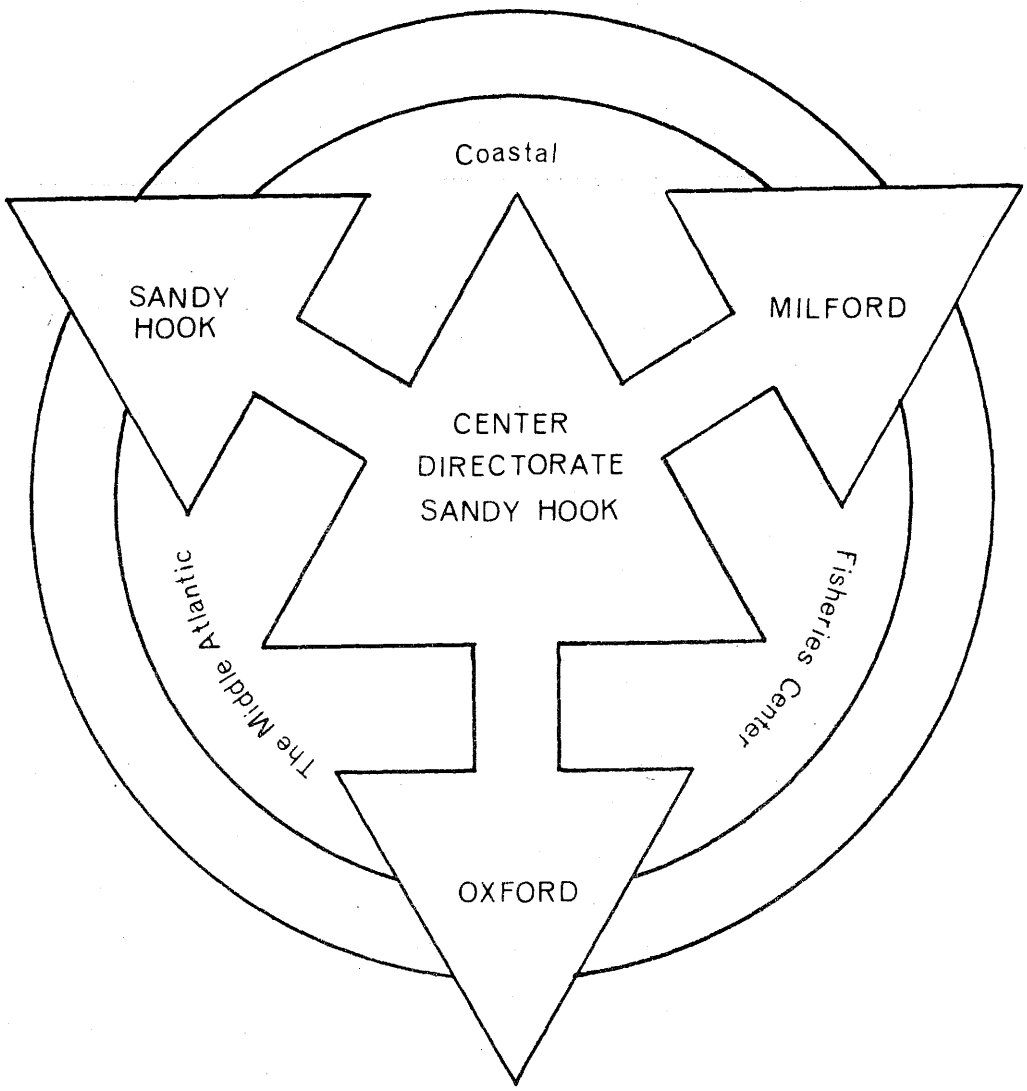
A MULTILABORATORY COOPERATIVE STUDY OF CONTAMINANTS IN THE COASTAL ENVIRONMENT AND THEIR EFFECTS ON LIVING MARINE RESOURCES: SUMMARY REPORT OF OPERATIONS FROM JANUARY 1, 1973 - December 31, 1974

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Region

MIDDLE ATLANTIC COASTAL FISHERIES CENTER



Informal Report
No. 73

MIDDLE ATLANTIC COASTAL FISHERIES
CENTER

NOAA--NMFS

A MULTILABORATORY COOPERATIVE STUDY OF CONTAMINANTS IN THE COASTAL ENVIRONMENT AND THEIR EFFECTS ON LIVING MARINE RESOURCES

SUMMARY REPORT OF OPERATIONS FROM

JANUARY 1 to DECEMBER 31, 1974

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NOTE:

The analytical findings reported herein are administratively restricted and are not to be published in any form.

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INTRODUCTION

A cooperative venture involving all NMFS laboratories of the Middle Atlantic Coastal Fisheries Center to study contaminants in marine ecosystems of the northeast and middle Atlantic coasts was initiated in the spring of 1971 and is continuing with the following objectives:

1. Delineate the contaminant levels (with particular emphasis on mercury) in living marine resources and their environment.
2. Determine the specific effects of waste disposal areas (i.e., ocean dump sites) in concentrating harmful contaminant materials.
3. Determine the pathological and physiological effects of contaminants on selected, typical marine organisms.
4. Develop a multilaboratory cooperative approach to the study of these problems and the essential methodology to conduct a long-range contaminant program.

The ultimate goal of the long-range study is to identify the impact of contaminants in relation to the abundance and distribution of living marine resources, to provide essential base lines for regulatory enforcement, and to provide specific information to balance waste disposal and economically valuable resources.

This report describes the efforts of the MACFC contaminant study from January 1 through December 1974; for previous information refer to Informal Reports, Nos. 5 and 26 of the Middle Atlantic Coastal Fisheries Center entitled "Cooperative Study of Contaminants in the

Coastal Environment and Their Effects on Living Marine Resources: Summary Report, 1971-1972" and "A Multilaboratory Cooperative Study of Contaminants in the Coastal Environment and their Effects on Living Marine Resources: Summary Report of Operations from May 1, 1972 to December 31, 1973." The cooperating laboratories and their area of specific responsibility and more general interest are listed below:

Milford	Ecosystems Investigations: Major responsibility for chemical analyses and for microbiological studies of the environment.
Oxford	Pathobiology Investigations: Responsibility for all pathological studies and for microbiological studies on or in the living organism.
Sandy Hook	Ecosystems Investigations: Major responsibility for ecological studies of waste disposal areas along the New York and New Jersey shore.
Milford	Experimental Biology Investigations: Responsibility for laboratory experiments to determine the physiological stress caused by contaminants on selected marine species.

ECOSYSTEMS INVESTIGATIONS

The principal thrust of Ecosystems Investigations is to understand the physical-chemical environment of the Middle Atlantic Bight and its relationships to the productivity and availability of living marine resources. Because the Bight is heavily impacted upon by the activities of man, it is essential that we understand how the various pollutants and physical disruptions affect the fisheries. The pollutants include discrete contaminants, such as heavy metals and hydrocarbons, as well as solid wastes (sewage sludge and dredging spoils) and liquid effluents which are complex, heterogeneous mixtures of sediments, organic matter and numerous, often unidentified specific contaminants.

Since NOAA and the NMFS have a legislated mandate to understand the effects of activities, such as ocean dumping, it is essential that our research be conducted in a manner that our data and results can be used to solve real management problems. Therefore, our studies are planned so that we can obtain information which will allow us to predict the impact of solid waste disposal, liquid effluents and contaminants on living marine resources. For instance, we are currently investigating the effects of dredging and spoiling in eastern Long Island Sound where we are determining the physical impact of dredging spoils on benthic communities and demersal fish. Our measurements are both direct and indirect; in addition to the

analysis of grab samples for changes in the biota and sediment, divers are observing and measuring how soils fall and spread from the dump site. Current structure and water column chemical measurements are also being made, again, to determine how the spoils react in the water column. Finally, the sediments and biota are being analyzed to determine how microorganisms, heavy metals and other contaminants resulting from dumping are accumulating in the physical environment and biota.

Similar measurements are being made in the New York Bight Apex where even larger quantities of waste materials are introduced as a result of ocean dumping, terrestrial runoff and point discharge of industrial and domestic wastes. Subtasks attached to Ecosystems Investigations are measuring the uptake of dissolved oxygen by contaminated sediments in the Bight, and adjacent estuarine waters, and relating the resulting data to other physical and biological factors. Other subtasks are concerned with the role of wastes in the carbon cycle, as it culminates in primary productivity, and with the nature of heavy metals in relation to binding with organic debris and the effects of these complexes on marine organisms. These subtasks are not concerned solely with static measurements but rather emphasize the dynamic aspects of the ecosystem. The principal questions include: "How are the various contaminants accumulating in the

system? At what rate are they entering and leaving the system? And, perhaps most important, how do they affect the living components of the total ecosystem?" The various subtasks have obvious relationships one to another and to the other subtasks and investigations of the Middle Atlantic Coastal Fisheries Center. The following brief resumés indicate progress made by the various investigations during the past six months.

ECOSYSTEMS INVESTIGATIONS

BIOLOGICAL OCEANOGRAPHY

J. Thomas, C. Evans, J. O'Reilly and W. Phoel

Phytoplankton and Primary Productivity

Raritan Bay and Lower Hudson Estuary:

As of March 1975, monthly field sampling for studies of phytoplankton primary productivity in the Raritan Bay and Lower Hudson estuary was completed. In all, sixteen monthly cruises occurred between November 1973 and March 1975. Station locations are shown in Figure 1. Primary productivity, photosynthetically active radiation, light extinction, temperature, salinity, dissolved oxygen, pH, alkalinity, phytoplankton nutrients (ammonia, nitrite, nitrate, phosphate, silicate, etc.), chlorophyll pigments, particulate organic carbon and nitrogen, hydrocarbons and particulate heavy metal concentrations were measured.

During the first seven months of our study, we observed an average of 11 percent of the photoassimilated carbon to be released as dissolved organic matter (< 0.45 micron). Table 1 presents a breakdown of percent release according to month.

Beginning in March 1973, we fractionated particulate production into two size classes, nannoplankton (< 20 microns) and netplankton (> 20 microns), so that we could assess the relative potential

Figure 1. Phytoplankton and Primary Productivity Sampling Stations in Raritan Bay and Lower Hudson Estuary.

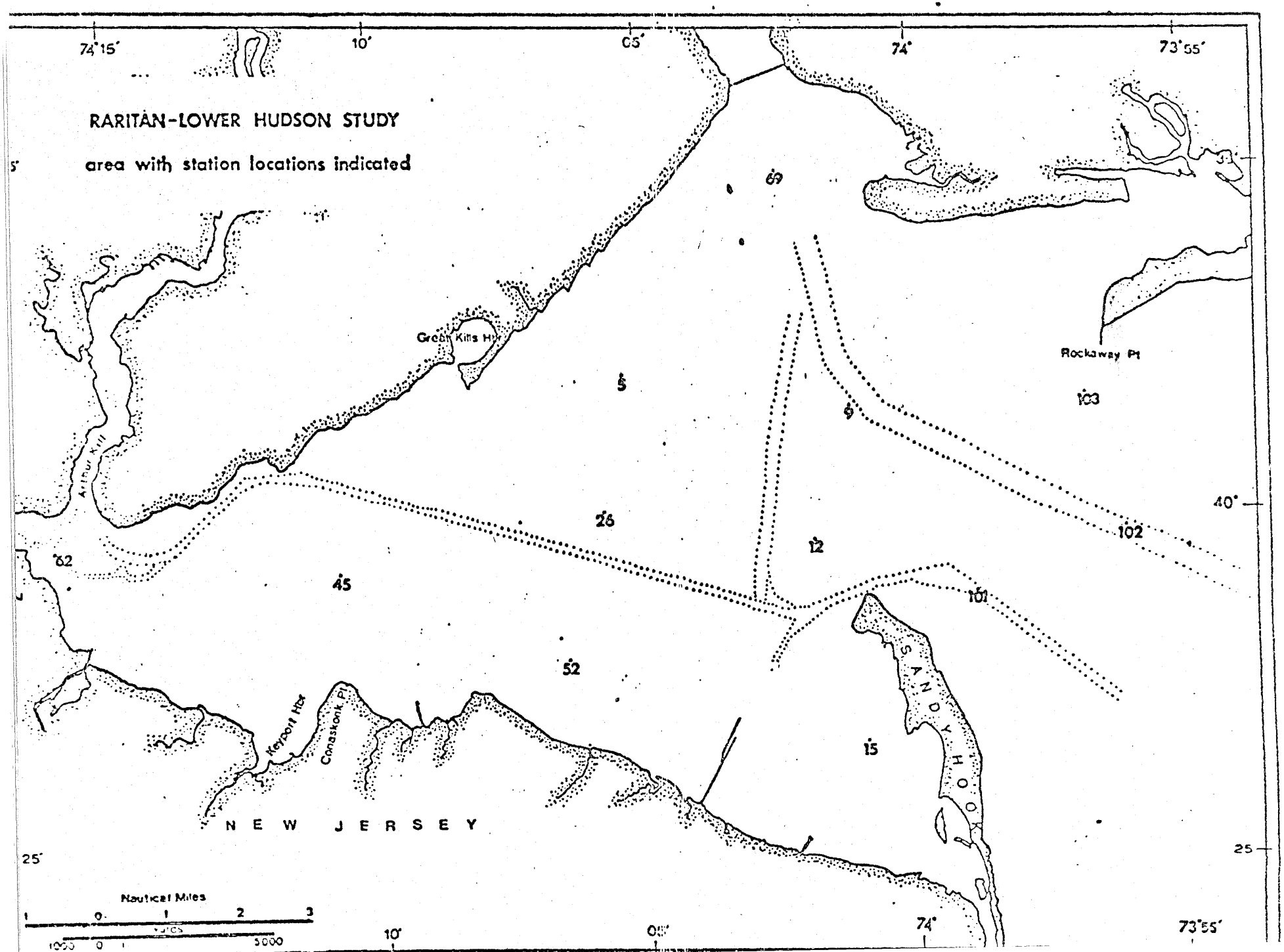

RARITAN-LOWER HUDSON STUDY
area with station locations indicated
74°15'
10'
05'
74°
73°55'
69
Great Kills Hbr
5
9
Rockaway Pt
103
Arthur Kill
26
12
102
40°
62
45
101
52
SANDY HOOK
Keyport Hbr
Conaskonk Pt
15
NEW JERSEY
25'
25
Nautical Miles
1 0 1 2 3
Yards
1000 0 1 5000
10'
00'
74°
73°55'

Table 1. Average Percent of Total Phytoplankton Primary Productivity Released as Dissolved Organic Matter ($<$ 0.45 micron) in Surface Water of Raritan-Lower Hudson Estuary

Month-Year	# of Stations Sampled	Average % DOM
Nov - 73	3	17.5
Dec - 73	3	11.1
Jan - 74	5	5.9
Feb - 74	11	9.2
Mar - 74	11	9.7
Apr - 74	11	11.5
May - 74	12	12.5

contribution of these two size classes to the food chain. Table 2 indicates that the nannoplankton/netplankton ratio progressively increased from 0.49 to 24.3. Or, in other words, nannoplankton were responsible for 33% of particulate productivity in March and 96% in August (Fig. 2).

Extreme spatial and temporal changes in the distribution of chlorophyll "a" concentration, and assimilation potential (total primary productivity at optimum light intensity and ambient sea surface temperature) have been observed. In February, assimilation potential (assimilation potential is highly correlated with phytoplankton biomass concentration) was fairly similar throughout the estuary, ranging between 20 and 60 mg C/ M^3/ hr. In March, during spring bloom of Skeletonema costatum, much spatial heterogeneity was observed. Values for assimilation potential ranged from 40 near Rockaway, Long Island, to 380 mg C/ M^3/ hr at station 15, Sandy Hook Bay. Similarly, in April and May, low assimilation values measured in Sandy Hook Bay and Raritan Bay (see Figs. 3, 4, 5 and 6). Maps of chlorophyll "a" concentration (Figs. 7, 8, 9 and 10) substantiate these trends. Samples of seawater, collected and preserved during our cruises, are presently being analyzed for phytoplankton species composition, density, and average cell size, so that we may causally link changes in these structural aspects of the phytoplankton community to concurrent changes in nannoplankton/netplankton productivity rations, release of dissolved organic matter, and total primary productivity.

Table 2. Average Ratio of Nannoplankton (< 20 microns)/to Netplankton (> 20 microns) Primary Productivity in Surface Water of Raritan-Lower Hudson Estuary

Month-Year	# of Stations Sampled	Average Nanno/netplankton Productivity Ratio
Mar - 74	11	0.49
Apr - 74	12	0.90
May - 74	12	1.57
Jun - 74	11	17.51
Jul - 74	12	23.40
Aug - 74	10	24.23

Figure 2. Percent Contribution to Total Primary Productivity by Netplankton, Nannoplankton and Dissolved Organic Matter.

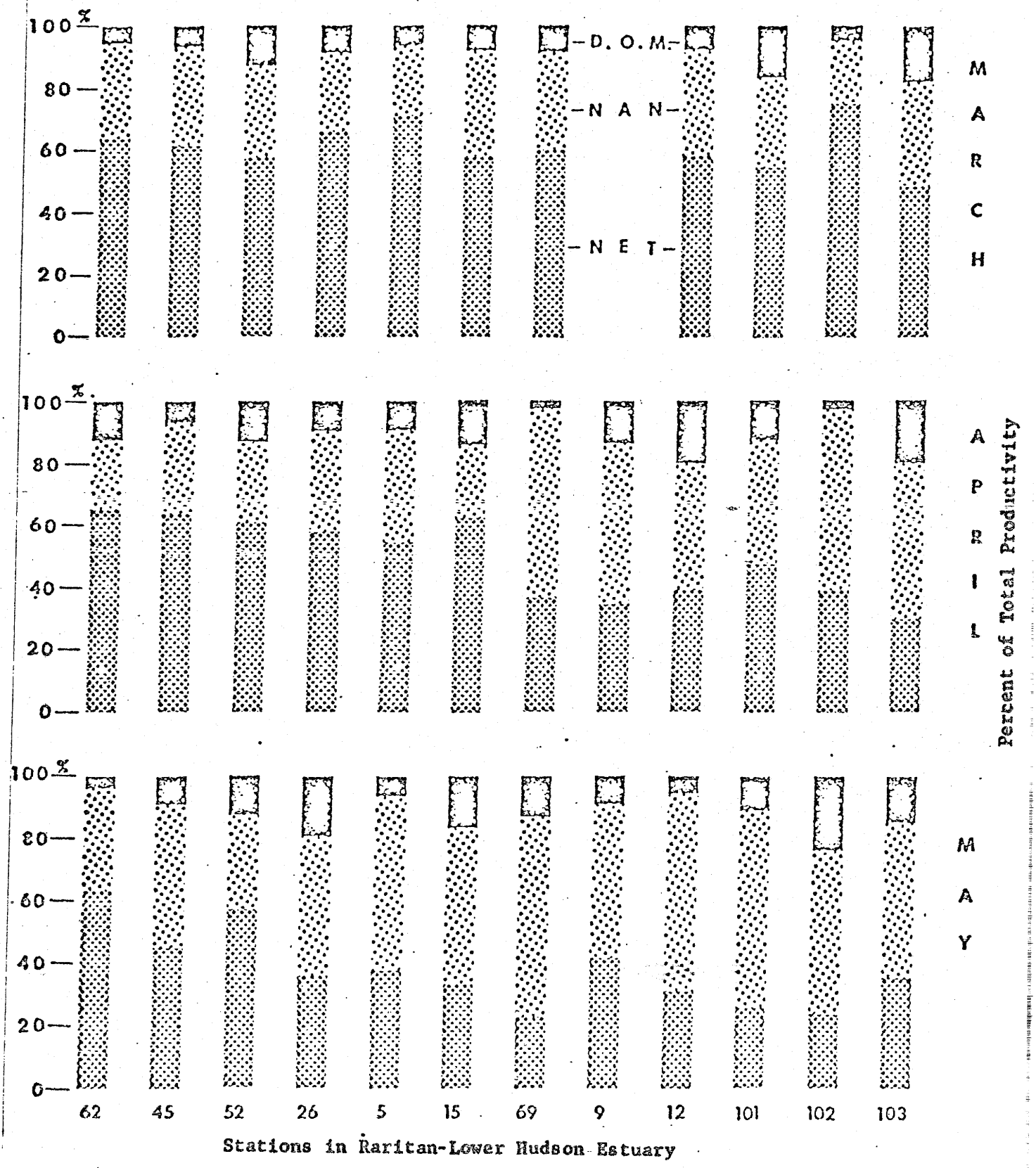


Figure 5. Total Primary Productivity Measurements in mg C/ M³/ hr in Surface Waters - February 1974.

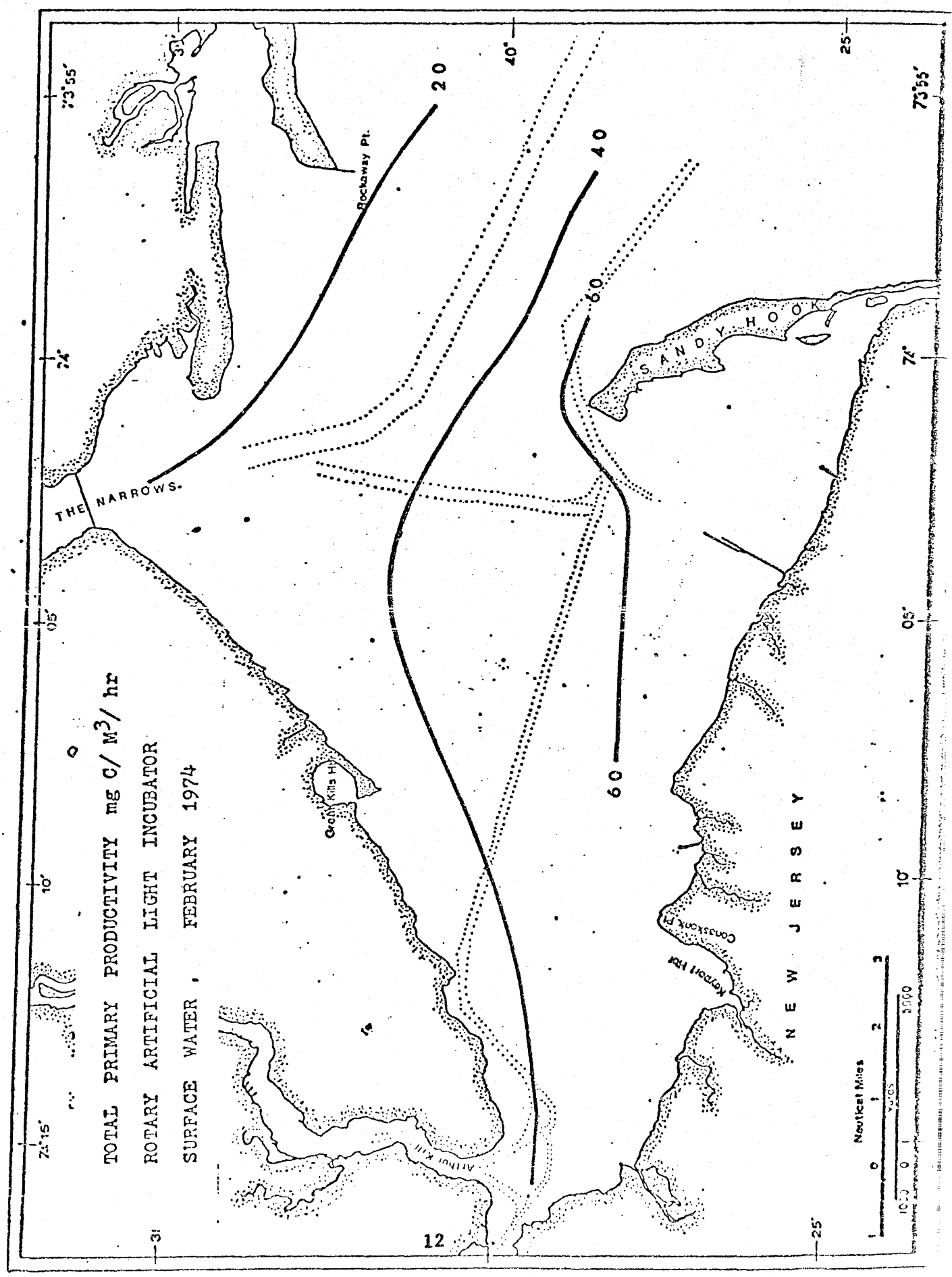


Figure 4. **Total Primary Productivity Measurements in mg C/ M^3/ hr in Surface Waters - March 1974**

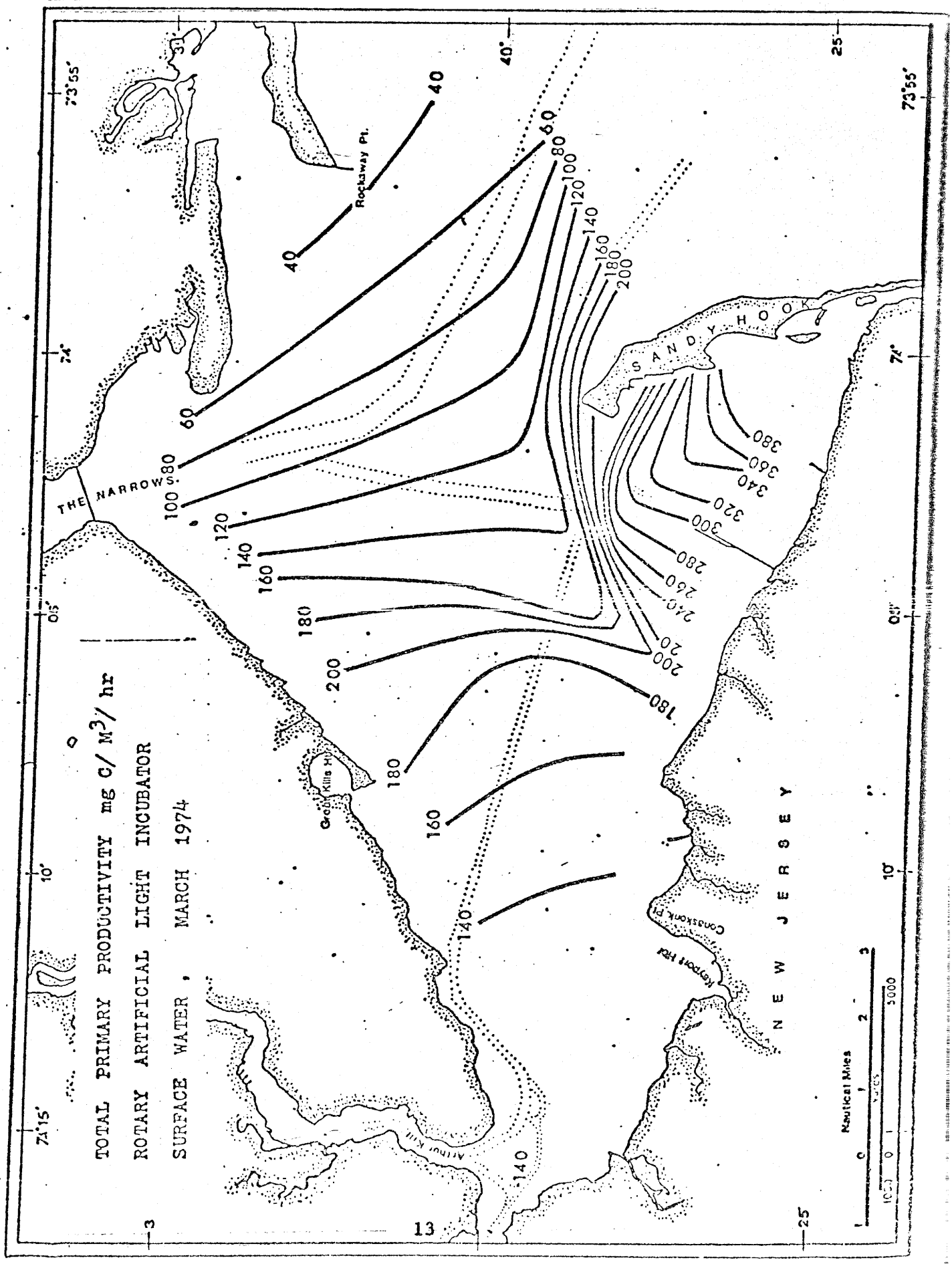


Figure 5. **Total Primary Productivity Measurements in mg C/ M^3/ hr in Surface Waters - April 1974**

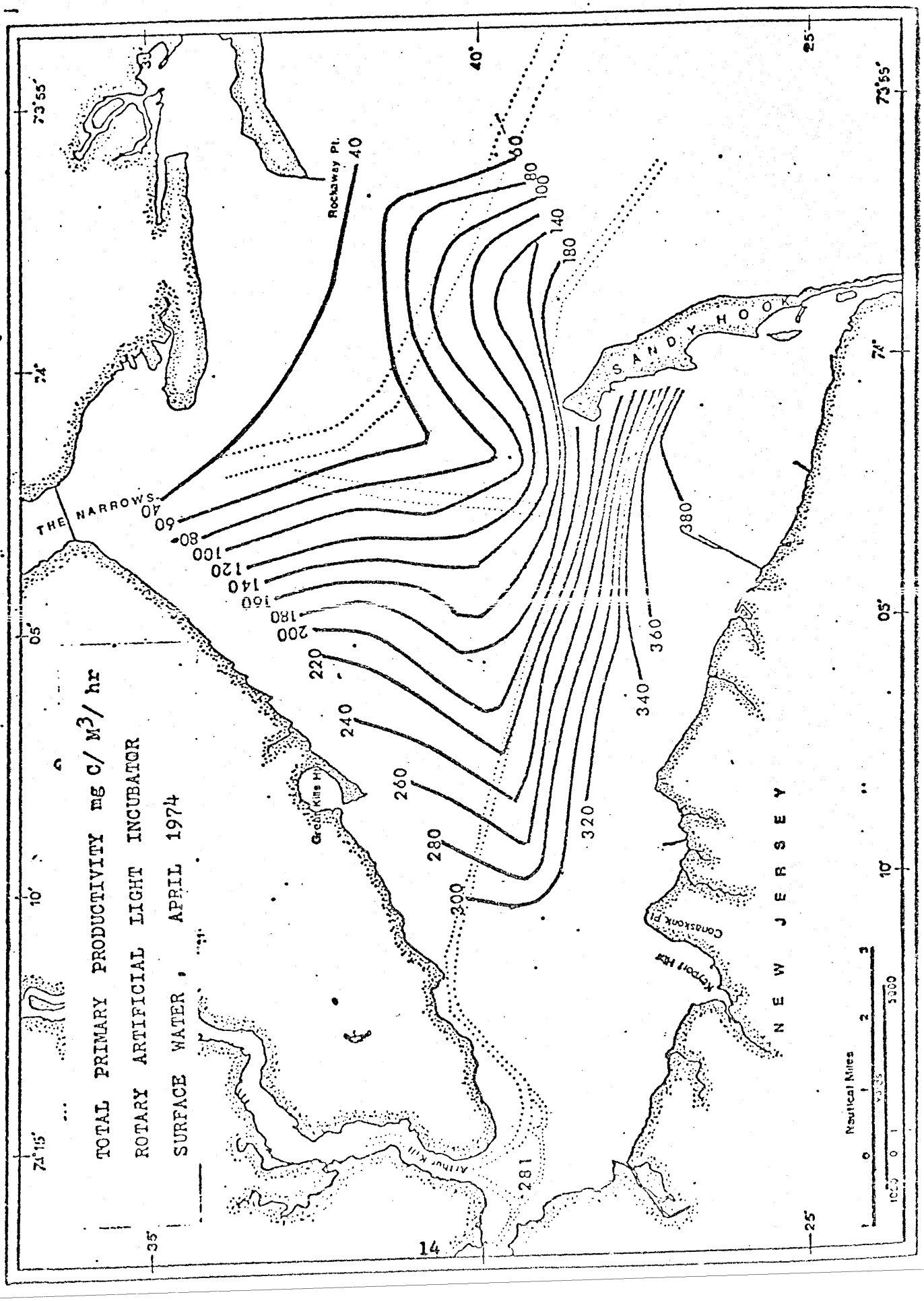


Figure 6. **Total Primary Productivity Measurements in mg C/ M^3/ hr in Surface Waters - May 1974.**

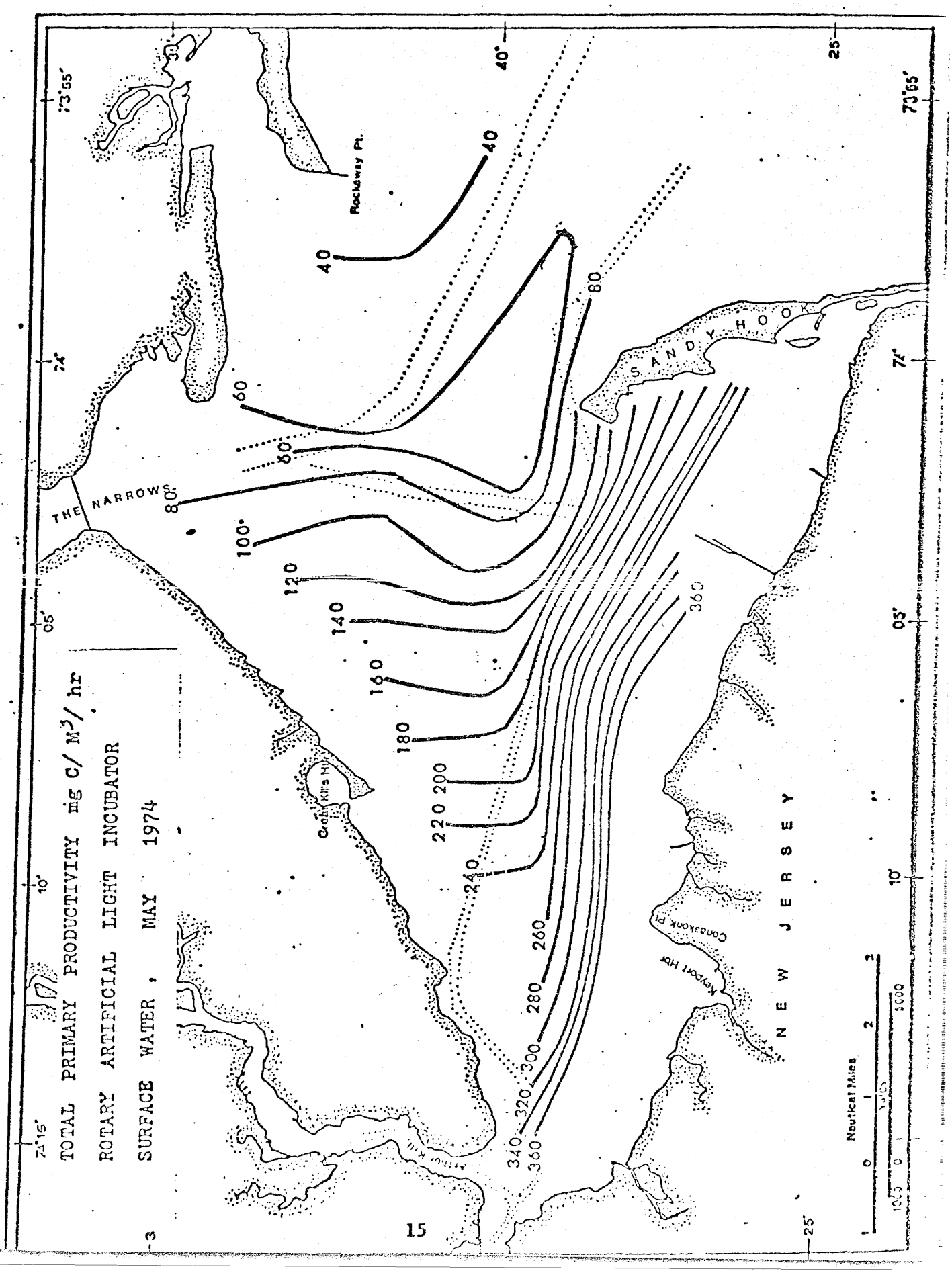


Figure 7. **Distribution of Surface Chlorophyll "a" (ug/L) February 1974**

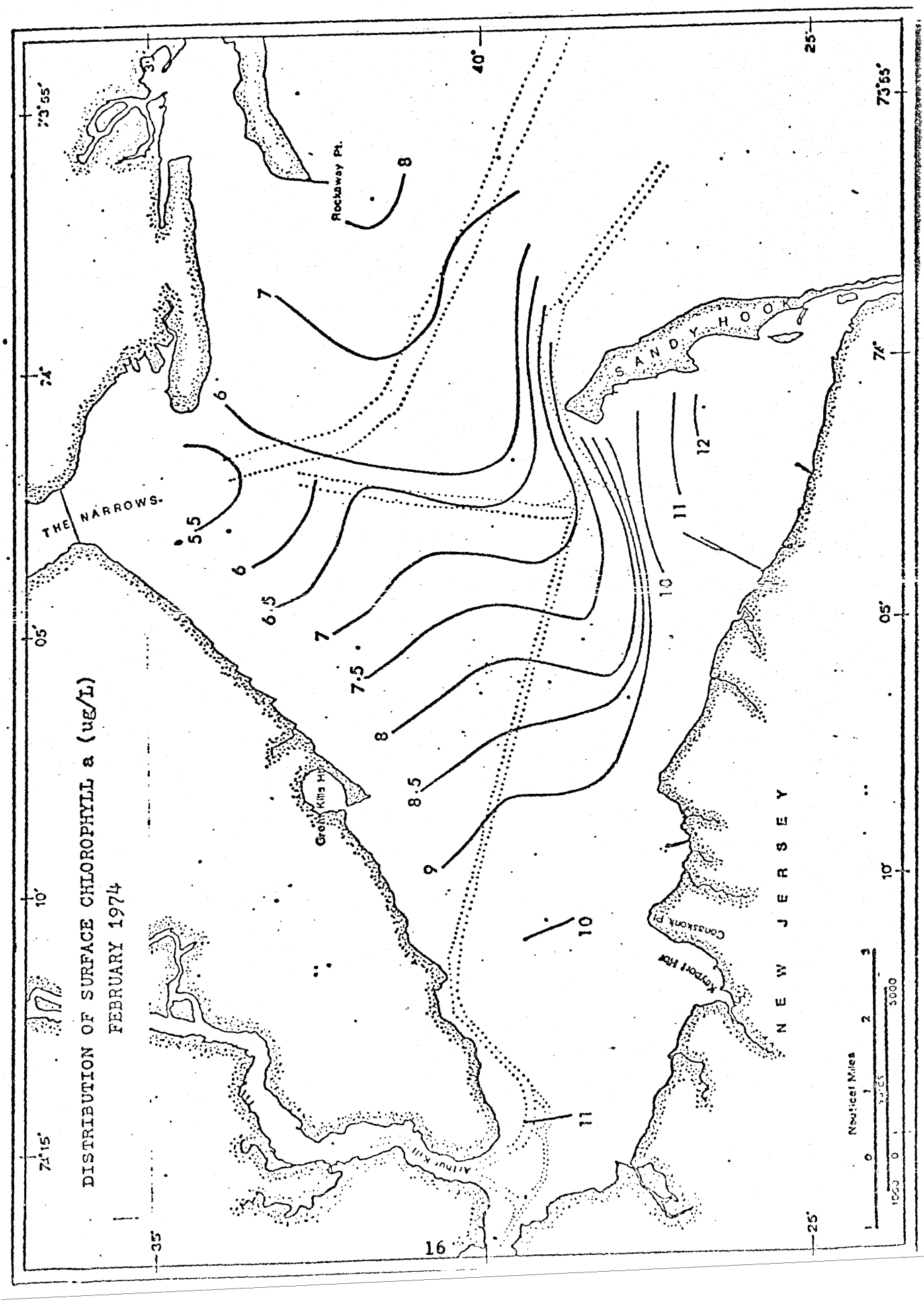


Figure 8. **Distribution of Surface Chlorophyll "a" (ug/L) March 1974**

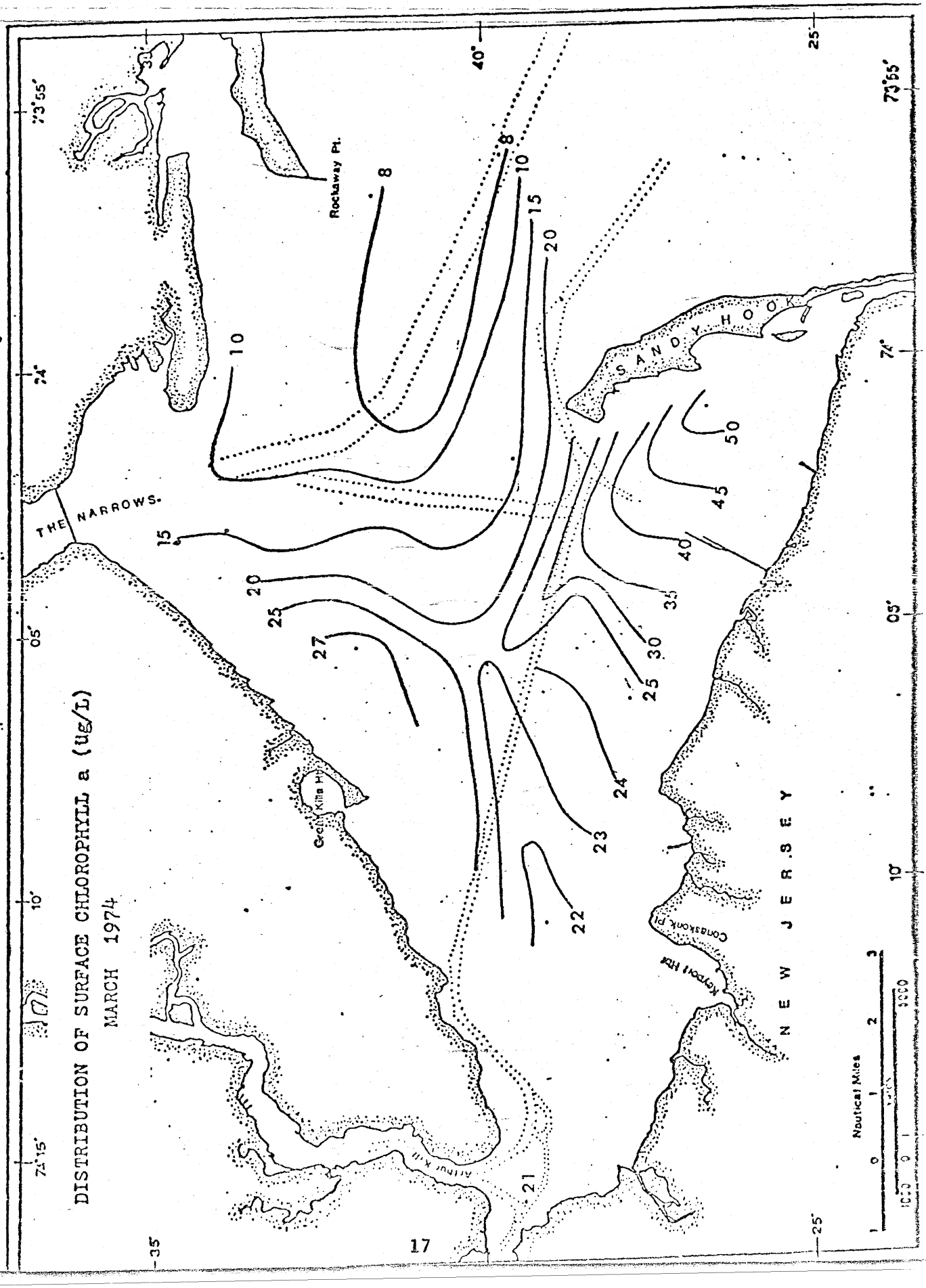


Figure 9. **Distribution of Surface Chlorophyll "a" (ug/L) April 1974**

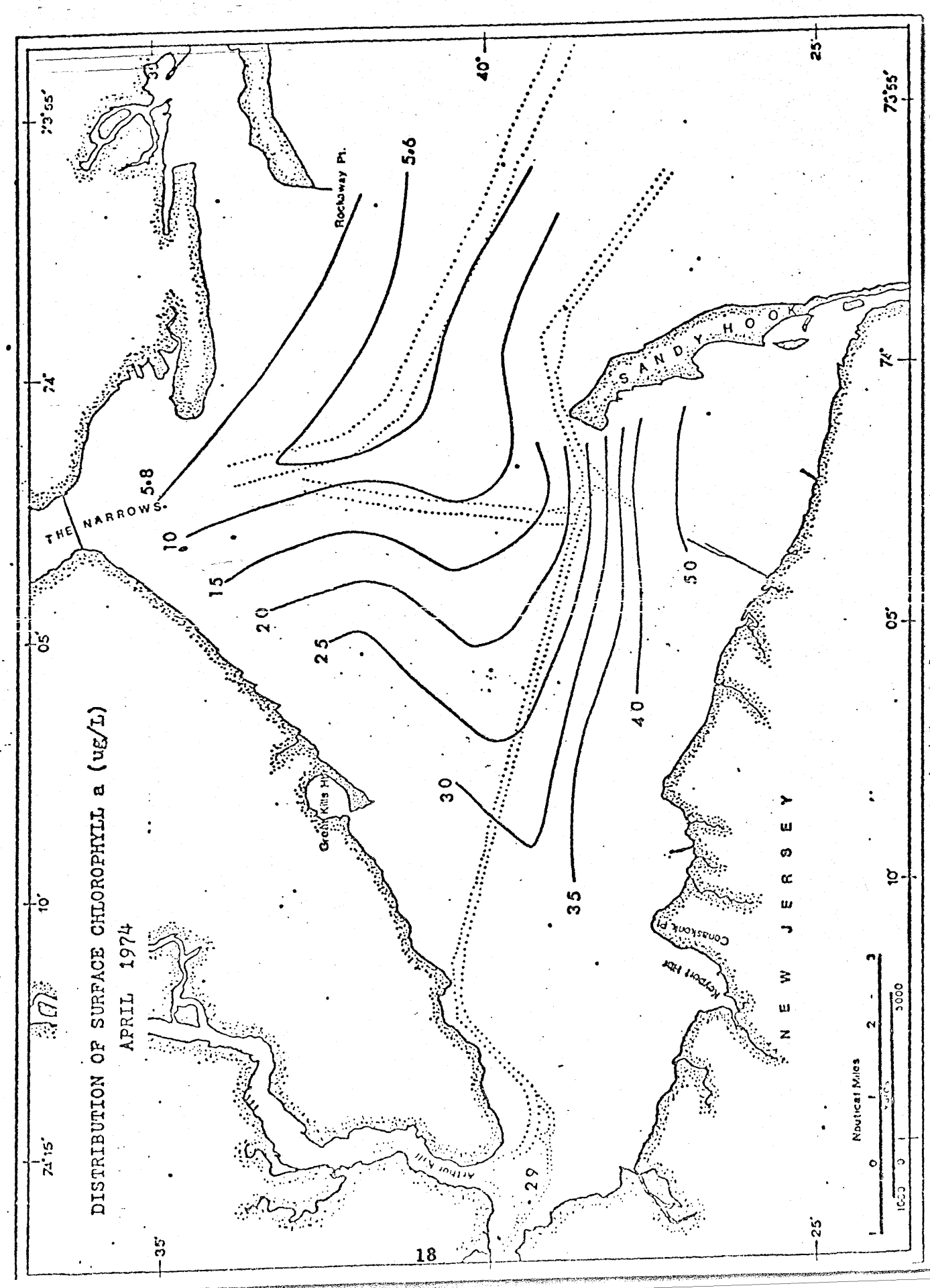
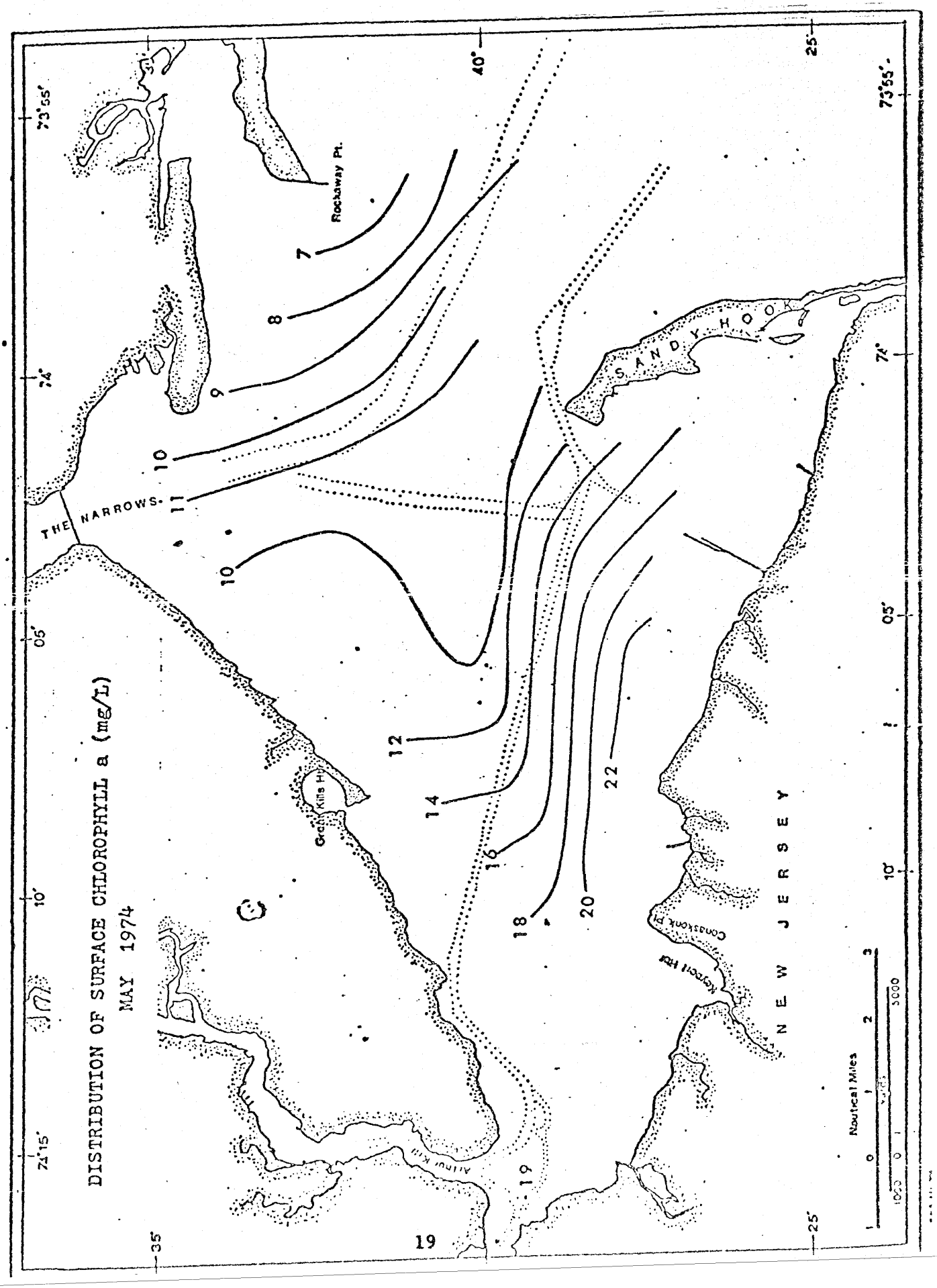


Figure 10. **Distribution of Surface Chlorophyll "a" (ug/L) May 1974**



Seabed Oxygen Consumption

New York Bight Apex:

The consumption of oxygen by the seabed was measured during cruises to the apex in January (45 stations), March (57 stations), August (73 stations) and December 1974 (63 stations) (Figs. 11-14) and in February 1975 (97 stations). The methodology was that of Pamatmat (Limnol. Oceanogr., 1971) for total oxygen consumption. Usually four samples per station were examined. Both live and formalin killed samples were investigated. The values are indicative of the quantity of organic matter (such as sewage sludge) oxidized per unit area of seabed per unit time. The data from the initial cruise (January) (Fig. 11) are difficult to interpret, primarily because of data gaps which were corrected on future cruises.

In March (Fig. 12) two areas of heightened seabed oxygen consumption can be identified. The area of elevated rates in the center of the figure is located over the upper Hudson Shelf Valley and Christiansen Basin (the so called "amphitheater") and would appear to define the area most affected by ocean dumping. No discernible effects on seabed oxygen consumption are observed near the acid area (40°20'N, 73°40'W) in March or during the other cruises. The second area of elevated rates at the lower left side of the chart is located off Asbury Park, N. J. This area exhibits elevated rates in both March and August (as high as are measured in the apex) but not in December. Values are highest nearest the coast and decrease to seaward indicating a probable source of organic matter (i.e. ocean outfall of sewage) near the coast in that region.

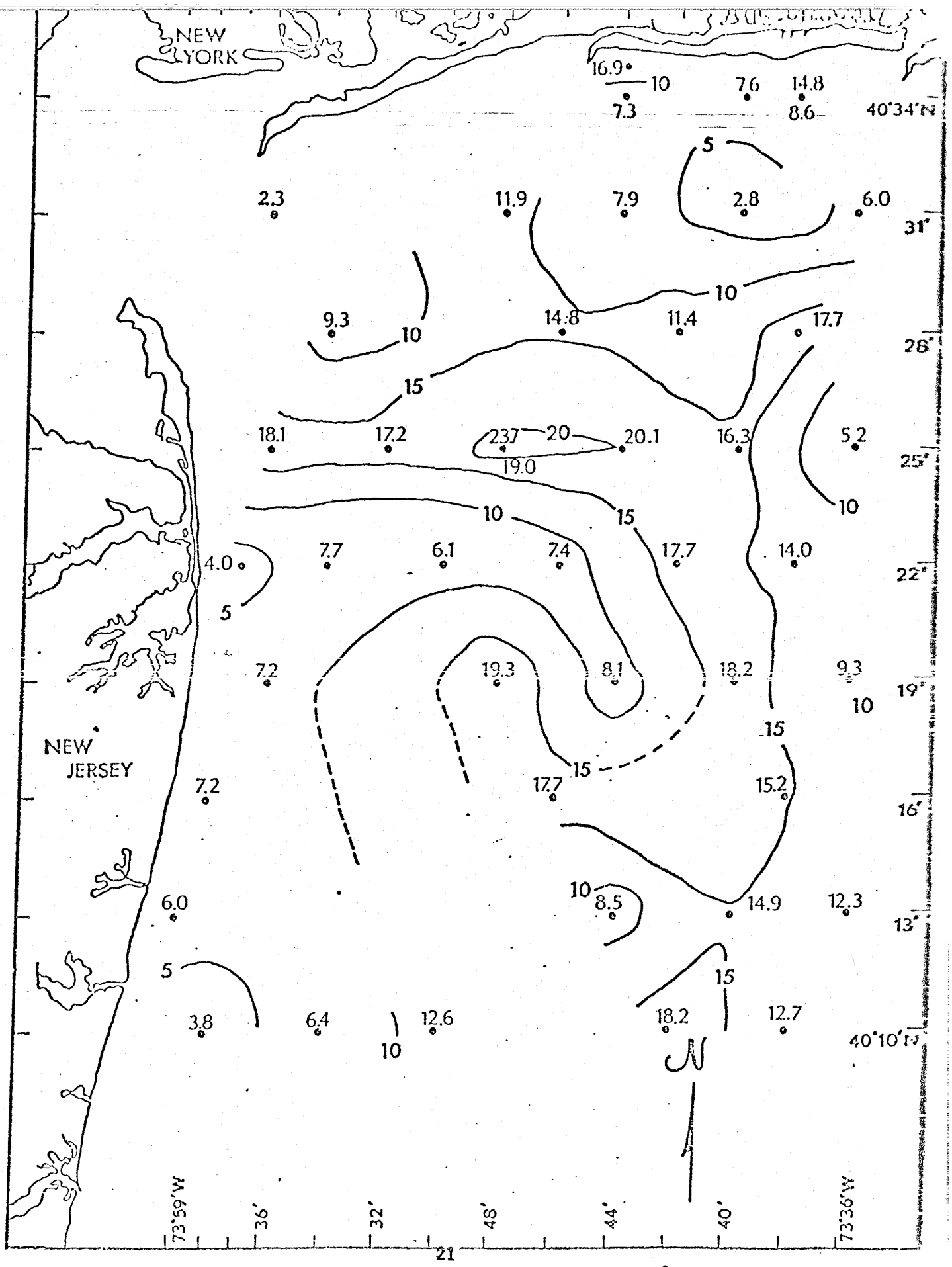


Figure 11. Total Seabed Oxygen Consumption (ml $O_2/m^2/hr$)
22 - 30 January 1974 Cruise A-74-1, FRS Albatross IV

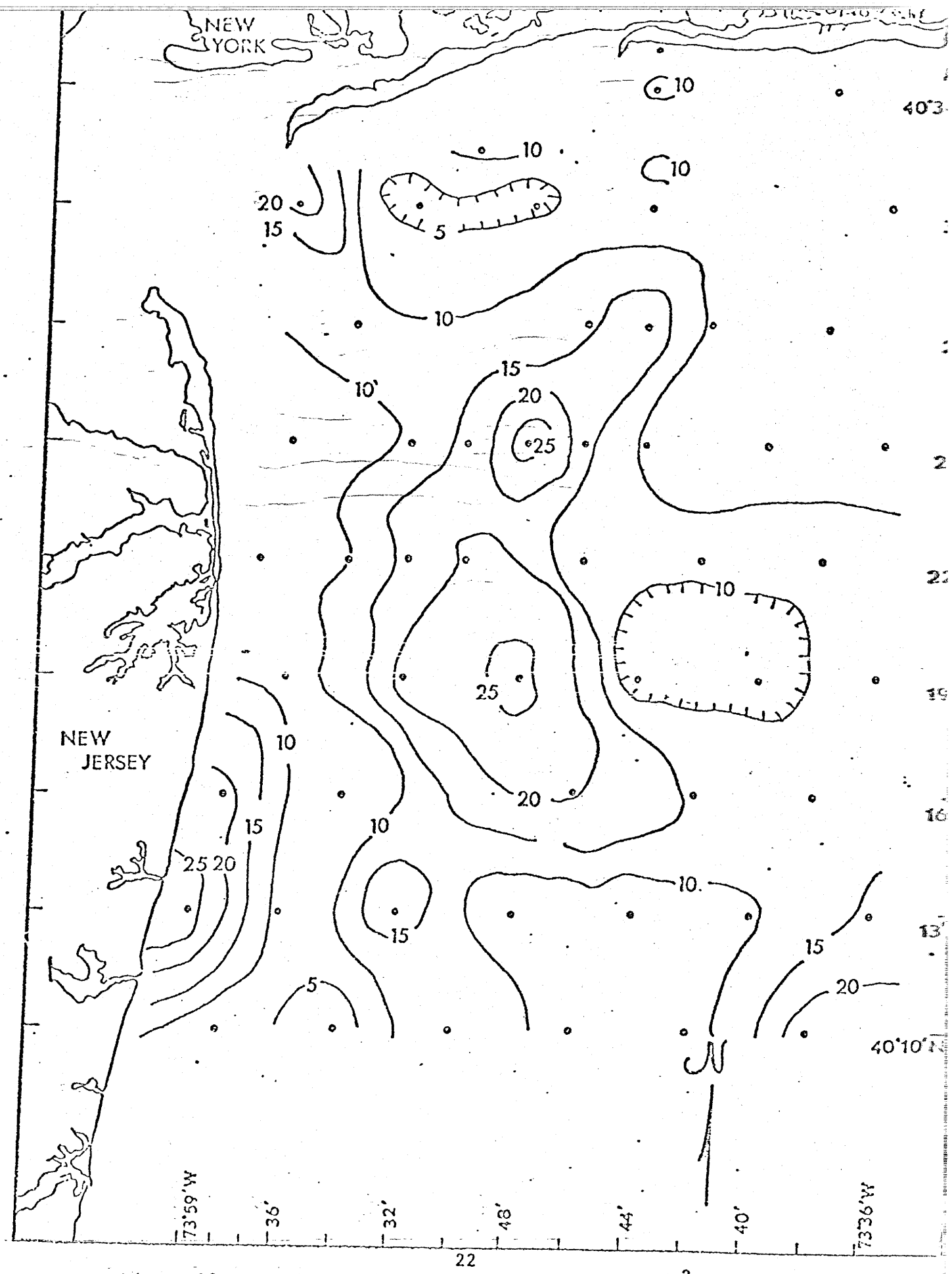


Figure 12. Total Seabed Oxygen Consumption (ml $O_2/m^2/hr$) 21 March - 4 April 1974 Cruise 0-74-1, FRS Oregon II

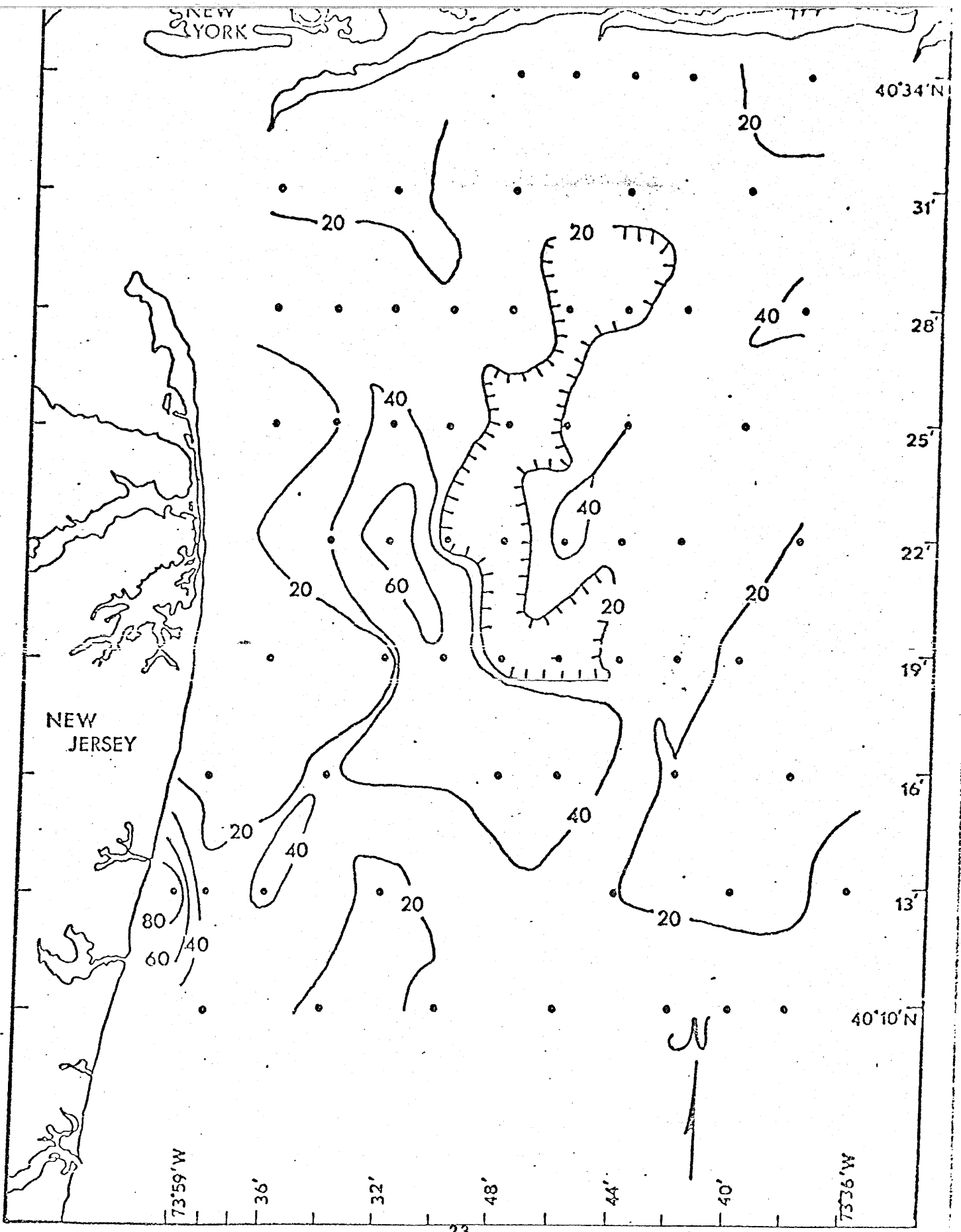


Figure 13. Total Seabed Oxygen Consumption (ml $O_2/m^2/hr$) 26 August - 6 September 1974 Cruise D-74-9, FRS Delaware II

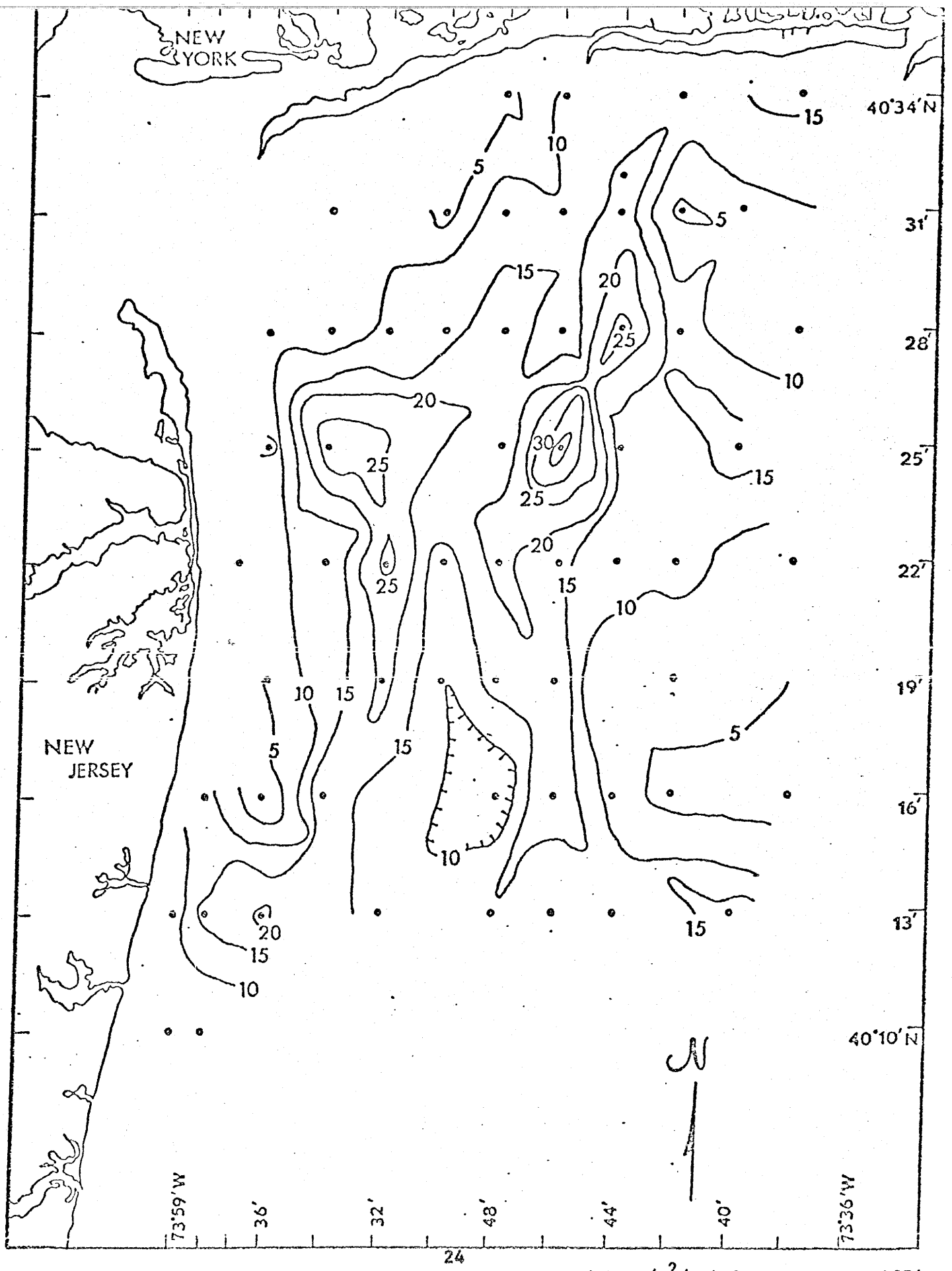


Figure 14. Total Seabed Oxygen Consumption (ml $O_2/m^2/hr$) 2-15 December 1974
Cruise D-74-16 FRS Delaware II

In August (Fig. 13), in response to higher water temperatures, seabed oxygen consumption rates are higher throughout the apex <u>except</u> for the area of the waste disposal grounds previously described as having elevated rates. Oxygen consumption rates in this area are not only not elevated compared with the surrounding area, but are equal to or depressed compared with values measured during March (Figs. 15 and 16). Such values are indicative of an overstressed area. The highest rates at this time of year occur on the periphery of the depressed area and decrease with distance from the impacted area.

In December (Fig. 14), the highest values are again found over the sewage sludge and dredge spoil disposal areas as in the previous March; greater detail is possible because of more data points.

The final cruise in the apex to measure seabed oxygen consumption rates for synoptic mapping of present conditions occurred 12-24 February 1975. During this cruise oxidation rates were also investigated in the Lower Hudson River, the Hudson Shelf Valley beyond the apex and in the two proposed alternate waste disposal areas. These data are presently being analyzed.

Measurements of salinity, temperature, heavy metals burdens in sediment, total organic carbon and dissolved oxygen are being examined as potential mechanisms mediating bottom oxygen consumption.

Figure 15. Total Seabed Oxygen Consumption Values Along Transect Across Apex of New York Bight from New Jersey Coast Due East Along 40°22'N.

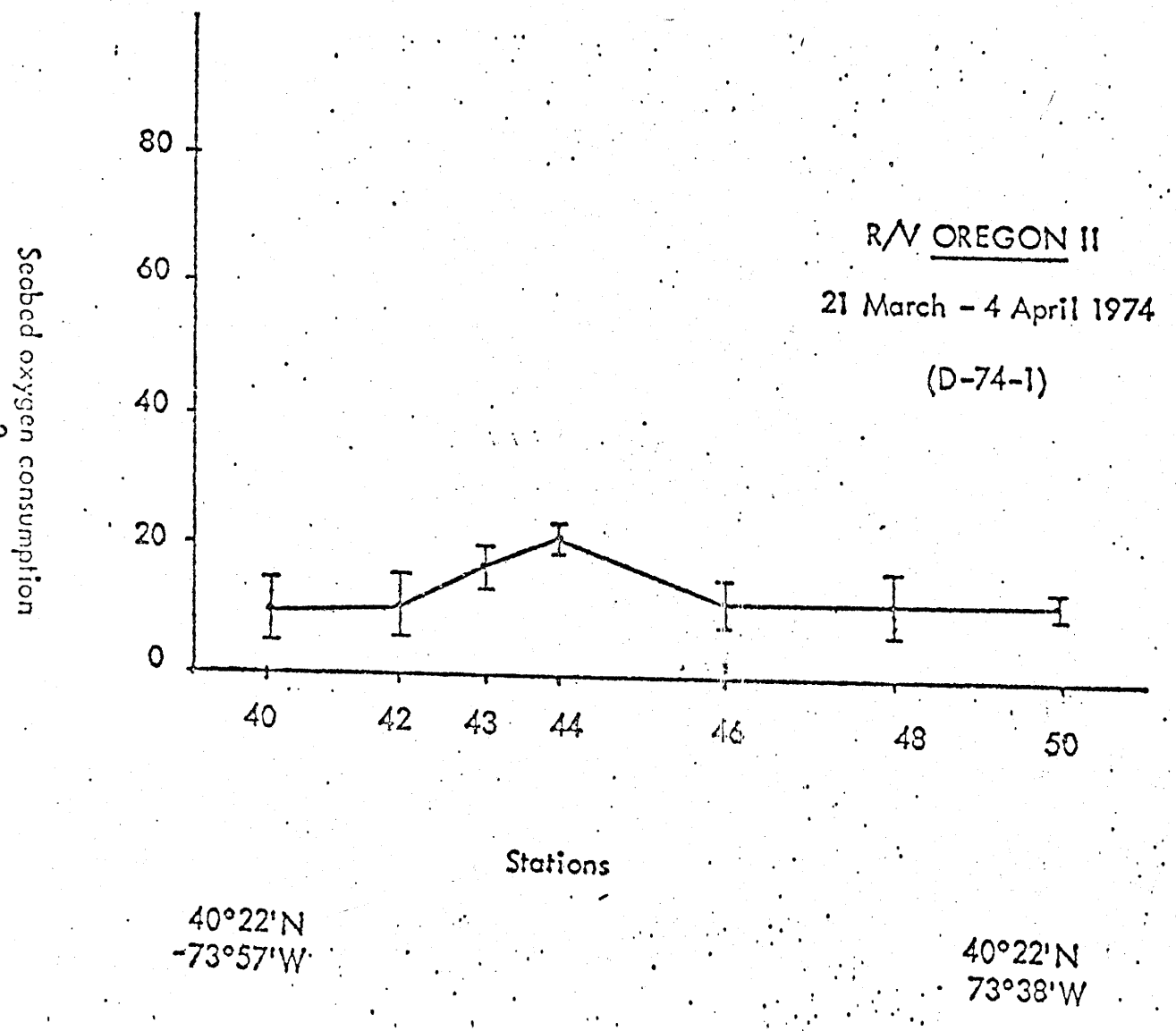
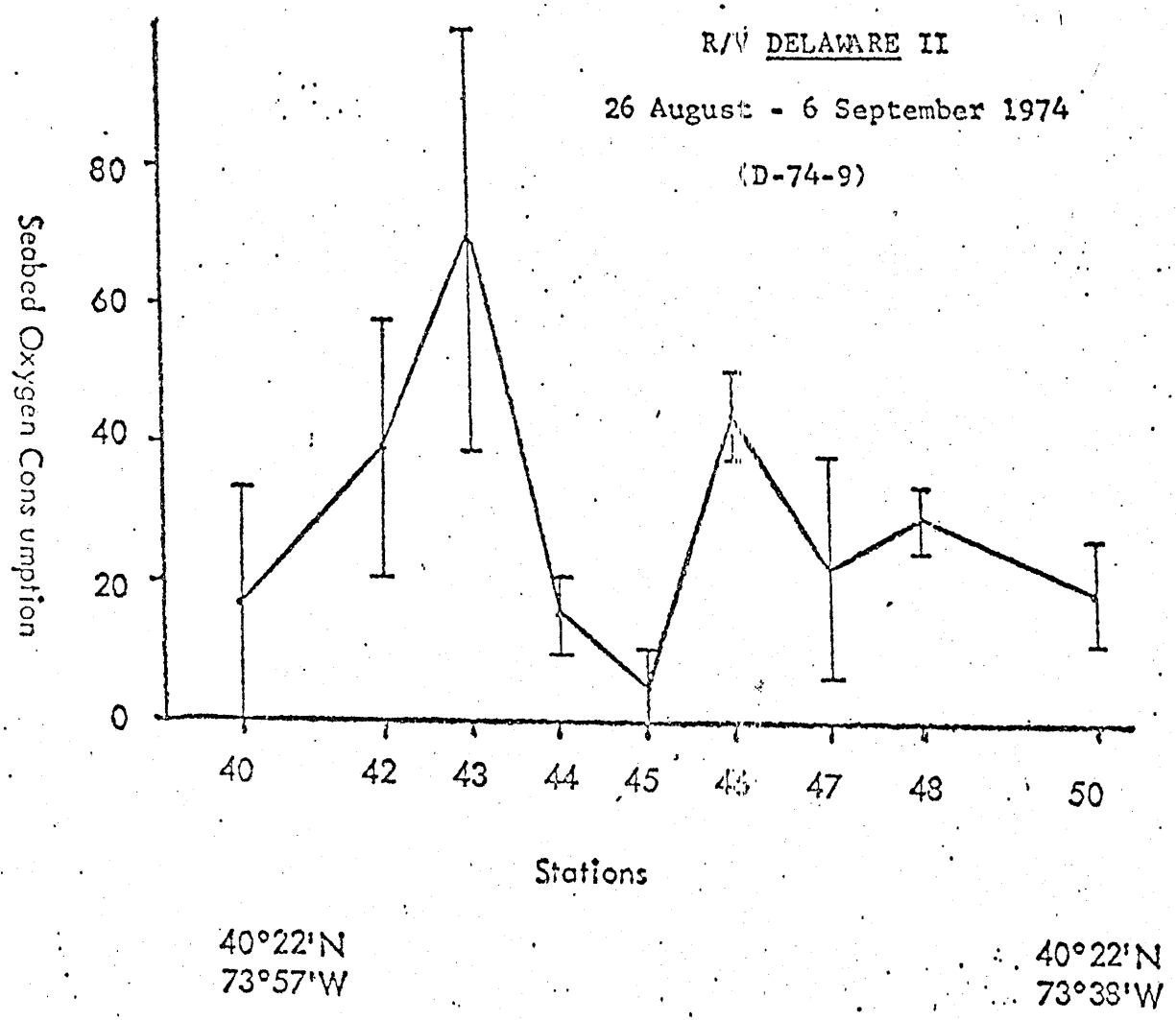


Figure 16. Total Seabed Oxygen Consumption Values Along Transect Across Apex of New York Bight from New Jersey Coast Due East Along 40°22'N.



ECOSYSTEMS INVESTIGATIONS

BIOCHEMICAL MODELING

R. Tucker, A. Draxler, A. Matte, R. Waldhauer and J. Young

Although some quantitative information is available concerning the amounts of metals in marine environments, especially in sediments, far too little is known about binding or chelation to organics, adsorption on particulate material or even inorganic combinations and states of the metals. Knowledge of the forms and combinations of contaminant metals is crucial in understanding their effects upon marine animals. Exchange between sediment and the water column, routes of uptake and rates of absorption through gut or other epithelia, and toxicity or other physiological effects could all very much depend on the chemical forms of the metals. The Biochemical Modeling Task is investigating a number of aspects of this problem. Metals are being determined in water samples from Raritan Bay by the very sensitive method of anodic stripping polarography. In this technique a voltage, more negative than the half-cell potential of any of the metals of interest, is imposed across a pair of electrodes immersed in the sample. Metal ions migrate to the anode and form an amalgam with a mercury drop or layer of mercury on a graphite electrode. After a set sampling period the voltage is gradually increased (a positive ramp) and as the half-cell potential for each of the metals in the sample is reached, the metal is stripped from the anode and the

resulting current differential is measured. By calibrating with known standards, current differential measurements can be converted to metal concentrations in the sample. In addition to determining quantities of metals in the parts per billion range, we have been subjecting aliquots of samples to ultraviolet irradiation ($\sim$220nm) to destroy organic moieties, and by running samples on the polarograph before and after irradiation some indication of organic binding can be obtained. Filtration, ion exchange, equilibrium dialysis, and chromatographic methods are also being investigated for their usefulness in determining organic binding of metals. Figures 17 and 18 are representative of some values for cadmium we have obtained in Raritan Bay. Figure 17 gives values from untreated samples, roughly corresponding to unbound cadmium, while Figure 18 shows concentrations after ultraviolet irradiation of samples and represents total cadmium. These values are preliminary, as the study is still in progress, but are in excellent agreement, where sampling stations overlap, with findings by investigators from Lehman College, CUNY, and from N.Y.O.S.L., Montauk. We are also analyzing samples for lead, zinc, and copper. The second major thrust of our investigation involves analysis of effects of metals, and of their organically bound forms, on living organisms and on biochemical systems within marine animals. We are presently setting up a system in which possible synergistic effects of stresses created by changes in temperature, salinity, and dissolved

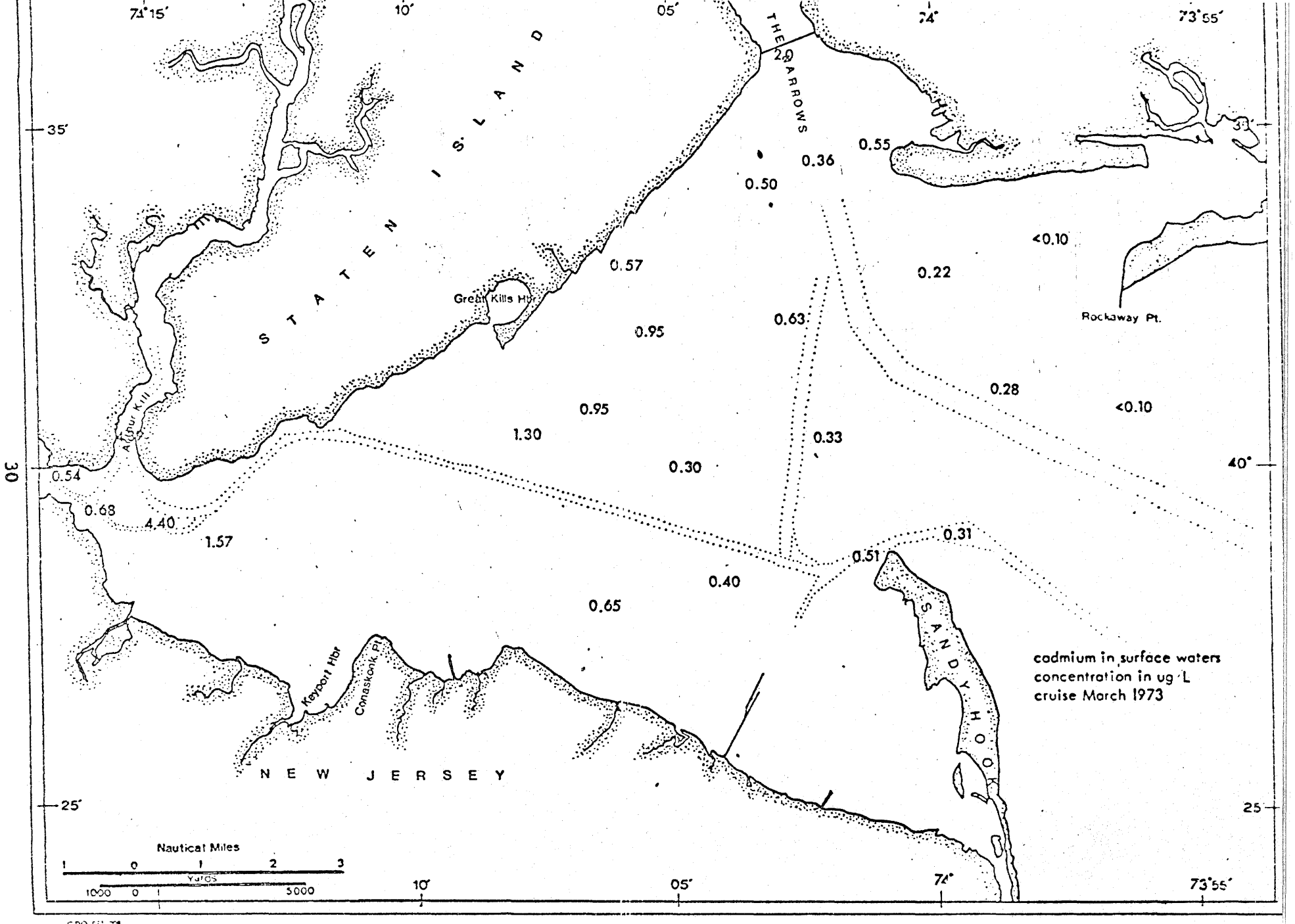


Figure 17. Levels of Unbound Cadmium in Surface Water Samples from Raritan Bay.

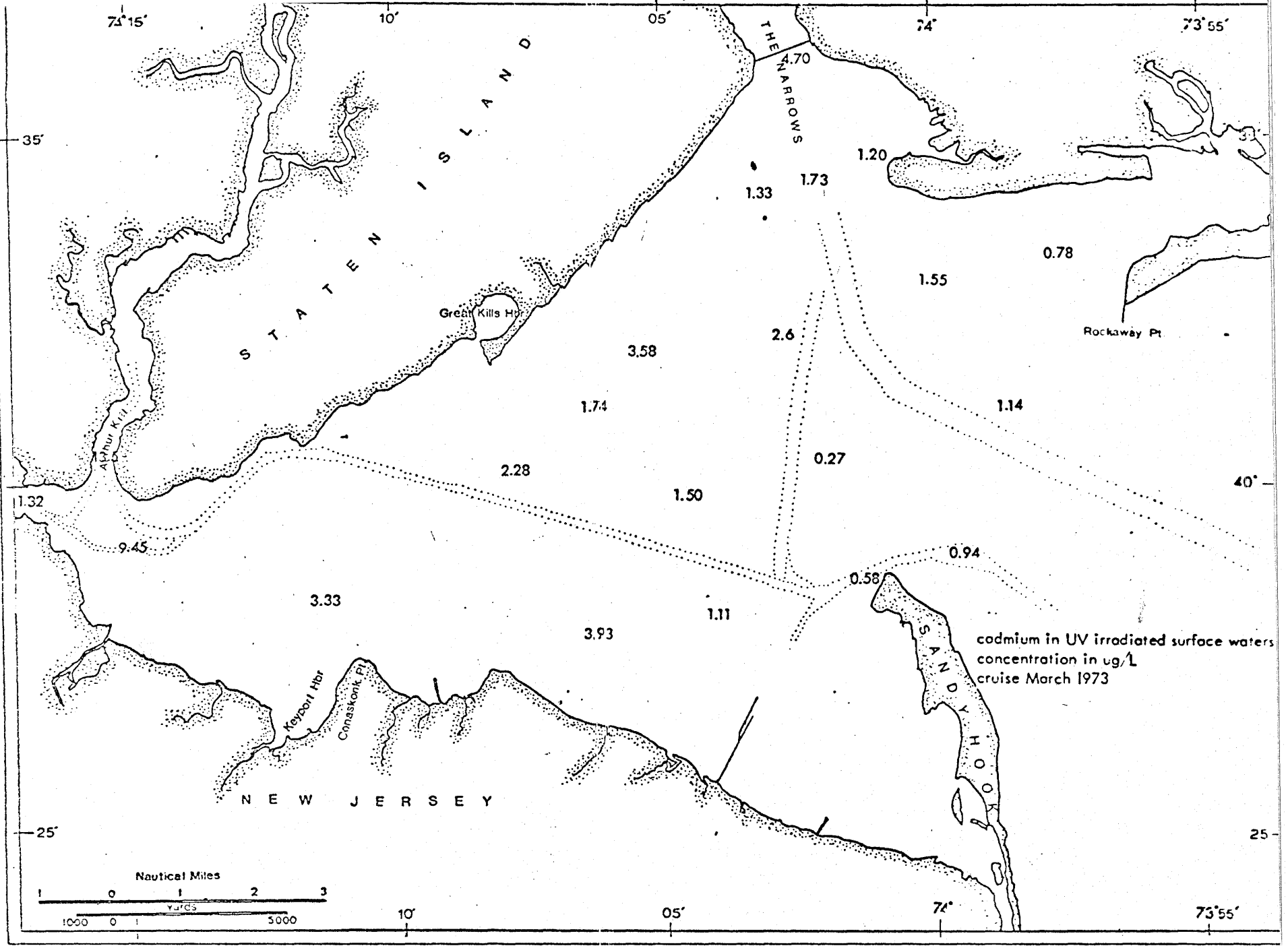


Figure 18. Levels of Total Cadmium in Surface Water Samples from Raritan Bay.

oxygen with simultaneous exposure to contaminant metals and metal-organics can be evaluated. *In vitro* studies of sodium-potassium - ATPase from gill of *Cancer irroratus* have shown slight, but not statistically significant, inhibition when cadmium is present at a concentration of 1 part per million. At 10 ppm Cd, inhibition is greater than 60% (significant difference from controls $P > 99\%$ confidence level). With lead, under the same conditions, an effect occurs on the magnesium - ATPase (probably mitochondrial), but little change is seen in Na-K-ATPase activity. We have recently participated in the center-wide cooperative study of lobsters exposed at the Milford exposure facility to cadmium in the range 0 - 6 ppb, for 30 to 90 days. Interestingly, there is a slight average increase in Na-K-ATPase activity in cadmium exposed animals, rather than the inhibition seen in our *in vitro* studies with crabs. However, the variability in these assays is great; the increases are only suggestive and not statistically significant. They do suggest, however, the desirability of longer term studies with perhaps slightly higher levels of cadmium. Other enzyme investigations we have initiated involve glutamic dehydrogenase and other amino acid metabolic pathway enzymes in larvae of *C. irroratus*.

By combining our chemical studies of metal-organic interactions with investigations of effects on organisms we hope to gain a better understanding of the real role of these contaminants in the marine ecosystem.

ECOSYSTEMS INVESTIGATIONS

COASTAL ECOSYSTEMS

R. Reid, R. McGrath, D. Radosh and A. Frame

This investigation concentrates on studies of baseline environmental conditions and effects of man's activities in local waters. The program's focus has changed considerably since the last marine contaminants reporting period. No further field activities were conducted in Long Island Sound or along the New Jersey coast during 1974. Sampling in Raritan Bay was curtailed after a February 1974 cruise. These surveys have measured, among other parameters, distribution of trace metals in sediments of the three study areas. The Long Island Sound work also included microbiological studies of sediments and the water column. Microbiological and trace metal studies were carried out in cooperation with the Environmental Microbiology and Chemistry Investigation; results and status of these analyses are discussed in the Environmental Microbiology and Chemistry section of this report. The Long Island Sound and Raritan Bay data are presently undergoing reduction and multivariate analysis to determine relationships among benthic macrofauna, sediment types and heavy metal contents. Detailed methods and findings to date are given in the individual project sections below.

The New Jersey Coast study has assumed an inactive status, with effort transferred to a baseline survey of possible oil exploration

areas in the Baltimore Canyon Trough. Most of Coastal Ecosystems' remaining resources are involved in an in-depth study of effects of dredging and spoil disposal at New London, Connecticut.

Long Island Sound: A final report on environmental baselines in the Sound for 1972-73 (MACFC Informal Report No. 42) was completed in December 1974. Portions of this study dealing directly with contaminant distributions will appear as MACFC Informal Reports Nos. 43 and 48, and are discussed in the Environmental Microbiology and Chemistry section of this report. Most significant among other contaminant-related findings are the following: 1) Such characteristics of the benthic macrofauna as numbers of individuals (N) and species (S), diversity (H') and equitability (J') did not appear to be strongly related to contaminant loads as measured by concentrations of heavy metals, nutrients, organics, dissolved oxygen, and fecal coliform bacteria. Correlation to sediment type was much closer. 2) More evident was a shift toward forms known to be stress-tolerant in the most heavily impacted areas. 3) This trend was also detected relative to data collected 20 years earlier for central Long Island Sound. Most notable was the change in dominant bivalves, from *Nucula proxima* to the more opportunistic *Mulinia lateralis*. (Workers from Yale University have also documented these faunal changes, in central Long Island Sound, and demonstrated a correlation to heavy metal contamination of sediments). Further analyses of relationships between contaminants and macrofauna, using polynomial regression and clustering techniques, are still in progress.

Raritan Bay: One sampling cruise was conducted in Raritan Bay in 1974. Fifteen stations were occupied near the Naval Ammunition Depot, Earle Pier and the site of the Environmental Protection Agency Oil-Spills Division Field Test Site. Sampling procedures follow those outlined in MACFC Informal Report No. 26. Core subsamples of sediment were taken for future heavy-metal analyses. No analyses have been performed as of this writing.

During 1974 results of the remaining heavy metal analyses from the RB I Cruise (Jan-Feb 1972) were received from R. Greig, Milford Laboratory. A paper is presently in preparation which will detail sediment characteristics and metal burdens in Raritan Bay.

Baltimore Canyon Trough (BCT): Baseline samples were collected in the Baltimore Canyon Trough area during a NOAA-USGS cooperative cruise 15-30 May 1974. Although the BCT covers an expansive amount of ocean bottom, the areas studied are USGS established and are believed to hold the greatest potential for petroleum exploration (Figure 19). Time restrictions allowed only single grab samples for benthic macrofauna to be taken at most of the 93 stations in Areas I (87) and III (6); 21 stations were replicated. Cores for heavy-metal analyses were taken at 33 stations and these are presently in storage at Sandy Hook Laboratory, as are the macrofaunal samples, pending a Bureau of Land Management (BLM) contract.

Future sampling will be conducted on a quarterly, monitoring-type basis, both temporally and areally to hopefully show any natural

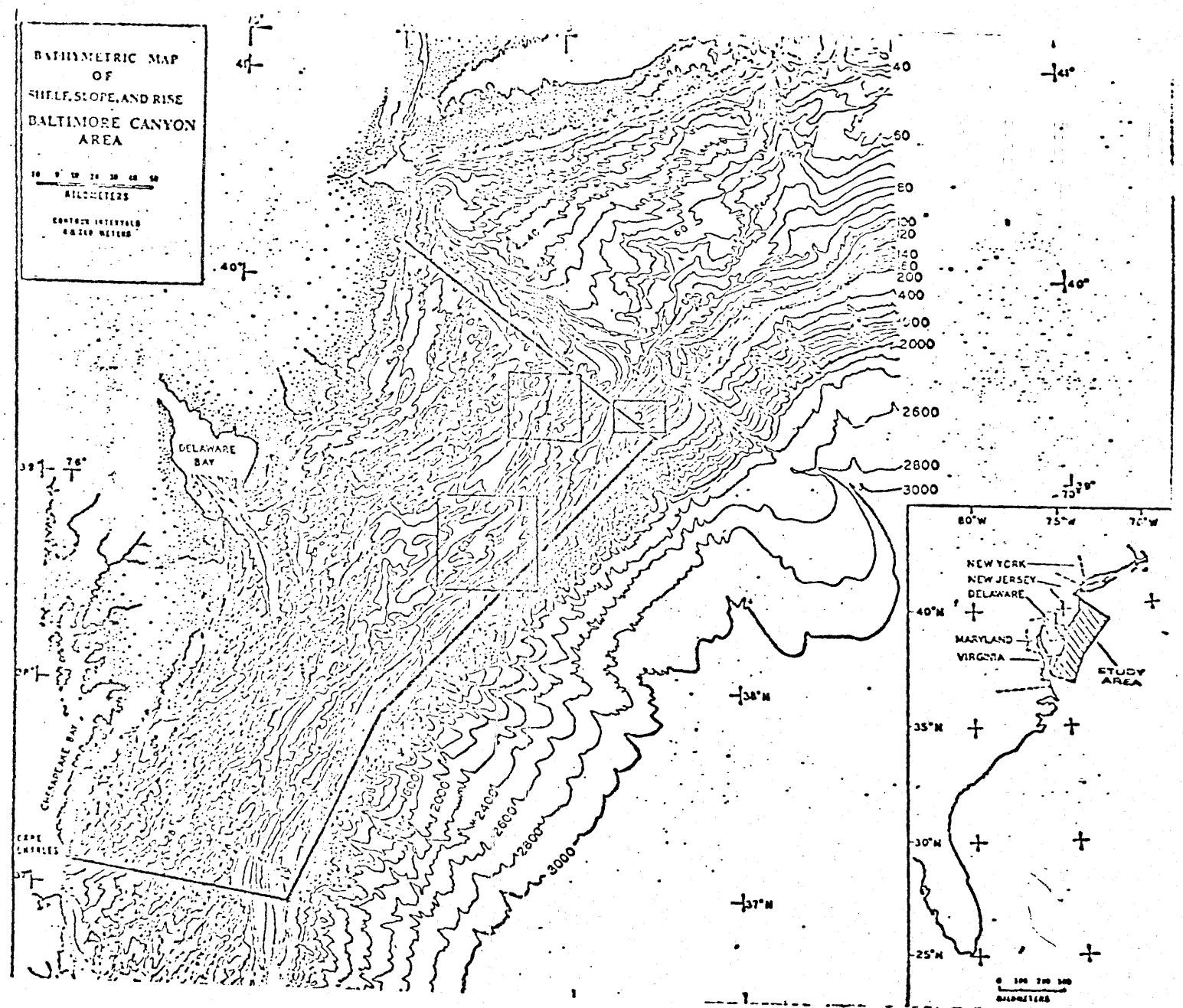


Figure 19. Baltimore Canyon Trough Area. Squares 1-3 represent areas of seismic study(USGS) and/or benthic sampling(NMFS).

variabilities induced by seasonal-related activities. This will also help us to view any possible impact from pollutants which may be carried out of the immediate exploration or drilling areas.

Also, specific benthic organisms, including important commercial species, will be collected for analyses for petrochemical and heavy metal contamination. These organisms will be re-collected during the monitoring phase to determine whether oil-related activities have added to contaminant body burdens.

All future plans for research conducted by MACFC in the Baltimore Canyon Trough are tentative and are awaiting federal approval of existing research proposals.

New London: Quarterly surveys to determine predredging benthic conditions and to monitor effects of dredging - disposal activities were begun in June 1974. Sediment and macrofauna samples are being collected from seven stations in the Thames River (Figure 20) and 38 in the New London Dumping Ground and vicinity (Figure 21). *In situ* studies of sedimentation rates and composition, and effects of spoils on benthic macrofauna, have been initiated. We are also holding *Mercenaria mercenaria* in cages at the disposal buoy and a control site in an attempt to determine whether spoil disposal is leading to increased heavy metal concentrations or gross pathology.

The Environmental Microbiology and Chemistry Investigation has been studying microflora of river and dump site, and heavy metals in dump site fish and shellfish. Dump site studies of heavy metals in

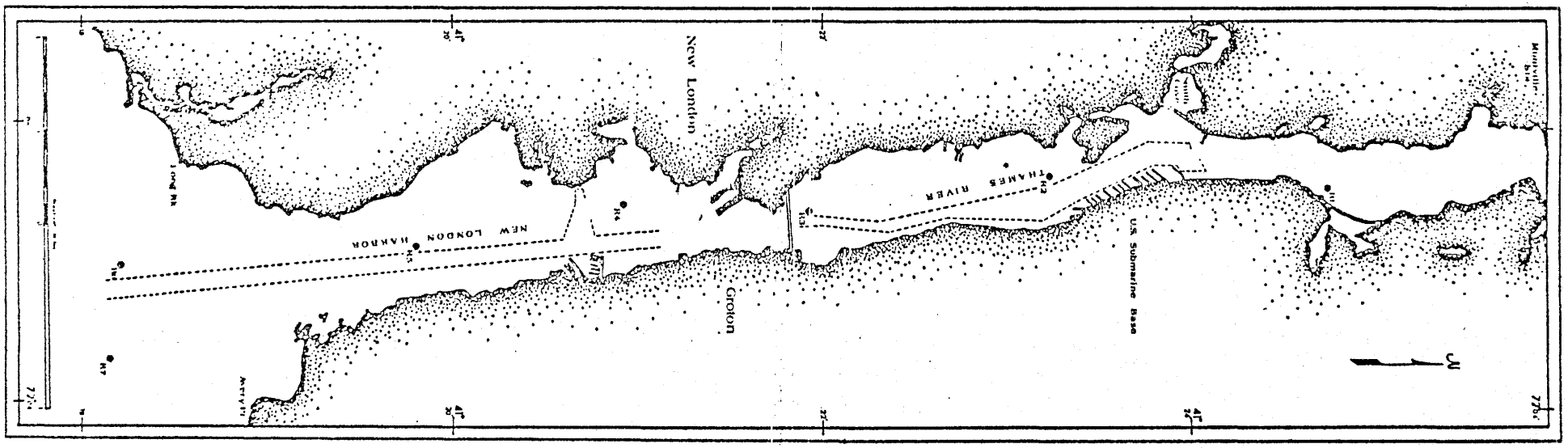


Figure 20. Thames River (Ct.) Station Locations for Study of Effects of Dredging on Benthic Macrofauna. Dashed Lines Indicate Areas to be Dredged.

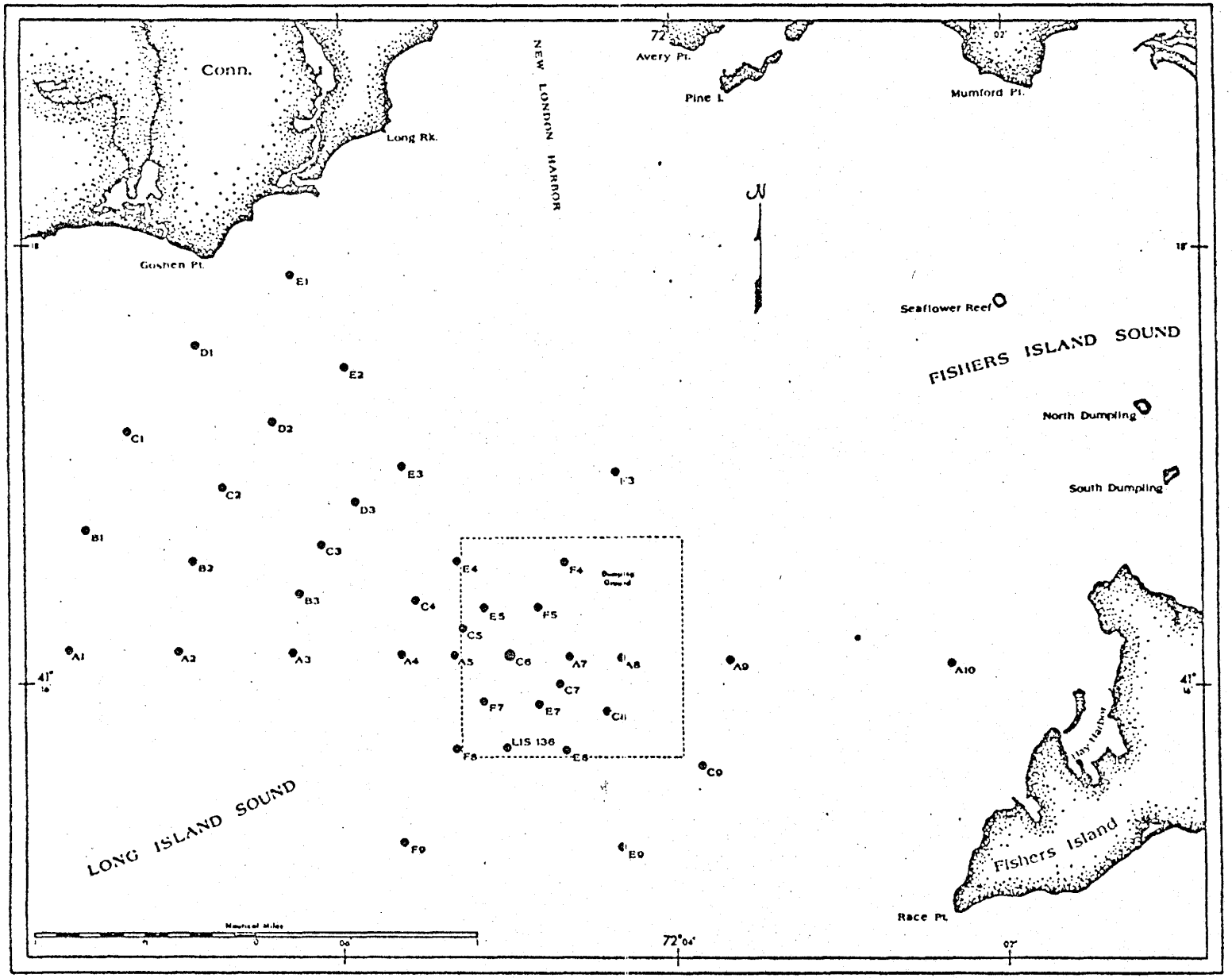


Figure 21. Station Pattern for Survey of Spoil Disposal Effects on Benthic Macrofauna, New London, Ct. Original Disposal Point was at C6; has Since Been Changed to Vicinity of C7.

sediments, benthic macrofauna and seston, as well as water column dissolved oxygen, pH, total organic carbon, suspended and volatile solids, are being carried out through contract with the New York Ocean Science Laboratory. The University of Connecticut is similarly studying heavy metals in the water column, sediments, and shellfish of the Thames River, and effects of dredging on concentration and composition of seston, as well as monitoring lobster populations in the disposal area.

As of 1 February 1975, the above studies had uncovered no significant changes attributable to spoil disposal outside of the immediate vicinity of dumping operations. Detailed objectives and methodologies for the overall monitoring survey are contained in MACFC Informal Report No. 25-A, and results to date are presented in the first and second quarterly reports (MACFC Informal Reports Nos. 40 and 49).

ECOSYSTEMS INVESTIGATIONS

ENVIRONMENTAL MICROBIOLOGY AND CHEMISTRY

Introduction

This Task is concerned with the effects of man-induced pollution, both chemical and microbial, on living marine resources and their environment. Various microbial species and chemicals have been identified in regard to diseases of food fishes, food poisoning outbreaks, and restricted use of the resource due to contamination and limitation on the use of the environment for recreation. The objective of this Task is to determine the presence of pollutant chemicals and disease-producing microbes, their persistence in the environment, their cycling through food-chain mechanisms and the possible inter-related microbial and chemical effects which act to the detriment of marine animals, their habitat and the use of the resource. The Environmental Microbiology and Chemistry Task is comprised of two subtasks - Microbiology Subtask and Chemistry Subtask.

One objective of the Microbiology Subtask in regard to coastal contamination is to ascertain the degree of fecal contamination in sediments of the mid-Atlantic coastal waters in order to determine the potential presence of disease-producing organisms which may induce diseases in marine animals or, by contamination, result in restricted use of the resource.

The objective of the Chemistry Subtask is to determine the levels of elemental metal contamination (Ag, Ar, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn) in the many animal species, water and sediments of the fisheries

environment and food-chain organisms. In addition to obtaining baseline levels of chemicals in the marine environemnt, the Chemistry Subtask has been working with the Physiological Effects of Pollutant Stress Task in determining the uptake and distribution of select heavy metals in several marine animals under controlled laboratory exposures.

In the past, the reports on the contaminants study have dealt, in general, with broader base data on the degree of metal concentrations in animals and sediments and fecal contamination of sediments of the mid-Atlantic region. The summaries for this report include the same type of information, but are concerned with more in-depth studies of localized areas in the region and, in particular, those that related to the deposition of dredge spoils in the inshore areas. The Chemistry Subtask is also continuing cooperation with the Physiological Effects of Pollutant Stress Task with employing additional metals and marine animals.

ENVIRONMENTAL MICROBIOLOGY AND CHEMISTRY:MICROBIOLOGY SUBTASK

John T. Graikoski, John A. Babinchak
Shearon Dudley, Maureen Nitkowski

As part of the Center Marine Contaminant Program, our objective has been to assess the degree of fecal contamination in sediments of mid-Atlantic coastal areas in order to determine the potential presence of disease-producing organisms. Sediments represent the areas of persistence and proliferation of bacteria in the environment, and the sources of contamination of food-chain organisms. In the previous MACFC Informal Report No. 26, data on the broad distribution of fecal coliforms in sediments from Long Island Sound and New York Bight Area were reported. For this report the same type of information is reported, but it is more restricted because of the area being studied.

Last year there was considerable concern relative to the possible migration of sewer sludge from the disposal area in the New York Bight to the beach areas on the south shore of Long Island and into unrestricted clamming areas. Several cruises to these areas were made to collect sediments for fecal coliform analyses to ascertain possible sewage migration by sediment transport.

Another area under study concerns the deposition of dredge spoils in Long Island Sound. In July of 1974, a two-year study was initiated regarding microbiological parameters during the dredging of the Thames River and deposition of the spoils in Long Island Sound. Baseline

data on the fecal coliform levels in water have been obtained and the fate and distribution is being followed at quarterly intervals. Ancillary information is being obtained on total microbial populations, marine vibrios and Chostridium perfringens in the sediments and select animals.

The fecal coliform information obtained from the studies of the areas of consideration has been compiled in several Informal MACFC Reports, Numbers 22 and 43. They are included as part of this report. In addition to these data, two cruises to the New York Bight in July and September of 1974 were made to obtain further information on fecal coliforms in sediment on transects from the disposal area to the beach. These data have not been presented elsewhere and are summarized for this report.

Fecal Coliform Densities in Sediments From The New York Bight Area - Atlantic Beach

Sediment samples were collected in July and September 1974 from an area extending from Atlantic Beach, Long Island to the sewage sludge disposal site. The sampling stations are shown in Figures 22 and 23. The fecal coliform counts are shown in Table 3 and represent MPN's per 100 cubic centimeters of the top centimeter of surface sediment.

The fecal coliform distribution agrees with earlier work presented in MACFC Informal Report No. 22, that the fecal contamination which may occur near Atlantic Beach is from a land-based source or short-dumps and not from sewage sludge migration from the disposal site.

Figure 22. Fecal Coliform Sampling Stations Inside East Rockaway Inlet

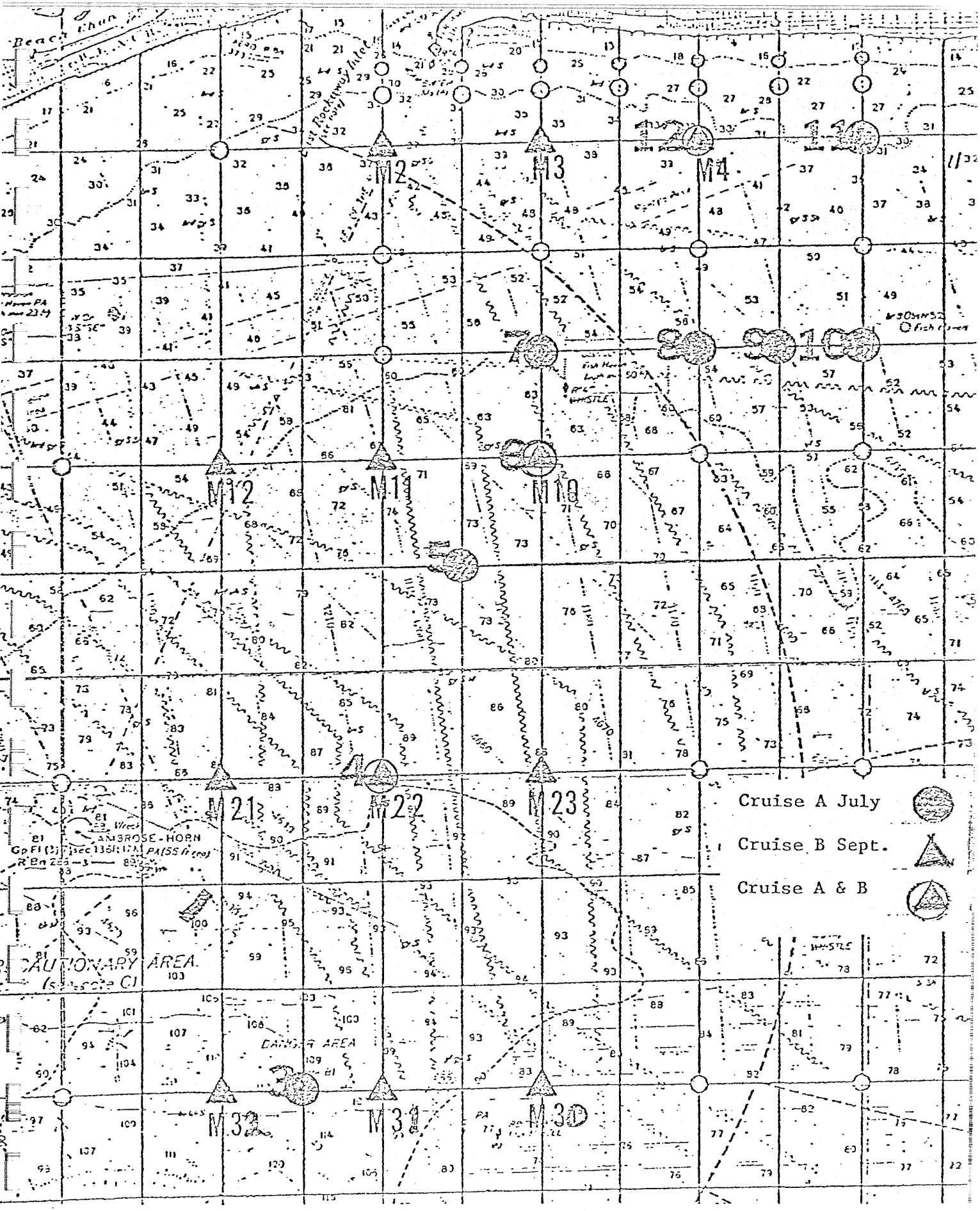


Figure 23. Fecal Coliform Sampling Stations Offshore of Atlantic Beach, Long Island.

Table 3. Fecal Coliform Densities in Sediments from New York Bight Area.

Cruise A July 1974 Stations	Fecal Coliforms/100 cc	Cruise B September 1974 Stations	Fecal Coliforms/100 cc
1	490	M2	2
2	3300	M3	2
3	7	M4	8
4	17	M10	0
5	11	M11	6
6	17	M12	130
7	17	M21	5
8	0	M22	17
9	2	M23	11
10	2	M30	790
10*	0	M31	330
12	5	M32	11

* Subsurface

Distribution of Fecal Coliforms in Sediments and Water from the Thames River and New London Dump Site - Long Island Sound

The following report constitutes data obtained on the distribution of fecal coliform bacteria and total aerobic plate count in the top layer of sediments obtained from the Thames River and the New London, Connecticut, dumping area prior to the commencing of dredging operations in August 1974. A limited number of water samples was also examined for the presence of fecal coliforms. For the initial baseline studies, sampling was conducted in June, July and August of 1974.

Sampling Plan

The three sampling areas designated for the bacterial studies were as follows:

Spoils (dumping) area - a circular area within a 1-mile radius from the designated point of dumping designated by the NL buoy in Long Island Sound.

Control area - the area outside the spoils area but within a circle of a 2-mile radius from the NL buoy.

River stations - areas of the Thames River to be dredged.

Forty stations located both in the spoils area and control area and five river stations were sampled for bottom sediments during four daily cruises in June and July 1974. These sediment samples were analyzed for total fecal coliforms and total aerobic bacteria. Three weeks later, 18 stations within the control and spoil areas and one river station were re-sampled for bottom sediments. Bottom water

samples were collected at six of these. All samples were analyzed for total fecal coliforms. In August, a spoils station at NL buoy (station C6), three control (spoils interface) stations located one mile from the NL buoy and one river station were sampled for bottom water once each during flood and ebb tides. These samples were analyzed for total fecal coliform counts.

Materials and Methods

Collection and Handling of Samples

Bottom sediments were obtained using a Smith-McIntyre grab. The top centimeter of the sediment surface was removed with a sterile tongue depressor and placed in a sterile 8 oz. French square bottle until full. The samples were then stored in refrigerated ice chests and examined within 24 hours at the laboratory. Each sample represented an approximate 100 cm^2 surface area of the sediment.

Water samples were taken in one-liter sterile plastic bags by means of a sterile polyethylene bag water sampler (Oceanics, Inc.). Water was aseptically transferred to sterile 8 oz. French square bottles and refrigerated for transport to the laboratory. All water samples were analyzed within 24 hours of collection.

Analysis for Fecal Coliform Bacteria

The procedure used for determining total fecal coliform MPN's follows Standard Methods for the Examination of Water and Wastewater (13th edition), modified for the dilution of sediment samples for inoculation of the culture tubes.

Results

The data obtained on the several cruises are presented in Tables 4-7 and plotted on outline maps (Figures 24-26). The plots on the maps also indicate the stations sampled during this study. For the convenience of analysis the fecal coliform ranges are grouped in the three areas as follows:

River Stations

	No. of Stations	Fecal Coliform Range
	3	10,000-172,000
	2	1,000- 10,000
Total	5	

Spoils Area

	3	1,000- 10,000
	13	100- 1,000
	4	0- 30
Total	20	

Control Area

	7	1,000- 10,000
	7	100- 1,000
	5	0- 30
Total	19	

The highest fecal coliform counts were obtained in the sediments from the river stations. Three samples had counts in the range of 10,000-172,000 fecal coliforms per 100ml of sediment. Except for the count in sediments from Station R1, furthest up the river, all counts on the river samples exceeded those obtained from the dump site and adjacent areas.

Table 4. Distribution of Total Bacterial Counts and Fecal Coliforms in Sediments from Thames River - New London Dump Site - June 26 - July 8, 1974.

Station	Initial Survey Total Plate Count X 10^4	Fecal Coliforms/100 ml
A1	69	1,300
A2	110	1,720
A3	140	11
A4	140	490
A5	70	240
A6	100	221
A7	72	790
A8	85	460
A9	160	1,410
B1	33	17
B2	26	14
B3	36	2
B4	26	11
B5	15	14
C1	130	172
C2	170	700
C3	160	4,900
C4	150	2,200
C5	95	3,300
C6	17	220
C7	95	330
C8	120	490
C9	93	490
D2	37	221
D3	72	172
D4	91	221
D5	37	130
E1	970	7,900
E2	260	2,400
E3	290	2,210
E4	160	1,090
E5	250	490
E7	310	26
E8	150	109
E9	130	22
F3	140	790
F4	200	460
F5	890	490
F7	170	26
F8	110	172
F9	37	172
R1	2,650	1,300
R2	2,200	4,900
R3	1,080	172,000
R4	6,500	22,100
R5	930	24,000

Table 5. Comparison of Fecal Coliforms in Sediments at Selected Stations Before Dredging and Dumping

Station	Initial Survey Fecal Coliforms/100 ml June 26 - July 8, 1974	July 30, 1974
A2	1,720	330
A3	11	5
A4	490	33
A5	240	330
B2	14	490
B3	2	170
B4	11	49
B5	14	70
C2	700	490
C3	4,900	240
C4	2,200	70
C5	3,300	49
C6	220	79
C7	330	130
C8	490	23
E7	26	0
E8	109	172
F7	26	17
R4	22,100	17,200

Table 6. Distribution of Fecal Coliforms in Sediments and Bottom Water - from New London Dump Site - July 29, 1974

Station	Fecal Coliforms/100 ml Sediment	Bottom Water
A2	330	33
C6(N1)	79	0
B3	170	8
C2	490	17
E8	172	0

Table 7. Distribution of Fecal Coliforms in Bottom Water at Ebb and Flood Tides Before Dredging and Dumping

Station	Fecal Coliforms/100 ml Ebb Tide 8/12/74	Flood Tide 8/19/74
A3	8	2
C3	49	5
E3	79	0
C6	2	0
R4	630	790

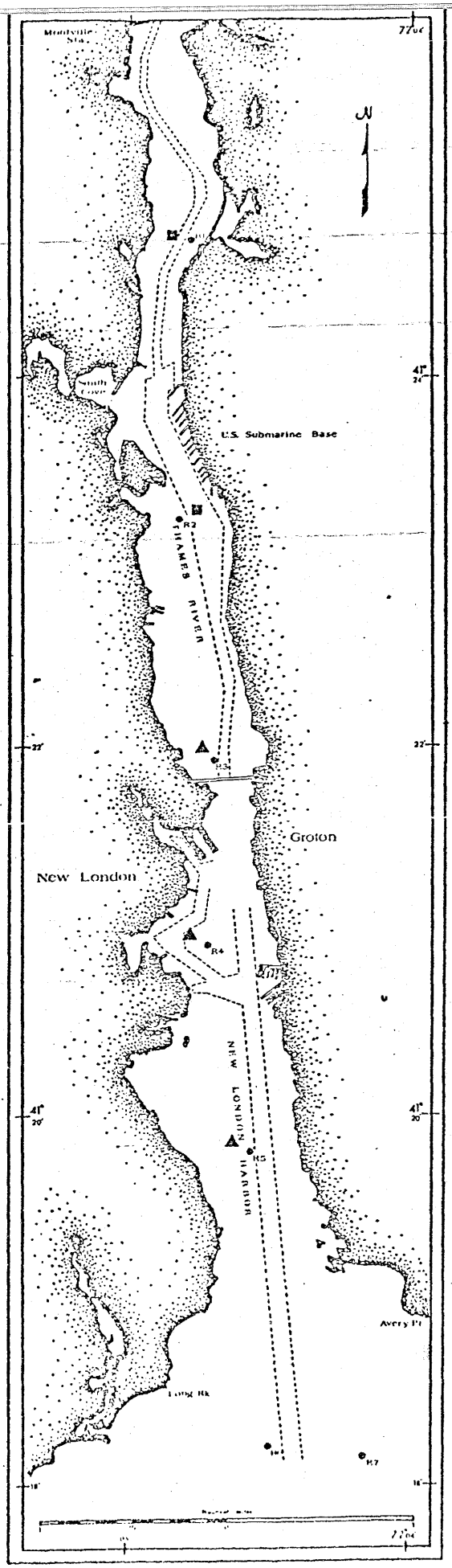


Figure 24. Distribution of Fecal Coliforms in Sediments - Thames River.

FECAL COLIFORMS / 100 ml

■ — 1,000 – 10,000

▲ — 10,000 – 200,000

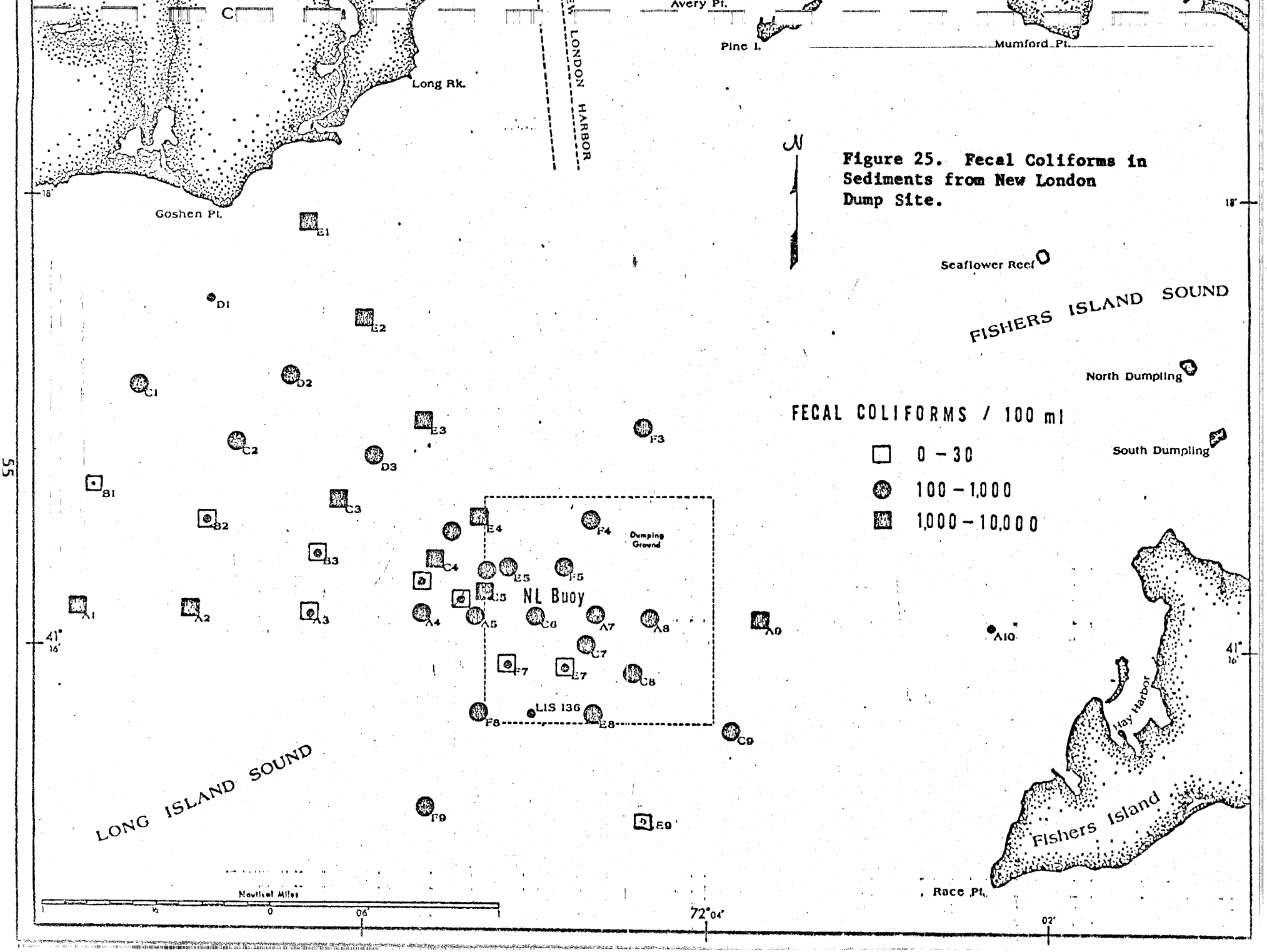


Figure 25. Fecal Coliforms in Sediments from New London Dump Site.

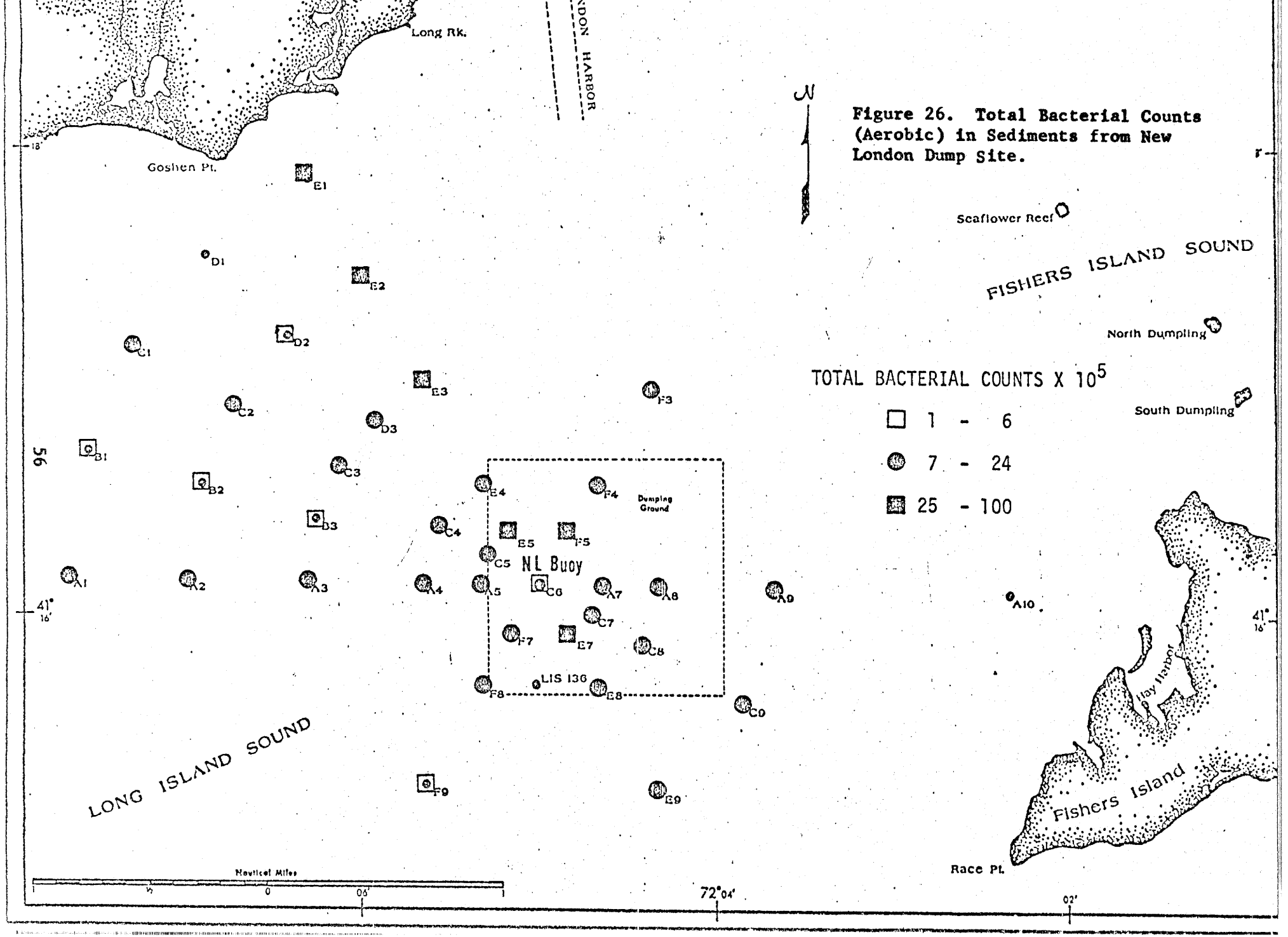


Figure 26. Total Bacterial Counts (Aerobic) in Sediments from New London Dump Site.

Fecal coliform counts in the top sediments from the dump site and adjacent areas were elevated, but lower than those obtained from the river samples. They also exhibited a variance in counts, a factor which would normally be expected.

In examining the grouping of counts, i.e., within the spoils and control areas, no set pattern of distribution was observed. The range of fecal coliform counts in the sediments from each of these areas were quite similar.

Resampling and analyses of sediments from 18 selected stations in the dump site area and one river station were made three weeks after the initial survey. These samples yielded fecal coliform counts of which 9 to 19 correlated well, 3 of 19 were comparable and 7 of 19 differed greatly. Considering the difficulty in obtaining sediments from the exact location at each station during resampling (and the variable distribution of bacteria in sediments), such results would be expected.

Grouping the total aerobic counts in the top sediments from the various stations as follows, showed no pattern of distribution:

	Number of Stations		
Count Range X 10^5	River	Control	Spoils
1.5 - 6.0	---	4	2
6.1 - 24.0	---	12	13
24.1 - 96.0	---	3	3
93.0 - 650.0	5	---	---

Counts in the river sediments were significantly higher than those obtained from the dump site and adjacent areas. Comparison of total counts with the fecal coliform counts did not have a high degree of correlation.

In general, the data would indicate that the fecal coliform and bacterial densities in sediments of the dump site were significantly higher in those from the northern section relative to the center. This would indicate that the contamination pressure of this area was exerted by the river outflow.

In the initial survey, sediments were selected for bacterial and coliform analysis to determine the dispersion of bacteria by dredging operations and the deposition of the spoils into surrounding waters. To determine the relationship between coliform densities in sediments and their overlaying, samples from five stations were simultaneously collected and analyzed for fecal coliforms. Although limited, the data in Table 6 show that no uniform ratios or correlating densities could be established between sediments and the overlaying waters.

To establish any variability in coliform densities in bottom water due to tidal flow, samples were collected from five stations at ebb and flood tides and analyzed for fecal coliforms. The data in Table 7 indicated that higher coliform densities were present in bottom water during ebb rather than flood tide, except at Station R4, the river station. This observation indicated that contamination pressure was exerted by the Thames River to the inshore areas in regard to increased bacterial and fecal coliform densities.

Future Studies

Sediments from 7 stations represented by triangles in Figure 27 will be sampled quarterly to monitor any increases in fecal coliform and total bacterial densities in the spoils and control areas.

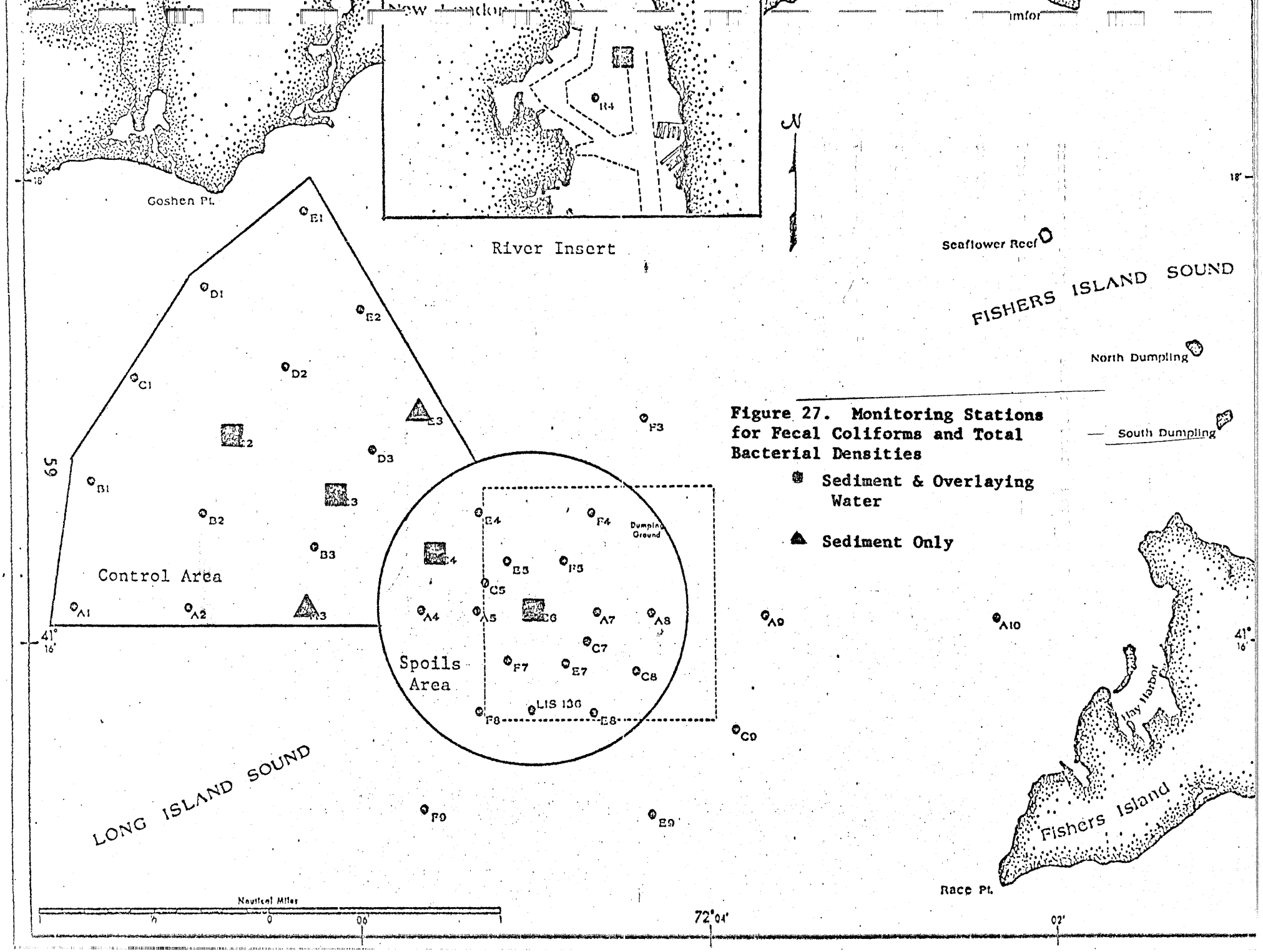


Figure 27. Monitoring Stations for Fecal Coliforms and Total Bacterial Densities

● Sediment & Overlaying Water

▲ Sediment Only

Bottom water and sediment samples will be collected simultaneously at five stations and analyzed to establish any correlation between coliform densities in the sediment and water. The bottom water will be sampled at ebb and flood tides during the spring, summer, and winter quarters.

July sediment samples from all stations in the spoils and control areas, as outlined in Figure 27, will be analyzed for fecal coliforms and total bacterial densities as done in the initial survey.

ENVIRONMENTAL MICROBIOLOGY AND CHEMISTRY - CHEMISTRY SUBTASK

R. Greig, D. Wenzloff and B. Nelson

Uptake and Clearance of Metals by Marine Organisms

Uptake of Silver by the Cunner (Tautogolabrus adspersus)

In a cooperative study with the Physiological Effects of Pollutant Stress Investigation cunners were exposed to 0.25 and 0.5 ppm of silver (as silver nitrate) for 96 hours in seawater. Red blood cells, serum, muscle, gill, liver and carcass samples were examined for the uptake of silver (Tables 8, 9). Only the gills, liver and carcass had measurable levels of silver in one study but not a second study. This was due to the fact that in Study I individual fish were analyzed, whereas in Study II pools from two fish were analyzed which provided better detectability of the metal. In Study II silver levels averaged 1.8, 0.8, and 0.2 ppm in gills for 0.5, 0.25, and 0.0 ppm exposure levels, respectively, while in Study I silver levels in gills were less than 1.9 ppm, the detection limit of the methodology employed. A measurable amount of silver was found in livers (mean value of 0.8 ppm of fish exposed to 0.5 ppm), whereas silver concentrations could not be measured in livers of cunners exposed to 0.25 and 0.0 ppm. Some differences in silver uptake by the carcass of cunners were noted between studies. At exposure levels of 0.25 and 0.5 ppm, silver concentrations in the carcass dropped from mean values of 0.32 and 0.46 ppm to 0.23 and 0.36 ppm for the two studies. Silver levels in the carcass of control fish averaged 0.17-0.19 ppm for both studies.

Table 8. Metal Content of Red Blood Cells, Serum and Muscle of Cunner Exposed to Various Concentrations of Silver in Seawater

Tissue	Study No.	Exposure Levels (ppm)	Metal Concentrations* Mean ± Std. dev. ppm, wet weight			
			Ag	Na	K	Mg
Red Blood Cells	I	0.0	<1.0	1632 ± 690	1513 ± 1000	135 ± 49
		0.25	< 2.6	3661 ± 1320	4880 ± 2293	154 ± 31
		0.50	< 2.6	2404 ± 539	* * *	162 ± 60
Serum	I	0.0	< 2	2951 ± 790	* * *	52 ± 17
		0.25	< 4	4398 ± 2182	* * *	* * *
		0.50	2	3730 ± 3102	306 ± 158	* * *
Whole Blood	II	0.0	< 0.26	2470 ± 154	1540 ± 196	98 ± 14
		0.25	< 0.16	2380 ± 71	1420 ± 85	87 ± 10
		0.50	< 0.22	2320 ± 139	1370 ± 44	85 ± 7
Muscle	I	0.0	< 0.15	396 ± 32	4134 ± 294	263 ± 67
		0.25	< 0.2	460 ± 49	5040 ± 254	335 ± 31
		0.50	< 0.2	490 ± 137	5280 ± 803	332 ± 56
Muscle	II	0.0	< 0.10	282 ± 51	4320 ± 561	228 ± 56
		0.25	< 0.10	308 ± 36	4730 ± 209	249 ± 58
		0.50	< 0.09	393 ± 140	4400 ± 307	242 ± 68

* Ten cunners were analyzed per study. Individual fish were analyzed for muscle, whereas pools of two fish were analyzed for red blood cells, serum, and whole blood.

*** Data on these samples were too variable to average. Values obtained were:

K - Red Blood Cells, 0.5 ppm exposure,	(3) at	3,500 - 7,550
	(2) at	13,600 - 19,300
K - Serum, 0.0 ppm exposure,	(3) at	28 - 63
	(1) at	291
0.25 ppm exposure,	(3) at	870 - 1050
	(1) at	3910
Mg - Serum, 0.25 ppm exposure,	(2) at	28 & 68
	(2) at	149 & 151
0.50 ppm exposure,	(3) at	22 - 75
	(1) at	149

Table 9. Metal Content of Gills, Liver and Carcass of Cunner Exposed to Various Concentrations of Silver in Seawater.

Tissue	Study No.	Exposure Levels (ppm)	Metal Concentrations* Mean ± Std. dev. ppm. wet weight			
			Ag	Na	K	Mg
Gills	I	0.0	< 1.4	2627 ± 283	3132 ± 452	493 ± 77
		0.25	< 1.6	3484 ± 632	3183 ± 369	622 ± 107
		0.50	< 1.9	2940 ± 538	6448 ± 1976	502 ± 59
Gills	II	0.0	0.20 ± 0.02	2750 ± 67	2152 ± 80	405 ± 90
		0.25	0.84 ± 0.09	3400 ± 546	1950 ± 62	418 ± 81
		0.50	1.8 ± 0.21	2980 ± 356	1960 ± 13	434 ± 44
Liver	I	0.0	< 2.0	685 ± 123	4620 ± 1018	179 ± 45
		0.25	< 2.5	948 ± 250	6330 ± 1645	239 ± 43
		0.50	< 2.5	809 ± 132	* * *	183 ± 39
Liver	II	0.0	< 1.0	649 ± 27	3050 ± 94	123 ± 49
		0.25	< 1.0	* * *	2500 ± 446	106 ± 49
		0.50	0.8 ± 0.15	688 ± 66	2902 ± 512	62 ± 12
Carcass	I	0.0	0.17 ± 0.02	1758 ± 187	2949 ± 1089	458 ± 80
		0.25	0.32 ± 0.05	1843 ± 124	2831 ± 133	536 ± 70
		0.50	0.46 ± 0.09	1875 ± 238	3934 ± 2614	515 ± 51
Carcass	II	0.0	0.19 ± 0.07	1620 ± 49	3040 ± 163	647 ± 55
		0.25	0.23 ± 0.04	1720 ± 198	3010 ± 84	563 ± 48
		0.50	0.36 ± 0.06	1700 ± 156	2800 ± 96	600 ± 39

* Ten cunners were analyzed per study. Individual fish were analyzed in Study I and pools from two fish were analyzed in Study II.

*** These data were too variable to average. Values obtained were:

K - Liver, 0.5 ppm exposure, Study I
(3) at 3720 - 7531
(7) at 11, 418 - 34, 048

Na - Liver, 0.25 ppm exposure, Study II
(2) at 371 & 451
(2) at 1110 & 1530.

In addition to silver analyses, the above samples were analyzed for sodium, potassium, and magnesium. In most cases, the sodium and magnesium levels did not vary substantially as a function of silver exposure levels, while potassium concentrations did in certain samples. There were conflicting results, however, between studies in the case of potassium. For example, in Study I the potassium level in red blood cells was much greater for fish exposed to 0.25 and 0.5 ppm compared to control fish. In serum the potassium level was greatest for fish exposed to 0.25 ppm. Whole blood was analyzed in Study II and the potassium level was about 1400-1500 ppm for all exposure levels. Somewhat similar results were obtained with gills and liver. In Study I the potassium level was twice as great in gills of fish exposed to 0.5 ppm compared to those at 0.25 and 0.0 ppm. In Study II there was little difference in potassium levels for all three exposure concentrations.

The results with livers were the same as with gills; substantial differences were seen between fish exposed to 0.5 ppm silver and those exposed to 0.25 and 0.0 ppm silver in Study I. In Study II no substantial difference in potassium levels was observed for all three exposure concentrations.

One possible explanation for increased potassium levels in the samples exposed to the highest level of silver is that the kidney, which is the prime route of potassium excretion, was damaged. However, damage to the kidney should have occurred in both Study I and Study II, thereby resulting in similar increases in potassium levels for fish exposed to the highest level of silver. The differences between these two studies are irreconcilable.

Uptake of Cadmium by the American Lobster (*Homarus americanus*)

This study was conducted in cooperation with the Physiological Effects of Pollutant Stress Investigation. Lobsters were exposed for 30 days to 0, 3, and 6 ppb of cadmium (as cadmium chloride). Measurements of cadmium, sodium, potassium, and magnesium were made on muscle, gill, and digestive diverticula of these lobsters.

Results showed a nearly twofold increase in cadmium levels for gills of lobsters exposed to 3 and 6 ppb compared to controls (Table 10). The variation of sodium, potassium, and magnesium concentrations was small for the 3 and 6 ppb samples compared to the 0 ppb samples. Cadmium levels were less than 0.12 ppm in all muscle samples. Little variation was found among the three exposure levels for concentrations of sodium, potassium, and magnesium.

The cadmium content of the digestive diverticula ranged from 16-30 ppm for the three exposure levels (Table 11). Levels in samples exposed to 3 and 6 ppb were not substantially different from controls. The sodium, potassium, and magnesium levels did not vary substantially for the three exposure levels.

Table 10. Metal Content of Gills and Muscle Tissue of Lobsters Exposed to Various Concentrations of Cadmium for 30 Days.

Test No.	Metal Concentrations (ppm, wet wt.)* Mean ± Standard Deviation			
	Cd	Na	K	Mg
		Gills - Controls		
1	1.5 ± 0.21	6742 ± 586	1565 ± 57	403 ± 51
2	1.5 ± 0.30	6438 ± 770	1603 ± 259	418 ± 82
		Gills - 3 ppb		
1	3.3 ± 1.36	7210 ± 433	1542 ± 144	423 ± 40
2	2.2 ± 0.46	6892 ± 488	1445 ± 160	471 ± 94
		Gills - 6 ppb		
1	3.4 ± 0.93	7145 ± 400	1472 ± 204	441 ± 33
2	3.3 ± 0.72	7442 ± 488	1498 ± 97	458 ± 125
		Muscle-Control		
1	< 0.12	1696 ± 181	4828 ± 258	227 ± 84
2	< 0.12	1657 ± 169	4498 ± 130	278 ± 100
		Muscle - 3 ppb		
1	< 0.12	1802 ± 205	4372 ± 298	353 ± 32
2	< 0.12	1642 ± 209	4490 ± 347	259 ± 86
		Muscle - 6 ppb		
1	< 0.12	1812 ± 353	4672 ± 75	316 ± 78
2	< 0.12	1783 ± 171	4593 ± 205	305 ± 67

* Six individual lobsters were examined per test.

Table 11. Metal Content of the Digestive Diverticula of Lobsters Exposed to Various Concentrations of Cadmium for 30 Days.

Test No.	Metal Concentrations (ppm, wet wt.)* Mean ± Standard Deviation			
	Cd	Na	K	Mg
			Controls	
1	24.3 ± 13.0	2083 ± 58	2918 ± 38	149 ± 60
2	16.3 ± 7.5	1945 ± 252	3383 ± 360	99 ± 57
			3 ppb	
1	24.8 ± 9.8	2130 ± 354	3725 ± 384	168 ± 45
2	20.2 ± 9.0	2088 ± 142	3750 ± 300	141 ± 59
			6 ppb	
1	30.3 ± 11.7	2357 ± 678	3527 ± 267	163 ± 68
2	16.7 ± 4.3	2060 ± 542	3575 ± 291	115 ± 42

* Six individual lobsters were examined per test.

Clearance of Silver by the American Oyster (*Crassostrea virginica*)

In a previous study, oysters were examined for uptake of silver after exposure to 0.5 ppm silver for 96 hours. In addition, some of these oysters were held for four weeks in clean seawater to determine whether oysters could clear the silver from their system during this period. It was found that oysters could not purge themselves of silver during this time period.

A second study was conducted, therefore, to determine whether oysters held for 25 weeks in clean seawater after a four-day exposure to 0.5 ppm silver would be able to purge themselves of silver. Results indicated that a substantial reduction in silver content occurred after 13 weeks and that the silver level returned to the same level as control oysters after 25 weeks. (Table 12).

Table 12. Clearance of Silver from Oysters Exposed to 0.5 ppm Silver for 96 Hours.

Weeks in "Clean" Seawater After Exposure to Silver	Silver Concentrations* (ppm, wet wt.)	
	Control Oysters	Test Oysters
0	3.3	22.8
7	2.6	15.9
13	1.9	5.0
19	3.4	5.6
25	2.1	3.5

* Ten individual oysters were examined at each test period.

Exposure of the Surf Clam (*Spisula solidissima*) and the American Oyster (*Crassostrea virginica*) to Polluted Sediment.

Methods. - Twenty surf clams and twenty oysters were placed in separate 20-gallon aquaria. In each case, one aquarium had natural seawater (Milford Harbor water) flowing through it, a second aquarium had a layer of polluted sediment covering the bottom to a depth of about 1/2-inch, and a third aquarium contained sand-filtered water that filtered through the polluted sediment of aquarium No. 2.

In one experiment conducted in April, 1974 surf clams and oysters were held for 30 days. In a second experiment oysters were held for 90 days from September through November, 1974.

All samples were analyzed for silver, cadmium, chromium, copper, nickel, lead and zinc by atomic absorption spectrophotometry.

Results - Metal concentrations found in the sediment were (ppm, dry weight, in ranges from 4 samples): silver - <2.5; cadmium - 3.2 to 4.0; chromium - 24 to 30; copper - 141 to 153; nickel - 9.8 to 13; lead - 75 to 85; zinc - 88 to 104. These levels of metals are similar in magnitude to those found in sediments at the New York Bight ocean dump sites (Greig, unpublished data).

In the first study, the metal concentrations of surf clams and oysters held for 30 days were not substantially different for the three experimental conditions (Table 13). Thus, the organisms did not take up metals from the sediment during this exposure period. The metal content of oysters held 90 days was also quite similar for the three experimental conditions and thus oysters did not take up metal from the sediment during this exposure period (Table 14).

Table 13. Metal Content of Surf Clams and Oysters Exposed for 30 Days to a Polluted Sediment and the Effluent Water After Passing Over the Polluted Sediment.

	Controls		I**		II***	
	Avg.	Std.Dev.	Avg.	Std.Dev.	Avg.	Std.Dev.
Surf Clams*						
Ag	0.04	--	0.05	--	0.1	--
Cd	0.09	--	0.15	--	0.1	--
Cr	<0.4		<0.4	--	<0.4	
Cu	0.7	0.4	3.0	2.1	0.95	0.69
Ni	<0.2	--	0.2		<0.2	--
Pb	0.44	0.09	0.7	0.55	0.4	0.12
Zn	2.2	2.70	7.0	5.90	4.4	4.22
Oysters*						
Ag	2.9	1.14	2.47	1.12	2.85	0.73
Cd	2.5	0.63	2.62	0.70	2.36	0.44
Cr	<0.8	--	<0.8	--	<0.8	--
Cu	231	89.7	2.24	1.02	2.41	0.86
Ni	<0.2	--	<0.2	--	<0.2	--
Pb	0.9	0.39	1.70	0.28	1.24	0.30
Zn	1330	360	1267	569	1.31	0.52

* Ten individual surf clams and oysters were examined; results are ppm, wet weight.

** Organisms exposed to a "polluted" sediment for 30 days.

*** Organisms exposed to effluent water that passed over "polluted" sediment for 30 days.

Table 14. Metal Content of Oysters Exposed for 90 Days to a "Polluted" Sediment and the Effluent Water After Passing Over the "Polluted" Sediment.

	Oysters*					
	Controls		I**		II***	
	Avg.	Std.Dev.	Avg.	Std.Dev.	Avg.	Std.Dev.
Ag	2.10	0.52	2.40	0.78	2.40	1.14
Cd	2.47	0.69	2.24	0.51	2.83	0.46
Cr	<0.8	--	<0.6	--	<0.68	--
Cu	117	39.6	124	37.6	122	52.7
Ni	<0.5	--	<0.3	--	<0.4	--
Pb	<0.7	--	<0.5	--	0.7	0.15
Zn	1610	428	1625	427	1575	350

* Ten individual oysters were examined; results are in ppm, wet weight.
** Oysters were exposed to a "polluted" sediment for 90 days.
*** Oysters were exposed for 90 days to sand-filtered water that passed over "polluted" sediment.

Trace Metals in Marine Biota and Sediments Collected from the New York Bight

For nearly a century solid waste materials from the New York Metropolitan Region have been disposed of by dumping them in designated areas within the New York Bight. Several investigators have examined the deleterious impact of these materials on the marine environment, as well as their sources, chemical nature, and disposal practices.

Recently, an alternate dumping site in relatively deep waters compared to the present dumping areas has been proposed. See Figure 28 for location of the current and proposed deep water dump sites. Baseline studies of the environmental conditions at this alternate dump site are being made to assess the impact of solid waste disposal at this site. In the present study, the baseline conditions for trace metals were examined for both bottom sediments and the indigenous species of fish in the areas. The samples were examined for cadmium, chromium, copper, lead, nickel, silver, and zinc.

Sample Collection and Preparation

Whole fish, tissues and organs of fish were taken aboard ship, packaged in plastic bags, and frozen until analysis. At the laboratory, the fish samples from each station were pooled by grinding with an electric blender using glass jars and stainless steel cutting blades.

Bottom sediment samples were obtained with a Smith-McIntyre grab. Plastic tubes 1-3/4 inches in diameter were inserted to the depth of the grab, then capped and frozen until analysis. At the laboratory

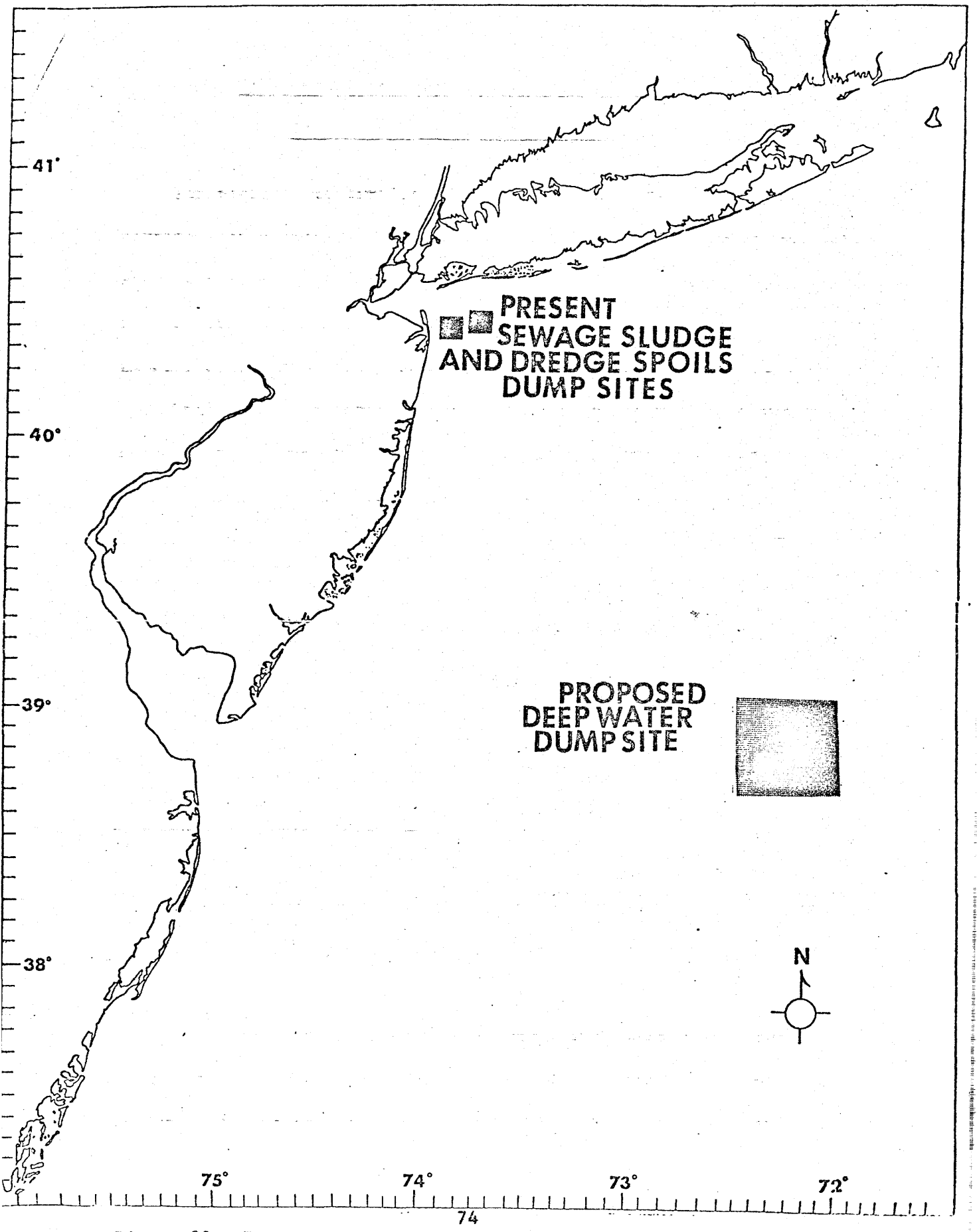


Figure 28. Present New York Metropolitan Area Ocean Dumping Sites and Proposed Deep Water Ocean Dump Site.

the top 1-1/2 inches of sediment were removed from the plastic tubes and dried at 60°C for 48 hours. Whenever possible, the dried samples were ground into a homogenous sample using a glass mortar and pestle.

Chemical Analysis

Trace metal analyses of fish samples were carried out as follows: up to 10 g of sample were placed in 250-ml glass beakers and dried overnight at 100-115°C. Sufficient concentrated nitric acid was added to the dry samples to cover them and the acid was evaporated by gentle boiling. About 3-6 ml of the concentrated nitric acid were again added and evaporated to dryness. This process, repeated until a fairly whitish residue was obtained, usually required 4-6 acid additions. Samples were then filtered through Whatman No. 2 filter paper and brought to a 25-ml volume with 10% nitric acid.

Metal analyses of sediment were conducted as follows: 2.5 g of material were placed in a 250-ml beaker; to this were added 10 ml of concentrated nitric acid and 0.5 ml of 30% hydrogen peroxide. The acid and peroxide were evaporated to dryness by gentle boiling. The following were added to the dried sample: 25 ml of acid mixture, consisting of 80 ml of concentrated nitric acid, 20 ml of concentrated HCl and 300 ml of distilled water; 8 ml of 10% NH_4Cl; and 0.4 ml of calcium nitrate (11.8 g/100 ml of $Ca(NO_3)2.4\ H_2O$). This mixture was heated to a gentle boil for 15 minutes and cooled for five minutes or longer. Samples were then filtered through Whatman No. 2 filter paper and diluted to 100 ml with distilled water. The filtered samples of sediment and fish were analyzed directly on an atomic absorption spectrophotometer (Perkin-Elmer Model 403).

Marine Fish

Silver, cadmium, and chromium levels in the muscle and liver of most fish examined did not vary greatly. However, copper, zinc and lead levels varied substantially. Lead showed the greatest variation of all the elements tested (Table 15). High levels of silver, cadmium, copper, and zinc encountered were in the liver of the deep sea slickhead (*Alepacephalus agassizi*). The levels of these metals in the liver are several orders of magnitude greater than metal concentrations found in livers of windowpane flounder (*Scophthalmus aquosus*) collected at the present New York Bight sewage sludge and dredge spoils disposal sites (Greig, unpublished data). The levels of these metals in the liver of the slickhead were: cadmium - 13.9 ppm; copper - 28.6 ppm; silver - 1.12 ppm; and zinc - 271 ppm; all values are on a wet weight basis. Additional liver samples were taken from the deep sea grenadier (*Nematonurus armatus*), rattail (*Nezumia bairdi*), whiting (*Merloccius bilinearus*), and *Halosaupsis macrochin* (no common name). Trace metal levels in the livers of these fish were fairly similar to those found in livers of the windowpane flounder.

The level of cadmium found in the liver of the deep sea slickhead was unusually high at 13.9 ppm. Other marine animals, such as the oyster (*Crassostrea virginica*) and the digestive glands of channeled whelk (*Busycon canaliculatum*), which have the ability to concentrate metals, contained only 1-5 ppm and 10-15 ppm of cadmium, respectively, for animals obtained from Long Island Sound.

Table 15. Trace Metal Concentrations in Marine Fish Collected in the New York Bight.

Common Name	Scientific Name	N	Tissue	Latitude	Longitude	Metal Concentrations (ppm. wet wt.) Ag	Cd	Cr	Cu	Pb	Zn
Butterfish	Prepilus triacantnus	5	Whole	39°30'N	72°34'W	0.14	0.23	0.54	4.6	1.1	28.8
Deep Sea Slickhead	Alepacephalus agassizi	3	Muscle	38°50'N	72°34.5'W	<0.09	0.09	<0.3	0.2	<0.3	11.6
Deep Sea Slickhead	"	11	Liver	38°50'N	72°34.5'W	1.16	13.9	0.6	28.6	0.5	271.
Deep Sea Slickhead	"	11	Gills	38°50'N	72°34.5'W	0.12	0.27	0.5	1.1	1.3	69.0
Deep Sea grenadier	Nematonurus armatus	3	Muscle	39°09'N	72°19.5'W	<0.08	<0.07	0.4	0.4	<0.4	3.7
Deep Sea grenadier	"	9	Liver	39°09'N	72°19.5'W	<0.1	<0.1	1.1	2.4	<0.6	18.5
Deep Sea grenadier	"	9	Gills	39°09'N	72°19.5'W	0.15	0.08	0.8	1.2	2.0	18.0
	Dicrolene intronigra	11	Whole	39°02'N	72°25'W	0.20	0.17	0.94	2.2	2.1	12.3
Eel	Synaphobranchus kaupi	11	Whole	39°02'N	72°25'W	0.11	0.51	0.58	4.2	1.7	14.7
	Halosaupsis macrochin	4	Muscle	38°38'N	72°06'W	<0.09	<0.07	<0.45	2.7	<0.4	4.3
	Halosaupsis macrochin	3	Liver	38°38'N	72°06'W	0.33	0.30	<0.67	2.0	<0.6	4.9
	Halosaupsis macrochin	3	Gills	38°38'N	72°06'W	0.16	0.13	0.75	1.4	2.3	15.5
	Halosaupsis macrochin	2	Gonad	38°38'N	72°06'W	<0.29	<0.23	1.4	3.6	<1.2	55.5
	Nematonurus armatus	3	Muscle	39°30'N	72°34'W	0.09	<0.07	<0.40	1.2	0.8	9.7
	Nematonurus armatus	3	Gills	39°30'N	72°34'W	<0.10	0.08	0.58	1.4	1.3	27.0
	Pontaster hebitus	18	Whole	38°38'N	72°06'W	0.54	0.83	1.00	4.0	8.0	59.4

Table 15 (Cont.). Trace Metal Concentrations in Marine Fish Collected in the New York Bight.

Common Name	Scientific Name	N	Tissue	Latitude	Longitude	Metal Concentrations (ppm, wet wt.) Ag	Cd	Cr	Cu	Pb	Zn
Rattail	Nezumia bairdi	60	Whole	39°09'N	72°19.5'W	0.17	0.26	0.95	3.0	2.7	32.9
Rattail	"	4	Muscle	39°02'N	72°25'W	<0.08	<0.07	0.45	0.6	0.6	5.8
Rattail	"	4	Liver	39°02'N	72°25'W	0.12	0.79	<0.5	10.9	0.8	59.9
Rattail	"	7	Gills	39°02'N	72°25'W	0.21	0.15	<0.7	2.2	4.2	32.0
Rattail	"	11	Gonad	39°02'N	72°25'W	<0.08	0.15	<0.4	6.5	1.1	56.0
Redcrab		1	Muscle	39°09'N	72°19.5'W	0.16	0.11	0.48	25.2	0.6	51.0
Redcrab		1	Digestive gland	39°09'N	72°19.5'W	0.62	1.14	<0.42	107.	1.2	19.6
Sea Cucumber	Benthedites giganteus	1	Whole	38°50'N	72°34.5'W	0.48	0.03	0.23	0.2	0.3	1.3
Whiting	Merloccius bilinearus	4	Muscle	39°30'N	72°34'W	<0.09	<0.08	0.49	1.0	<0.3	4.2
Whiting		4	Gills	39°30'N	72°34'W	<0.09	0.15	0.54	1.8	2.4	26.8
Whiting		4	Liver	39°30'N	72°34'W	0.08	0.29	0.52	14.7	<0.4	12.2
Whiting		4	Gonad	39°30'N	72°34'W	<0.09	0.07	0.45	1.2	<0.4	12.7
Witch Flounder	Glyptocephalus cynoglassus	58	Whole	39°09'N	72°19.5'W	0.13	0.20	0.73	1.8	3.3	14.7

For comparison with the present study the data of Windom on cadmium, copper, and zinc levels in about 35 species of fish obtained from North Atlantic waters can be used. Cadmium levels in the livers of these fish generally were less than 1.7 ppm, although the liver of one species of fish contained 5 ppm. Cadmium levels in other organs and whole fish mainly were less than 1 ppm, although some species had levels as high as 2.6 ppm. Since these data were expressed on a dry weight basis the values presented here would have to be reduced by about one-fifth, assuming 80% moisture in the fish samples. The highest cadmium level of 5 ppm dry weight found by these investigators would be about 1 ppm on a wet weight basis. Copper levels were in most cases less than 10 ppm in these fish. The copper level in livers of the deep sea slickhead in our study was 28.6 ppm on a wet weight basis, which is more than triple the highest level of about 9 ppm on a wet weight basis reported by Windom. Copper concentrations in other species of fish obtained in the present study were similar to the levels in fish examined by Windom.

Zinc levels in North Atlantic fish were reported by Windom and co-workers to be in the range of 10-80 ppm on a dry weight basis. However, a high level of zinc of 397 ppm was obtained in *Anchoa mitchelli*. This level would be about 80 ppm on a wet weight basis, similar to zinc levels found in this study.

Heavy Metals in Sediments

Sediment samples collected at stations shown in Table 16 were examined for chromium, copper, nickel, lead, and zinc. With one exception, the metal concentrations in sediments were quite similar for all stations sampled; chromium, copper, nickel, and lead levels were in the range of 20-30 ppm, dry weight basis, whereas zinc levels were 30-60 ppm. For the exception, which was sediment collected at the station with the coordinates 39°32'N and 72°32'W, the values for the four metals were in the range of 3-8 ppm, and the zine level was about 14 ppm.

The levels of chromium, copper, lead, and zinc in these sediments were about one-half to one-seventh lower than typically found at the present New York metropolitan ocean sewage sludge and dredge spoils dumping areas. Surprisingly, the nickel levels in the majority of the above sediments were about the same magnitude as found at the present New York ocean dumping areas.

Table 16. Trace Metal Concentrations in the Top 1-1/2 Inches of Sediment Samples Collected from Deep-Water Stations of the New York Bight.

Field Code	Latitude	Longitude	Concentrations (ppm, dry weight)				
			Cr	Cu	Ni	Pb	Zn
D-74-2-1G1	39°32'N	72°32'W	6.8	3.0	3.2	6.	14.8
D-74-2-1G2	39°32'N	72°32'W	6.6	3.0	4.2	8.	13.2
D-74-2-2G1	39°11'N	72°15'W	26.2	28.2	24.4	26.	60.
D-74-2-2G2	39°11'N	72°15'W	23.2	24.0	19.0	28.	53.
D-74-2-St2G1	39°10'N	71°50'W	27.0	22.8	22.2	20.	64.
D-74-2-St2G2	39°10'N	71°50'W	26.0	21.0	22.4	22.	63.
D-74-2-3G3	38°57'N	72°29'W	34.2	23.2	25.4	28.	64.
D-74-2-3G1	38°57'N	72°29'W	25.6	28.0	22.8	26.	59.
D-74-2-3G2	38°57'N	72°29'W	23.4	19.6	17.8	24.0	58.
D-74-2-4G1	38°51'N	72°16'W	27.4	29.6	28.4	32.	63.
D-74-2-4G2	38°51'N	72°16'W	24.2	26.6	24.4	32.	56.
D-74-2-5G1	38°42'N	72°22'W	26.8	31.2	29.6	32.	61.
D-74-2-5G2	38°42'N	72°22'W	28.0	30.6	28.4	26.	64.
D-74-2-St6G1	38°30'N	72°06'W	28.6	37.2	32.4	26.	65.
D-74-2-6G1	38°30'N	72°06'W	27.8	34.8	31.8	30.	53.
D-74-2-St6G2	39°30'N	72°06'W	24.6	33.6	30.6	30.	58.
D-74-2-St6NG1	38°34'N	72°06'W	26.8	32.0	28.6	26.	56.
D-74-2-6NW	38°33'N	72°10'W	26.6	34.6	33.6	28.	58.
D-74-2-W of 6G1	38°30'N	72°11.5'W	27.4	34.0	27.8	30.	57.
D-74-2-SW6G1	38°26.5'N	72°11'W	26.4	32.6	26.4	28.	54.
D-74-2-6S	38°26.5'N	72°07'W	23.4	29.0	29.8	24.	52.
D-74-2-E of St6G1	38°29'N	72°00'W	24.6	36.0	28.2	26.	56.
D-74-2-7G1	39°05'N	71°40'W	25.4	26.2	23.0	26.	56.
D-74-2-7G2	39°05'N	71°40'W	22.6	23.6	22.4	20.	53.
D-74-2-WH-1G1	39°46'N	70°40'W	16.8	13.4	12.6	16.	33.2
D-74-2-WH-1G2	39°46'N	70°40'W	21.8	21.6	20.6	24.	45.

Metal Concentrations in Channeled Whelk and the American Lobster Collected from Dump Sites in Long Island Sound

Channeled whelks and lobsters were collected from two dump sites in Long Island Sound over a several-months period. The channeled whelk was selected for this study because of its abundance in the Sound, while the lobster was selected because of its commercial importance. Lobsters and whelks were collected with lobster pots at two dump sites, one located off New Haven, Connecticut, and the other off New London, Connecticut. Samples of muscle, digestive diverticula, and gills were taken from these animals.

Digestive diverticula were freeze-dried and ground into a homogenized sample with a teflon pestle and bowl. Frozen samples of muscle and gill were analyzed.

Chemical analyses were conducted as follows: up to 10 g of wet sample or 2 g of freeze-dried sample were placed in 250-ml beakers. Ten ml of concentrated nitric acid were added to the samples and then heated to dryness on a hot plate. About 2 ml of nitric acid were added to the samples and again evaporated to dryness. This process was repeated several times until a whitish residue was obtained. The residue was dissolved in several small volumes of 10% HNO_3 and filtered through Whatman No. 2 filter paper. All samples were brought to a final volume of 25 ml. These solutions were analyzed as is by atomic absorption spectrophotometry for metal content.

The levels of silver, cadmium, copper, and zinc in the digestive diverticula of these animals were 2-3 times higher for the New Haven dump site animals as compared to the New London dump site animals (Tables 17 and 18). The levels of metals in the digestive diverticula for animals collected at New Haven were about 30-50 ppm (wet weight basis) for silver and cadmium and about 3,000 ppm for copper and zinc. The chromium, nickel, and lead levels generally were below the detection limits of the method employed. The concentrations of silver, cadmium, copper, and zinc in the muscle of whelks and lobsters were considerably lower than in the digestive diverticula. Levels of chromium, nickel, and lead generally were below the detection limit of the method employed.

Analyses were conducted on lobster gills obtained from one location - the New Haven dump site (Table 19). With the exception of copper, the metal concentrations in this tissue were about the same magnitude as found in muscle.

Table 17. Metal Concentrations in Channeled Whelk and Lobster Muscle* Collected from Two Dump Sites in Long Island Sound.

Metals	Metal Concentrations (ppm, wet wt.)			
	Channeled Whelk**			
	New London Dump Site		New Haven Dump Site	
	Avg.	Std. Dev.	Avg.	Std. Dev.
Ag	0.14	0.040	<0.18	-
Cd	0.16	0.079	0.13	0.041
Cr	1.0	0.715	<0.5	-
Cu	16.1	4.80	20.4	6.35
Ni	<0.5	-	<0.5	-
Pb	0.8	0.24	<0.7	-
Zn	23.7	2.47	22.5	3.82
	Lobster**			
	New London Dump Site		New Haven Dump Site	
	Avg.	Std. Dev.	Avg.	Std. Dev.
Ag	<0.2	-	0.23	0.059
Cd	<0.1	-	<0.1	-
Cr	<0.5	-	<0.5	-
Cu	6.3	2.0	17.5	4.62
Ni	<0.5	-	<0.5	-
Pb	<0.7	-	<0.7	-
Zn	12.8	1.6	18.6	1.24

* Lobster muscle consisted of the tail meat only.

** Ten individual whelks and lobsters were analyzed per dump site.

Table 18. Metal Concentrations in the Digestive Diverticula of Channeled Whelks and Lobsters Collected from Two Dump Sites in Long Island Sound.

Metals	Metal Concentrations (ppm, wet wt.)			
	Channeled Whelk*			
	New London Dump Site		New Haven Dump Site	
	Avg.	Std. Dev.	Avg.	Std. Dev.
Ag	12.7	4.18	38.7	17.56
Cd	28.3	9.97	50.0	12.54
Cr	3.0	1.24	< 2.	-
Cu	448.	180.	2935.	837.
Ni	< 0.5	-	< 2.	-
Pb	5.7	1.04	< 4.	-
Zn	2377.	1380.	3068.	914.
	Lobster*			
	New London Dump Site		New Haven Dump Site	
	Avg.	Std. Dev.	Avg.	Std. Dev.
Ag	8.2	1.07	22.1	5.77
Cd	3.5	1.32	15.6	6.24
Cr	< 0.6	-	< 1.0	-
Cu	775.	275.	2308.	658.
Ni	< 0.7	-	< 1.	-
Pb	< 0.7	-	< 2.	-
Zn	36.2	15.5	61.	33.2

* Ten individual lobsters were analyzed per dump site.

Table 19. Metal Concentrations in Gills of Lobsters Collected from the New Haven Dump Site in Long Island Sound.

Metals	Metal Concentrations* (ppm, wet wt.)	
	New Haven Dump Site	
	Avg.	Std. Dev.
Ag	0.66	0.21
Cd	1.2	0.51
Cr	<0.5	-
Cu	65.3	25.3
Ni	< 2.	-
Pb	< 0.8	-
Zn	14.3	3.40

* Ten individual lobsters were analyzed.

Trace Metal Content in Adults and Eggs of the American Oyster, *Crassostrea virginica*

Information about the transfer of pollutants from adult aquatic animals to their progeny is relatively unknown. The present study was designed to measure the transfer of metals from adults to eggs of the American oyster, *Crassostrea virginica*. Two groups of oysters containing significantly different levels of copper and cadmium were induced to spawn, the eggs were collected and trace metal contents measured. The eggs from both groups of oysters contained the same amount of copper, while cadmium levels were below detectable limits in both groups of eggs. Silver, lead, and zinc concentrations also were determined. The concentrations of these metals were similar for both groups of adults and eggs; thus, no conclusion could be made about the transfer of these three metals from adults to eggs of oysters.

Trace metal composition of many marine organisms has been reported in the literature. These data have been obtained on adult animals and little is known about trace metal burdens in immature stages. In the present study the spawned eggs from two different groups of adult American oysters were examined for trace metal levels. One group had considerably higher levels of copper and cadmium than the second. The purpose of the study was to determine to what extent copper and cadmium would be transferred from the adult female to the eggs in both high and low copper and cadmium concentration groups.

Oysters were obtained from the Housatonic River, Connecticut, and off Branford, Connecticut. Adult oysters were induced to spawn by raising the temperature of the water in which they were held from 20° to 28°C. When spawning began, the oysters were transferred to individual dishes of clean seawater for collection on a 20 μ filter after they were passed through a 75 μ filter to remove detrital and other large materials. The eggs were washed by centrifugation in a small volume of seawater and the supernatant decanted. Eggs from two females were pooled for chemical analyses.

The eggs were prepared for chemical analyses as follows: the oyster eggs were dried in the centrifuge tubes at 105°-115°C for 18 hours. Three ml of concentrated nitric acid were added to the dried sample and the tubes were heated at 70°C until the samples were completely dissolved. Fat globules were removed by paper filtration (Whatman No. 2) and samples were brought to a final volume of 5 ml.

The adult oysters were prepared for chemical analyses as follows: the entire oyster, including shell liquor but minus the shell, were placed individually in 250-ml glass beakers and dried at 105° to 115°C for 18 hours. These oysters did not exceed 25 g in weight. Sufficient concentrated nitric acid was added to cover the dried sample and then evaporated to dryness by careful boiling. This process was repeated as many times as necessary to oxidize the organic material completely and to end up with a whitish residue. The residue was transferred with 10% nitric acid by repeated additions of small volumes of acid to the beakers and paper-filtered (Whatman No. 2) into graduated cylinders, then brought to a final 25-ml volume with 10% nitric acid.

In both cases standards of metals and blanks were carried through the same procedures as the samples. Analyses of all samples were done on a Perkin-Elmer atomic absorption spectrophotometer (Model 403).

As shown in Table 20, the levels of copper and cadmium in adult oysters obtained from the Housatonic River were nearly double those found in oysters obtained from Branford. The mean concentrations of copper and cadmium for these two groups of oysters were statistically different as measured by the Student's t-test (P=0.01). The eggs from both collections of oysters had very similar levels of copper at about 28 ppm and cadmium at less than 1.6 ppm. Although copper and cadmium were of particular interest, levels were also obtained on lead, silver, and zinc. Zinc levels in eggs from the Housatonic River oysters averaged 82.4 ppm compared to an average of 65.9 ppm for eggs from Branford oysters; compared to these values were the 10,400 ppm and 8,300 ppm levels in adults for these same two locations, respectively (Table 20). The average values for both eggs and adults, however, were not statistically different for the two groups as measured by the Student's t-test (P=0.01). Silver levels were about 12-16 ppm for both groups of adult females, while levels of silver were below detectable amounts in both groups of eggs. Lead concentrations were below detectable amounts in all but one of the samples; the lead content for this sample was 7.1 ppm for adults obtained from Branford. The detection limit for adult oysters was lower at 4.1 ppm than for eggs at about 10 ppm; this was due to the fact that a greater sample weight could be analyzed for adults than for eggs. The conclusion drawn from

Table 20. Trace Metal Concentrations in Eggs and Adults of the American Oyster.

Metal	N*	Avg.	Std. dev.	N*	Avg.	Std. dev.
		Metal Concentrations (ppm, dry weight)				
		Housatonic River, Connecticut				
		Adult oysters			Oyster eggs	
	12			4		
Ag		12.1	4.45		< 1.6	-
Cd		28.1	7.49		< 1.6	-
Cu		2208.	793.		28.9	3.96
Pb		< 4.2	-		< 10.0	-
Zn		10460.	2483.		82.4	8.05
		Branford, Connecticut				
		Adult oysters			Oyster eggs	
	13			4		
Ag		16.4	6.02		< 1.2	-
Cd		15.6	3.46		< 1.2	-
Cu		1260.	415.		27.8	7.80
Pb		7.1	2.45		< 9.9	-
Zn		8300.	1800.		65.9	19.6

* Number of individual oysters examined for adults and number of pools of eggs from two oysters for the eggs.

the above data is that although fairly substantial differences in cadmium and copper concentrations can exist in adult American oysters, the eggs from these oysters contain similar concentrations of these metals. This suggests that the amount of metal transferred from adult to egg is fairly constant and not dependent on the amount of metal available in the adult oyster. To confirm this latter hypothesis, however, adult oysters with a much greater range of concentrations of these metals would have to be examined. No conclusion could be drawn from the silver, lead, and zinc concentrations because the levels of these metals were quite similar for adults and eggs for the two groups of oysters examined.

ECOSYSTEMS INVESTIGATIONS PUBLICATIONS

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EXPERIMENTAL BIOLOGY INVESTIGATIONS

There exists, at all levels of both the public and the private sectors, considerable alarm that the living marine resources of the estuarine, coastal and offshore waters of the Middle Atlantic Bight are being adversely affected by extensive offshore dumping of untreated wastes and by run-offs of highly polluted waters. The mode and intensity of such adverse physiological effects is largely unknown. Baseline findings of marine environmental quality cannot be interpreted without such knowledge nor can rational water quality standards be established or enforced when such knowledge is lacking. Quantitative, controlled exposure experiments, both static and chronic, on living organisms, and involving all stages in their life histories, followed by a battery of analytical tests are necessary to permit evaluation, stardards-development, successful enforcement, and resource conservation. The nucleus of such a research team, expert in hatching and rearing of marine invertebrates, culture of phytoplankton for pollutant studies, and in the physiology and cyto-genetics of marine animals comprises the Experimental Biology Investigations.

The objectives of these investigations, therefore, are as follows: 1) determine lethal effects of a large variety of known pollutants on the larval, juvenile and adult stages of molluscs, crustaceans, and finfish as well as on marine phytoplankton and benthic fauna inasmuch as these form the base of the food chain for all living marine organisms;

2) determine the long-term sub-acute effects of exposure to a large variety of known pollutants on the larval, juvenile and adult stages of molluscs, crustaceans and shellfish; 3) define the physiological and biochemical pathways affected and relate them to the metabolic disorders, tissue abnormalities, etc., which result in death or permanent damage to the living marine organisms; 4) determine effects of marine pollutants on the chromosomes, and genetic development of the American oyster, C. virginica, initially, and on other fish and shellfish. Evaluate findings in terms of specific pollutants and of population genetics.

The four Investigation reports that follow, discuss the results of these studies.

EXPERIMENTAL BIOLOGY INVESTIGATIONS

REARING OF MARINE ORGANISMS

W. Landers, E. Rhodes and W. Cable

The development of culture methodology for use in the study of pollutant effects on all stages of development of selected marine species was expanded this year to include marine decapods along with bivalve culture techniques. Species selection was confined to those organisms having a real or potential commercial value.

The ocean quahog (*Arctica islandica*)

Efforts were continued to find a technique for spawning ripe ocean quahogs. Most of the environmental manipulations used last year were repeated; these included constant high temperature of the water in which the clams are pumping, rapid fluctuation of the water temperature, variations in salinity of the spawning water ranging from ambient (26-27 ppt) to 35 ppt (somewhat higher than that over the beds from which the clams were obtained). Combinations of the above factors were tested in standing water, in flowing water, with animals taken directly from conditioning trays, with animals kept in moist refrigeration overnight, with gamete stimulation and without gamete stimulation. In all cases but two, no clams spawned. In the two exceptions, a single male released large quantities of sperm but the presence of this material in the common spawning tray had no effect on the other clams present. Failure of the other clams to spawn was not due to unripeness

of the gonads; subsequent examination of several individuals showed them to be full of ripe gametes. This is the third consecutive year that our attempts to spawn both sexes of this clam have failed.

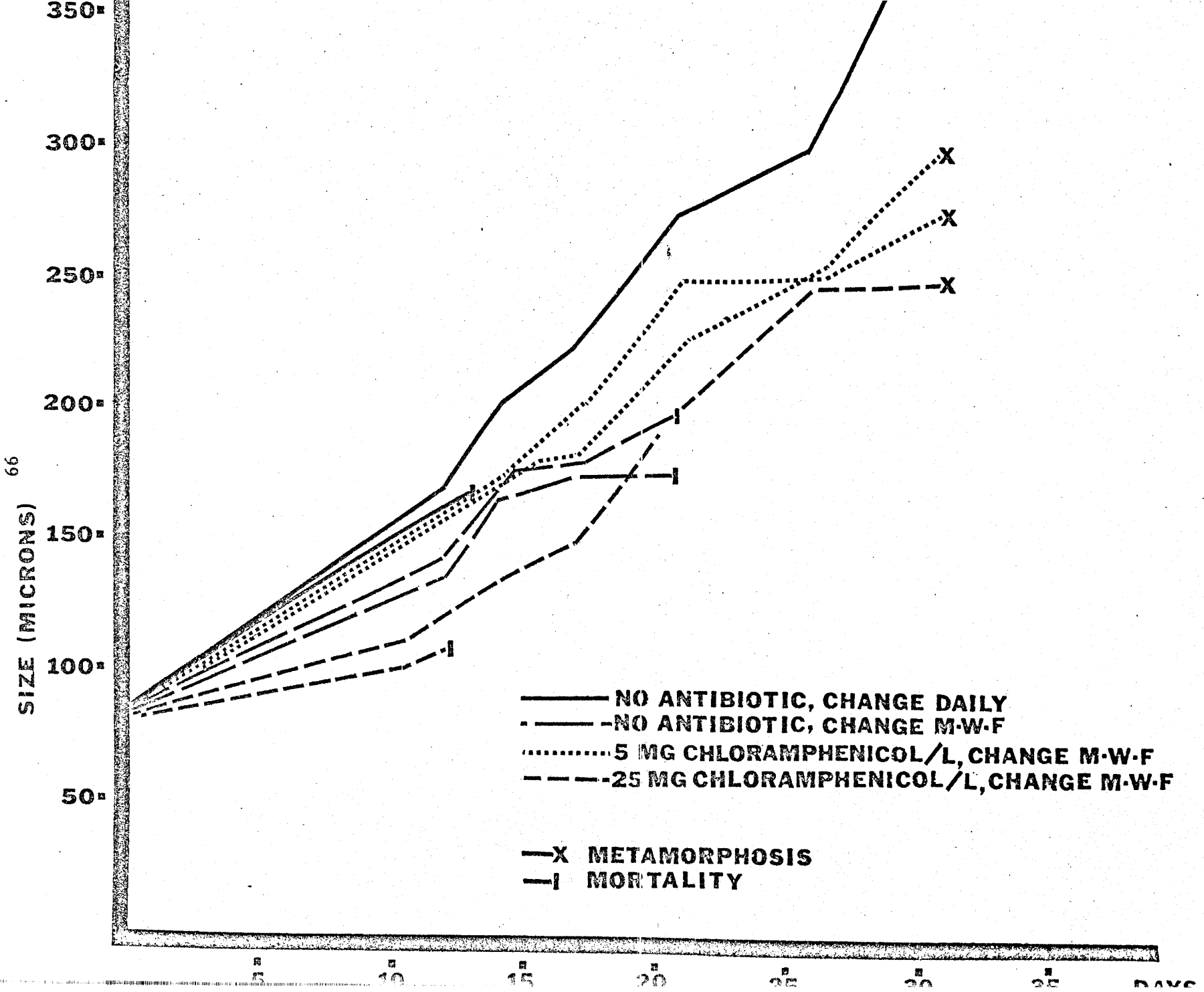
Attempts were made to repeat the successful technique used last year of rearing of stripped eggs using the ammonium hydroxide treatment of the eggs prior to fertilization; these trials were also failures. After three years of failure to produce a dependable culture method for the ocean quahog, we have decided to abandon further work with this animal.

The surf clam (*Spisula solidissima*)

Progress was made in developing culture methods for the surf clam. Earlier work on gametogenesis induced out-of-season in the laboratory showed that ripe animals could be produced readily from late March to November. Experiments are in progress that may lead to a method for winter ripening of the surf clam also.

An elusive goal in the development of methodology for any species is reproducibility of results, i.e., the ability of the culturist to repeat his successes with a high degree of consistency. Figure 29 shows the results of one experiment in which clam larvae and the subsequent post-set clams were grown for 30 days in different environments. Most apparent is the difference in survival and growth obtained in the different culture treatments. The most consistently good results were obtained with a 5 ppm chloramphenicol prophylactic treatment each time the culture water was changed. This treatment has been made standard in our larval cultures.

Figure 29. Growth of Larval and Post-set Surf Clams in Different Antibiotic Treatments.


350
300
250
200
150
100
50
SIZE (MICRONS)
NO ANTIBIOTIC, CHANGE DAILY
NO ANTIBIOTIC, CHANGE M·W·F
5 MG CHLORAMPHENICOL/L, CHANGE M·W·F
25 MG CHLORAMPHENICOL/L, CHANGE M·W·F
X METAMORPHOSIS
MORTALITY
5
10
15
20
25
30
35
DAYS

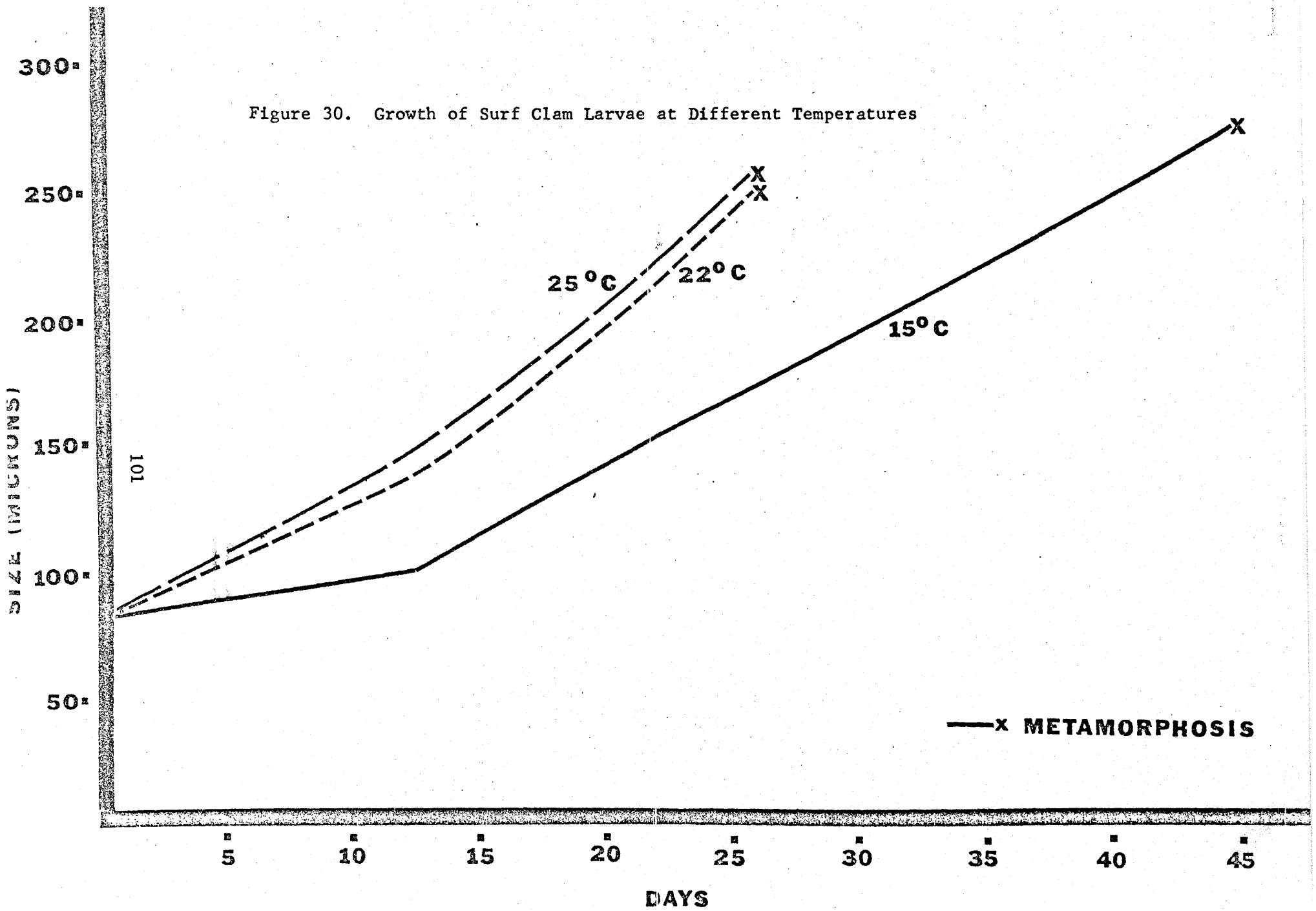
We find that chloramphenicol also helps to produce consistency of embryonic development. The summary below shows the percent development of several lots of surf clam eggs in different concentrations of the antibiotic. Above 100 ppm the chemical apparently becomes toxic to the eggs. The data for 0 concentration of the antibiotic are given as the range of percentages obtained rather than the average to illustrate the great inconsistency of results in untreated cultures. The averages in the following table at the other concentrations represent closely grouped means:

ppm	0	5	50	75	100	200	300	400	500
% development	0-65	60	63	65	65	<3	<1	0	0

Growth of clam larvae in relation to water temperature was investigated at three temperatures: 15°C, 22°C and 25°C. Figure 30 shows that growth and days to metamorphosis were virtually identical at the two highest temperatures. At 15°C the larvae needed almost twice the time to reach metamorphosis.

Several environments for rearing recently-set clams are being evaluated. These include standing water, recirculated water and flow-through systems. In one comparison of growth in standing water and in a filtered flow-through system having the same amount of food available, growth was slightly better in the standing water. This may have been due to a more efficient use of the cultured algae used as food. Probably a significant part of the algae dripped into the flow-through

Figure 30. Growth of Surf Clam Larvae at Different Temperatures



system escaped out the overflow. The overall growth of clams in both systems was good; average length increased from 0.8 mm at the beginning to 13.5 mm three-and-one-half months later. Figure 31 shows the growth trend during this period.

A comparison of growth of post-set clams fed equal amounts of different species of phytoplankton was made. Figure 32 shows the results. There was no significant difference between growth on Dunaliella, mixed Isochrysis and Monochrysis, or Phaeodactylum. Chlorella did produce slower growth.

When surf clams reach a size at which they can burrow well into the substrate they can be placed in large tanks of raw flowing seawater, such as the tank farm at Milford Laboratory, for further growth with a minimum of maintenance. We are assessing the importance of such factors in this system on the rate of clam growth as the type of substrate (fine sand, medium grain sand, coarse sand, no sand), density of planting, vertical position in the water column, horizontal placement in the tank and rate of water flow. To date, water flow appears to be by far the most influential factor. Growth increases as the flow rate is increased from 25 to 100 liters per minute. From mid-July to late October clams averaging about 23 mm at the start of the observations grew on the average 20 mm in length. This is about equal to the growth of siblings in field plots in Milford Harbor. One may conclude from the growth data already accumulated that the surf clam is a fast-growing species, a fact which implies a rapid rate of metabolism and a quick response to contaminant stress. Figure 33 shows the pattern of growth during the study period.

Figure 31. Growth of Juvenile Surf Clams in Static and Flowing Systems.

length (mm.)
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
9-18-74
10-25-74
11-5-74
12-5-74
1-7-75
static
flowing

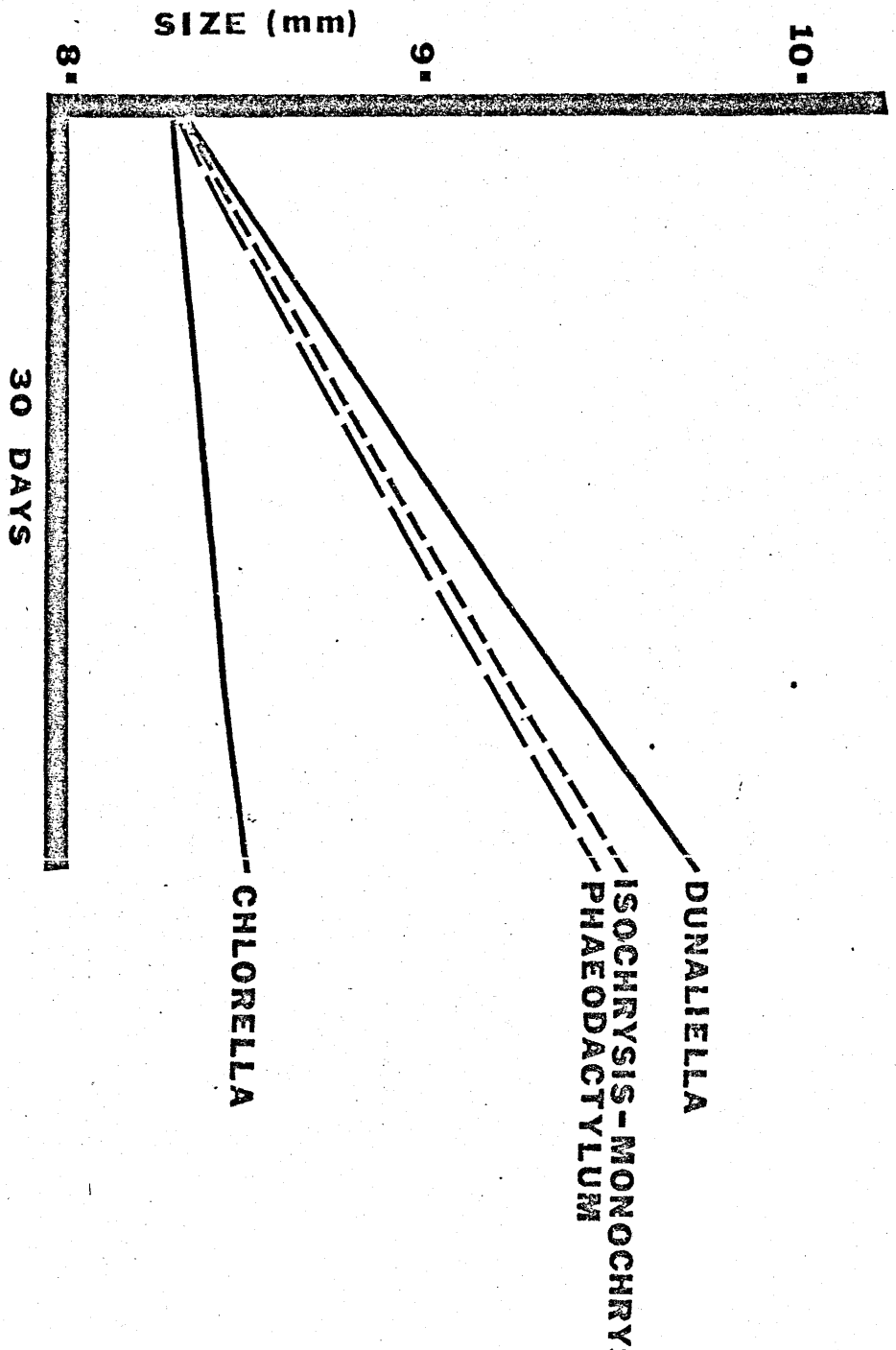
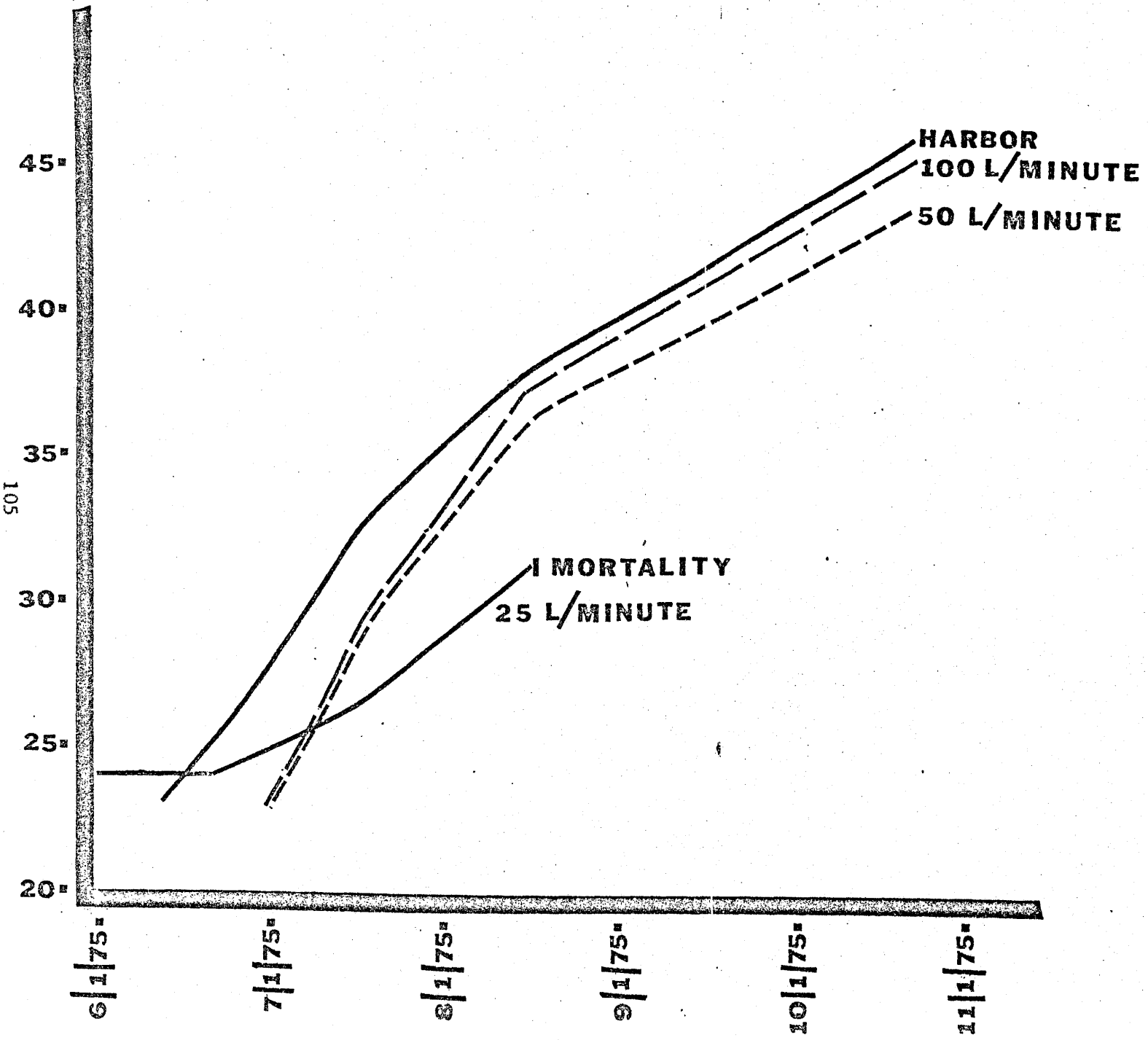


Figure 32. Comparison of Growth of Post-set Surf Clams Fed Different Species of Phytoplankton.

Figure 33. Growth of Juvenile Surf Clams in Different Environments.


HARBOR
100 L/MINUTE
50 L/MINUTE
I MORTALITY
25 L/MINUTE
45
40
35
30
25
20
6/1/75
7/1/75
8/1/75
9/1/75
10/1/75
11/1/75

The rock crab (*Cancer irroratus*)

The rock crab is of minor economic importance in the Middle Atlantic area although its potential for supporting a larger fishery is considerable. Observations on the culture of this species were made in conjunction with similar observations on the blue crab.

Rock crabs are year-round residents of Long Island Sound and, consequently, are in ready supply at any time with the proper collecting gear. They adapt easily to captivity and live practically indefinitely in our holding facilities. Berried females are available in the field from February through July, but copulation and egg-laying have taken place at ambient temperatures in the laboratory at various times of the year. We have also stimulated reproduction in the laboratory by manipulation of temperature. Reproductive activity of these crabs in the laboratory has, consequently, freed the culturist of total dependence on the natural supply of egg-bearing females.

Berried crabs held in warmed flowing seawater readily hatched zooea in large numbers as early as late February. The zooea, fed recently-hatched brine shrimp, passed through all of the larval stages, of which there are four. Survival of larvae in relation to culture density was studied. Survival to the megalops stage (first crawling stage) exceeded 90% at the two larval concentrations observed - 50 and 250 per liter - in a flow-through system. As soon as the megalops stage was reached, mortality rose sharply in the 250-per-liter cultures and less sharply but steadily in the 50-per-liter cultures. No

megalops in the 250-per-liter cultures reached the crab stage and only about 10% did so in the 50-per-liter cultures. The reason for this sudden onset of mortality is not proven but we suspect cannabalism and possibly new dietary demands that were not met.

Crab larvae that survived through the megalops stage were then observed for survival and growth in two kinds of flow-through culture systems. In one, the young crabs were kept in a common container and fed as a group, and in the other each crab was kept and fed in a separate container. In the first system, cannabalism was rampant on recently-molted crabs resulting very soon in only one live, but large and vigorous crab. In the other system there was no mortality and growth was excellent. This experiment shows the absolute necessity for segregating young crabs during this period in their development when individual crabs may molt every two or three days.

The blue crab (*Callinectes sapidus*)

The blue crab is a summer inhabitant in Long Island Sound. Its abundance and distribution during this season of the year is not predictable; consequently, local sources of this crab, particularly egg-bearing females, are erratic at best. Chesapeake Bay is a far more reliable source of experimental animals and berried adults are available in large numbers in season. Unfortunately, during the warm months of the year when these crabs are accessible it is difficult to transport them to Milford in good condition. Many individuals fail to survive the trip and most of those that do, die within a week or so thereafter. Local crabs do well in our seawater system and on one occasion a female produced an egg sponge in captivity.

As a result of the constraints described above, our attempts to culture blue crab larvae have been limited. Even the few attempts that we have made have failed. On one occasion two females from Chesapeake Bay liberated larvae just before succumbing to the trauma of being transported to Milford during the summer. Later in the summer two locally-collected crabs also liberated larvae. In both cases the larvae, reared according to the Costlow method, lived for about a week, failed to molt and finally died. Considering the imminent end of this Investigation's participation in contaminants studies, we plan to do no more work with this species.

The American lobster (*Homarus americanus*)

Considerable time was spent during 1974 in testing and refining the standard lobster rearing techniques developed at the Massachusetts State Lobster Hatchery with the objective of providing the Physiological Effects of Pollutant Stress Investigation with developmental stages of the lobster on demand.

Lobsters are available locally the year-round and egg-bearing females can be found during much of the year. They adapt readily to the laboratory environment and will live for months with no apparent stress in small containers as long as the water flow is adequate.

Hatching takes place in the field when the water temperature warms sufficiently, but in the laboratory this can be hastened by heating the seawater. Using this technique, individual lobsters obtained in March were induced to hatch their eggs by early May when the ambient temperature was still too low to stimulate hatching. It should also be

possible to delay hatching by keeping the egg-bearing female in cold seawater. Our only attempt to do this was inconclusive. Two berried females were obtained locally in July and were immediately placed in cold seawater. Hatching soon took place, however, suggesting that development of the eggs had already proceeded too far in the field to be stopped.

Lobsters have been reared to the fourth (crawling) stage several times in our facilities. We have used a modified Hughes culture container and one of our own design. The standard Hughes system uses a once-through water flow; our modification was to convert this into a recirculating system(Fig. 34) to conserve food (newly-hatched brine shrimp). Our own flow-through system (Fig. 35) is designed to retain the food organisms, as well as the lobster larvae. In both systems survival to the fourth stage was about 60%.

Shortly after the crawling stage was reached, the lobster populations began to sustain significant mortalities from Leucothrix disease which occurs during molting and is characterized by the heavy production of mucous and the inability of the lobsters to free themselves from the old exoskeleton. This was controlled by the application of 1 ppm streptomycin sulfate. Mortality reached about 25% before the disease was brought under control.

In August we initiated a test of the survival and growth of juvenile American lobsters in various seawater systems. The lobsters averaged 11 mm in length and 0.25 gm in weight. The systems being evaluated include various flowing seawater devices, a recirculating

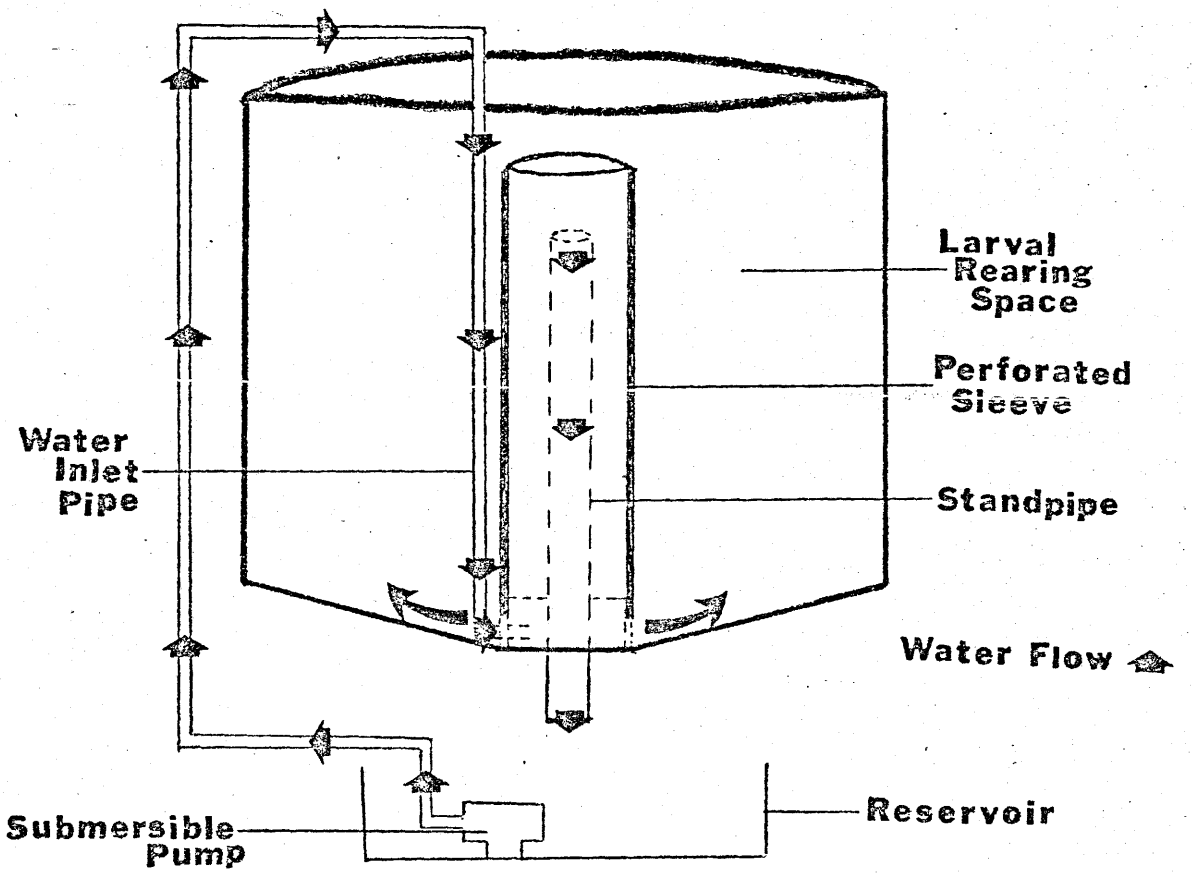


Figure 34. Modified Larval Lobster Culture Tank.

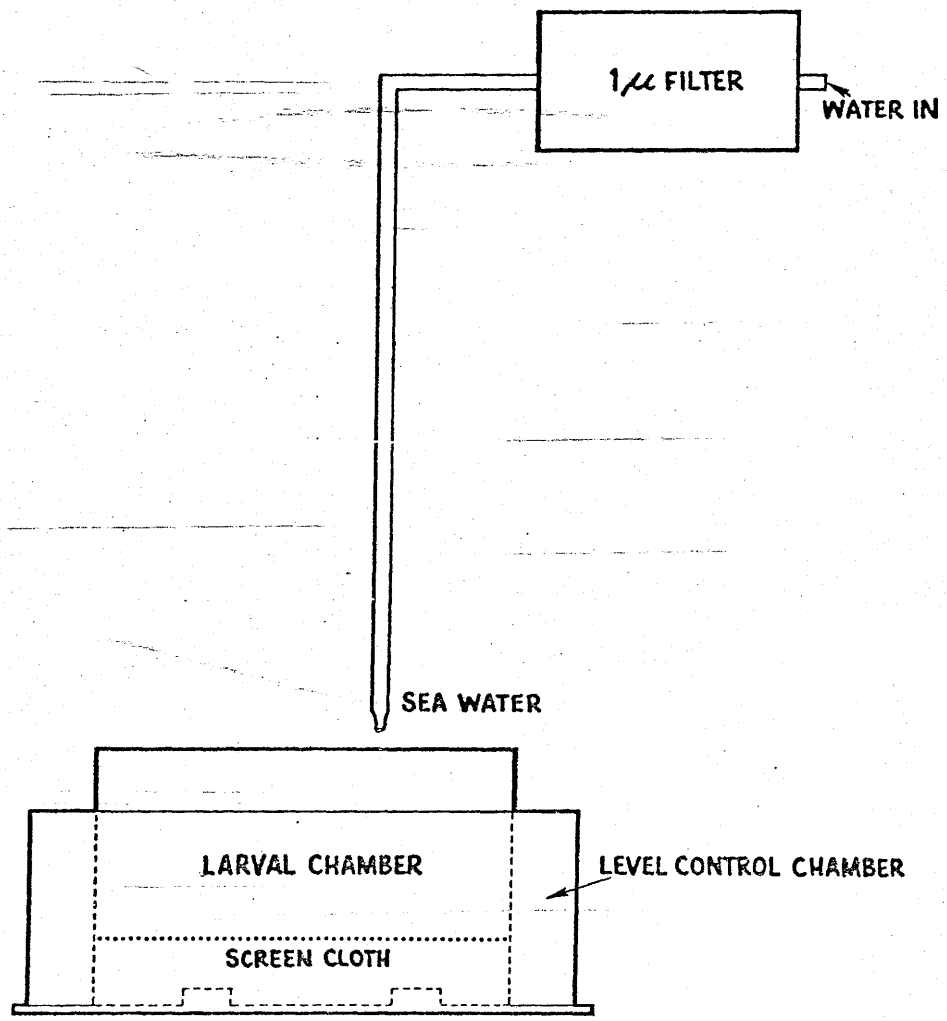


Figure 35. Flow-through System for Rearing Marine Invertebrate Larvae.

system, and a static system all at ambient temperature. The lobsters were fed frozen adult brine shrimp and it is now apparent that the use of a non-living food such as this makes it mandatory to have good water circulation. Water stoppage for less than 12 hours can lead to high mortality of the juvenile lobsters. At the end of three months, the experiment was terminated and growth of juvenile lobsters in the various environmental systems was compared. Lobsters reared in a single large outdoor tank with PVC (polyvinyl chloride pipe) habitats grew best, increasing 96% in carapace length and 10-fold in weight. Those in the laboratory grew an average of 78% in length and 5-fold in weight. No significant size differences were noted between laboratory-held groups that had been provided with water exchange by various methods. Mortality in the outdoor common environment reached 50%, compared to a range of 13-75% for those held in the several laboratory systems. The wide range of mortalities in the laboratory can be attributed to random accidental water shutdowns.

The juvenile lobsters reared at ambient temperatures during the summer and early fall were subsequently grown for three months in an experiment to assess the effects of heated seawater on growth compared to that of ambient water during its cooling off season. The effect of container size was also measured. The lobsters averaged slightly under 20 mm carapace length and 2 grams in weight at the start of the experiment. At the end of three months many of the lobsters held at 20°C had molted two or more times. The average increases in length and weight in the large containers (1100 cm^2) were roughly 11 mm and 7 grams,

respectively. In the small containers (124 cm^2) the increases in length and weight were about 8 mm and 5.5 grams, respectively. Only one of the animals grown at ambient temperatures (7°-4^oC) molted and, consequently, the increases in length and weight of the populations were insignificant. The diet for all animals consisted of a mixture of brine shrimp, clams and fish. Figure 36 shows the patterns of growth during the experiment.

Much of our experimentation on growth of lobsters has used frozen adult brine shrimp as food because it is the standard source of nutrition of the Hughes method for young stages. We find that live adult brine shrimp are superior largely because the decomposition problem is eliminated and the cultures are far cleaner. Lobster mortality is also greatly reduced, especially when mechanical or other problems shut down the water flow.

Our work with lobster rearing during this year has demonstrated that one can have larvae at least 10 months out of the year by manipulating hatching water temperature. This is possible because female lobsters carry their eggs, once laid, up to 11 months. As a result, berried females are present in nature virtually the year-round.

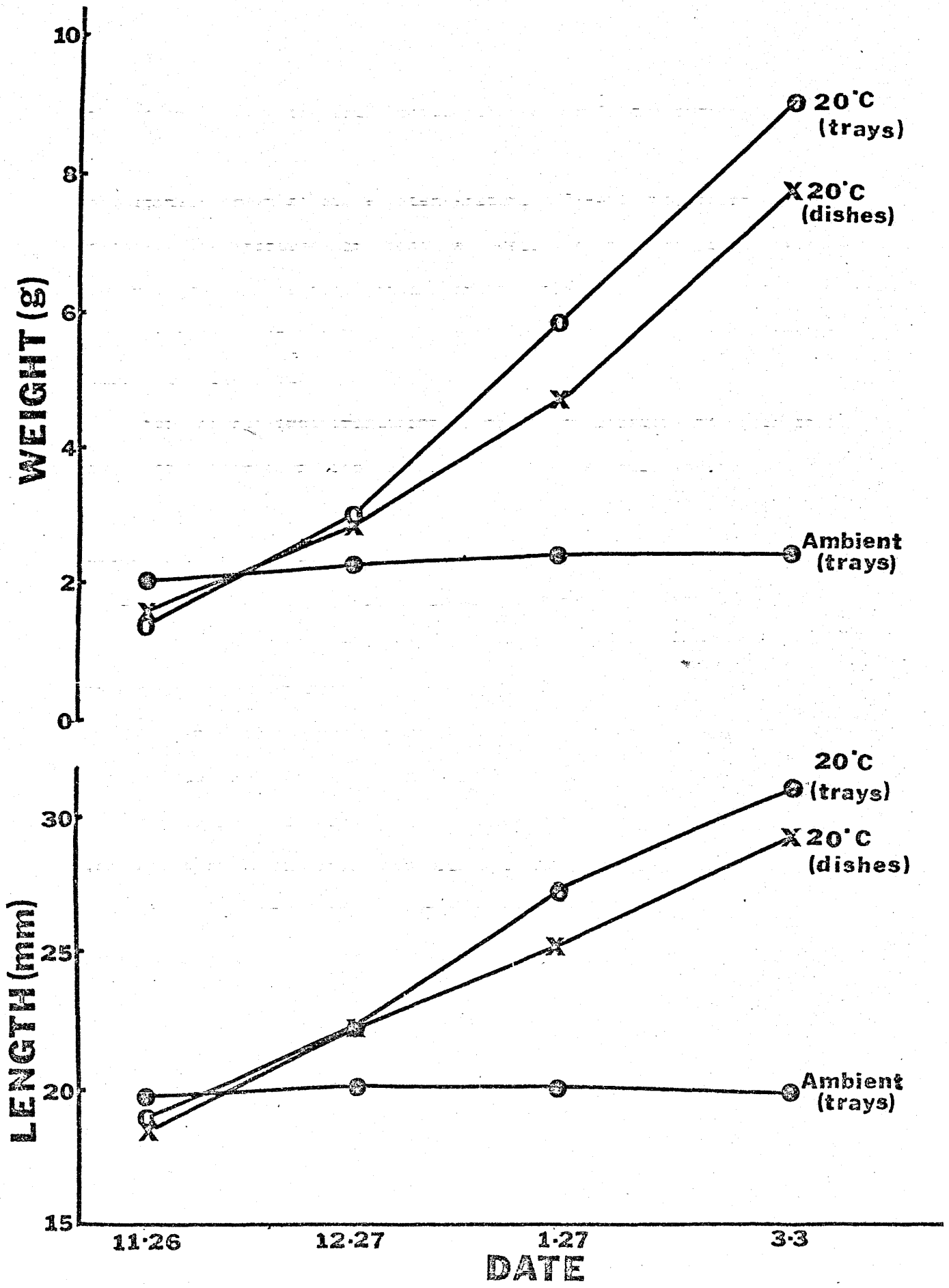


Figure 36. Growth of Juvenile Lobsters at Ambient Temperature (7° to 4°C) and 20°C.

PHYSIOLOGICAL EFFECTS OF POLLUTANT STRESS - PHYSIOECOLOGY SUBTASK

A. Calabrese, R. Collier, D. Nelson and J. Miller

Experimental Studies

Laboratory experiments in this Investigation were directed toward the evaluation of physiological stress on marine organisms induced by contaminants in the environment. A number of different experiments were conducted and are summarized in this Subtask report and those of the Physiological Effects and Biochemical Effects Subtasks which follow. Reference to exposures conducted by this Subtask will also be made by various investigators in this Informal Report. This Subtask has the major responsibility of exposing marine animals to pollutants in both static and chronic systems because of the facilities developed at Milford over the past two years. Animals are exposed on request from other MACFC investigators and also as a function of this Investigation. For results of previous studies by this Investigation refer to MACFC Informal Report No. 5 entitled, "Cooperative Study of Contaminants in the Coastal Environment and Their Effects on Living Marine Resources: Summary Report 1971-1972" and Informal Report No. 26 entitled, "A Multilaboratory Cooperative Study of Contaminants in the Coastal Environment and Their Effects on Living Marine Resources: Summary Report of Operations from May 1, 1972 to December 31, 1973.

Acute Studies

Rock Crabs, *Cancer irroratus*

A multi-metal-anion exposure to rock crabs, *Cancer irroratus*, was conducted for biochemical and physiological testing. A series of tests each of 96 hours' duration was conducted using cadmium chloride, cadmium acetate and cadmium nitrate as test pollutants. The results of these tests will be summarized in the Biochemical Effects and Physiological Effects Subtasks which follow.

Cunners, *Tautogolabrus adspersus*

A series of 96-hour exposure tests was conducted on the cunner, *Tautogolabrus adspersus*. Cunners were exposed to silver nitrate and silver acetate for biochemical effects studies and to silver nitrate for physiological effects and chemical uptake studies. The results of these tests will be summarized in the Biochemical Effects, Physiological Effects and Environmental Chemistry Subtasks in this report.

Mud crabs, *Eurypanopeus depressus*

A study was initiated to determine the physiological effects of mercury on mud crabs, *Eurypanopeus depressus*, held at various salinities and temperatures. Although these crabs are of no commercial importance, they were used because of their availability and small size, a determining factor necessary in a study of this type when several environmental parameters are being examined at one time. Experiments were set up at various sublethal concentrations of mercury at salinity and temperature

combinations of 10, 20 and 30 ppt and 15, 20 and 25°C, respectively. The intent of this study is to determine changes in oxygen consumption rates at the various mercury, salinity and temperature combinations. The data collected are still preliminary and will not be discussed here.

Bay Scallops, *Argopecten irradians*

Acute toxicity studies with juvenile bay scallops, *Argopecten irradians*, (20 to 30 mm) have been completed. Scallops were exposed to Ag (as silver nitrate), Cd (as cadmium chloride), Hg (as mercuric chloride) and As (as sodium arsenite) for 96 hours in static cultures. All cultures were changed each day of the test and both water and metal were renewed. Each test was conducted at five different metal levels in triplicate using 21 scallops per concentration. Each test was repeated three times and a total of 1134 scallops was used, including controls. Using the Litchfield-Wilcoxin method of analysis, the following LC_{50} values in decreasing order of toxicity were determined: Ag - 0.033 ppm, Hg - 0.089 ppm, Cd - 1.48 ppm and As - 3.49 ppm. These data are summarized in Table 21. It is apparent from these tests that juvenile scallops are extremely sensitive to silver, although this level of silver is probably higher than that found in most natural environments. If juvenile scallops are made available to us again, it would be desirable to expose them to silver for a longer period of time, i.e., 30 days.

All of the scallops exposed in these tests were saved and prepared for metal uptake analysis by the Environmental Chemistry Subtask.

Table 21. Toxicity of Heavy Metals, as Inorganic Salts, to Juvenile Bay Scallops, *Argopecten irradians*, at 20 ± 1°C in Natural Seawater (26 ± 2 ppt) in 96 Hours. The Concentrations are in Parts per Million of Metal Ion Added Each Day Causing Mortality of 5, 25, 50, 75 and 95%. These Values were Determined by the Litchfield-Wilcoxin Method of Analysis.[1]

Metals as Inorganic Salts	LC_5	LC_{25}	LC_{50}	LC_{50} 95% Confidence Limits	LC_{75}	LC_{95}	Slope Function
Silver nitrate	0.014	0.022	0.033	0.030-0.036	0.048	0.086	1.811
Mercuric chloride	0.052	0.062	0.089	0.054-0.147	0.11	0.150	1.370
Cadmium chloride	0.94	1.23	1.48	0.95 -2.31	1.78	2.33	1.295
Sodium arsenite	2.22	2.86	3.49	2.11 -5.81	4.17	5.41	1.330

1 These concentrations do not include background concentrations of heavy metals in natural seawater test medium. Concentrations were (in ppb): Ag - 2.8, Hg - <1, Cd - <1, As - <20.

Another phase of this experiment is to determine oxygen consumption rates of scallops exposed to these same metals at the estimated LC_5 and LC_{25} concentration values as determined from the toxicity tests above. This phase of the experiment has just recently been initiated and no data are available.

Chronic Studies

Oysters, *Crassostrea virginica*, and Hard Clams, *Mercenaria mercenaria*

Oysters, *Crassostrea virginica*, and hard clams, *Mercenaria mercenaria*, were exposed to silver nitrate at concentrations of 0.01, 0.05 and 0.1 ppm for 30 days and then kept in clean water for an additional 30 days for recovery. During this 60-day period samples were taken at 30 and 60 days by the Physiological Effects Subtask for studies of changes in oxygen consumption rate and the data derived will be summarized by that Subtask in the following section.

Oysters were also exposed to cadmium and silver in separate experiments for the Mutagenics Effects of Pollutants Task. Oysters were exposed to cadmium for two weeks at 0.05 and 0.1 ppm and to silver for one month at 0.5, 1.5, 2.5, 3.5 and 4.5 ppb. These animals were sampled at intervals and examined for mutagenic damage.

Rock Crabs, *Cancer irroratus*

Rock crabs, *Cancer irroratus*, were exposed for 30 days to cadmium chloride and cadmium nitrate in separate experiments for biochemcial testing and the results will be discussed in the Biochemical Effects Subtask which follows.

Cunners, *Tautogolabrus adspersus*

Cunners, *Tautogolabrus adspersus*, were exposed to cadmium as cadmium chloride at 0.05 and 0.1 ppm for 60 days in one test. Exposed fish were removed at 30 and 60 days for physiological examination.

Striped Bass, *Morone saxatilis*

Four chronic tests with striped bass, *Morone saxatilis*, have been completed. Bass were exposed to cadmium at 0.5, 2.5 and 5 ppb for 90 days in two tests and were left for 30 days in clean water. Bass were also exposed to mercury in one test at 1, 5 and 10 ppb for 120 days and then left in clean water for an additional 30 days. Exposed fish were removed at 30, 60, 90, 120 and 150 days and examined for respiratory changes, biochemical changes and metal uptake. Representative samples were fixed for histopathological examination by the Oxford laboratory.

A fourth experiment to determine immune response of bass exposed to cadmium at 5 and 10 ppb for 60 days was set up and completed for the Fish Immunology Subtask.

About 1000 bass were exposed in these experiments and the results will be discussed elsewhere in this report.

Blue Crabs, *Callinectes sapidus*

Blue crabs, *Callinectes sapidus*, were exposed to cadmium at 0.1, 0.5 and 1.0 ppm in two tests, one of 30 days and the second of 60 days' duration. These experiments were conducted primarily for histopathological examination by the Oxford laboratory and the results are summarized in a later section of this report.

Lobsters, *Homarus americanus*

Lobsters, *Homarus americanus*, were exposed to mercuric chloride and cadmium chloride in six individual tests. They were exposed to each of these metals in two tests of 30 days' duration and one of 60 days. In all tests lobsters were exposed to metal concentrations of 3 and 6 ppb, which is a level near that found in certain polluted areas of the natural environment. These lobsters were exposed for studies of changes in osmoregulation and oxygen consumption rates, Na-K ATPase, heart transaminase, metal uptake and mutagenic effects. During the course of these experiments over 600 lobsters less than one pound in size were exposed. The lobsters were distributed to five different subtasks for these studies.

Winter Flounder, *Pseudopleuronectes americanus*

A multidisciplinary experiment was initiated using the winter flounder, *Pseudopleuronectes americanus*, as the test organism and cadmium (as cadmium chloride) as the test pollutant. This experiment was conducted from December 1974 to April 1975 using approximately 180 animals in three chronic exposure diluter systems. The test concentrations were 0, 5 and 10 parts per billion as cadmium ion and each test was of 60 days' duration.

The following Tasks or Subtasks participated in this experiment:

Physiological Effects of Pollutant Stress Task:

Physioecology Subtask - A. Calabrese

This Subtask set up the experiment, maintained all systems and distributed the exposed animals as necessary.

Physiological Effects Subtask - F. Thurberg.

This Subtask examined fish for any possible cadmium-induced changes in gill-tissue oxygen consumption using a Gilson Respirometer (F. Thurberg). Fish were sampled from each of the three diluter systems as required. Also, blood samples were taken from the above fish and measurements of hematocrit, hemoglobin, osmolality and osmotic fragility were made and other hematological tests were conducted as appropriate (M. Dawson).

Biochemical Effects Subtask - E. Gould

This Subtask examined transaminase (AAT) activity in heart and liver, with and without Mg challenge *in vitro*; glucose-6-phosphate dehydrogenase (G6PdH) in liver; Mg-linked malic enzyme (ME) in skeletal muscle; and carbonic anhydrase (CA) in male gonadal tissue. AAT reflects demand on energy reserves, and in metal-stressed animals, may show lowered K_m (molecular change, more efficient activity with low substrate concentration). G6PdH reflects activity of glycolytic shunt pathway (direct oxidative), that provides pentoses for nucleic acids and nucleotides (biogenetic activity, energy turnover) and also provides reduced NADP (as does malic enzyme) for several biosynthetic processes. ME reflects sensitivity to divalent cations (metals/Mg) and provides reduced NADP. And CA is a Zn enzyme particularly susceptible to attack by Cd.

Disease and Environmental Stress Task:

Immunity in Marine Fish Subtask - R. Robohm

Thirty winter flounder exposed to 0, 5 or 10 ppb cadmium for 30 days were injected intraperitoneally with a bacterial suspension in Freund's complete adjuvant. Thirty control fish, under identical cadmium exposure, were injected with Freund's complete adjuvant alone. After an additional 30 days of cadmium exposure, the fish were bled and the serum examined for cadmium-caused differences in antibody titer using bacterial agglutinin tests. Hematocrit levels were also tested in cadmium-exposed and non-exposed fish.

Fish Pathology Subtask - R. Murchelano

Winter flounder tissues (gill, liver, spleen, kidney, gut, and muscle) were prepared for histopathological examination.

Environmental Microbiology and Chemistry Task:

Environmental Chemistry Subtask - R. Greig

This Subtask determined uptake of cadmium by liver, gills, gonads, blood and flesh of flounder. In addition, sodium, potassium, and magnesium were analyzed in the blood of these same fish. All samples analyzed by atomic absorption spectrophotometry.

Mutagenic Effects of Pollutants Task: - A. Longwell

This subtask spot-checked gonadal material taken from flounder for mutagenic effects of cadmium.

PHYSIOLIGICAL EFFECTS OF POLLUTANT STRESS - PHYSIOLOGICAL EFFECTS SUBTASK

F. Thurberg, M. Dawson and J. MacInnes

Physiological studies during the last twelve months have been directed toward an examination of marine animals exposed for extended periods of time to cadmium and mercury. Lobsters, striped bass, flounder and cunners were examined after 30-to 120-day exposures in our exposure laboratory. In addition, a comprehensive examination of the effects of silver on all principal life stages of the surf clam was completed. Detailed descriptions of these studies follow. Several red-tide toxin studies were also completed and prepared for publication. The balance of the year was spent constructing a new laboratory for respiratory studies of swimming marine animals, and in routine animal collection and maintenance duties.

Respiratory Response of Larval, Juvenile, and Adult Surf Clams, *Spisula solidissima*, to Silver

Larval, juvenile, and adult surf clams, *Spisula solidissima*, were exposed to sublethal concentrations of silver. At the end of each exposure period, oxygen consumption measurements were made on clams from each of these life stages. Silver-induced elevations in oxygen consumption were observed for each life stage. Valve-movement studies were conducted on adult clams, and more rapid rhythmic activity was observed in exposed clams than in control animals. Silver-uptake studies demonstrated rapid accumulation of silver in gill and body tissues, with gills concentrating four times as much silver as other body tissues.

Respiratory Studies:

The mean length of larvae and their corresponding respiration rates are shown in Figure 37. Two regression lines, one for the controls, the other for animals exposed to 50 ppb silver, are expressed by the following equations, respectively:

$$y = -3.768 + 0.085x \text{ and}$$

$$y = 0.181 + 0.082x$$

where

x = mean length of larvae (μ)

y = respiration rate (μl O_2/hr/1000 larvae).

The correlation coefficients of both equations are identical (0.74). According to the analysis of covariance of the regression lines, the slopes of the lines do not differ significantly from each other, but the elevations of the lines do differ. This finding indicates that the experimental larvae respired at a higher rate throughout the larval stage and the difference between the exposed and control larvae was constant. Calculation from the equations showed that when the larvae reached the length of 150μ, the controls respired at 9.4 μl O_2/hr/1000, a rate slightly higher than that reported for oyster larvae of the same size. The silver exposed larvae respired at 12.5 μl O_2/hr/1000 or about 1/3 higher than the controls.

The oxygen consumption rates of juvenile surf clams exposed to silver are presented in Figure 38. The mean oxygen consumption rate increased with increasing silver concentration. Statistical analysis

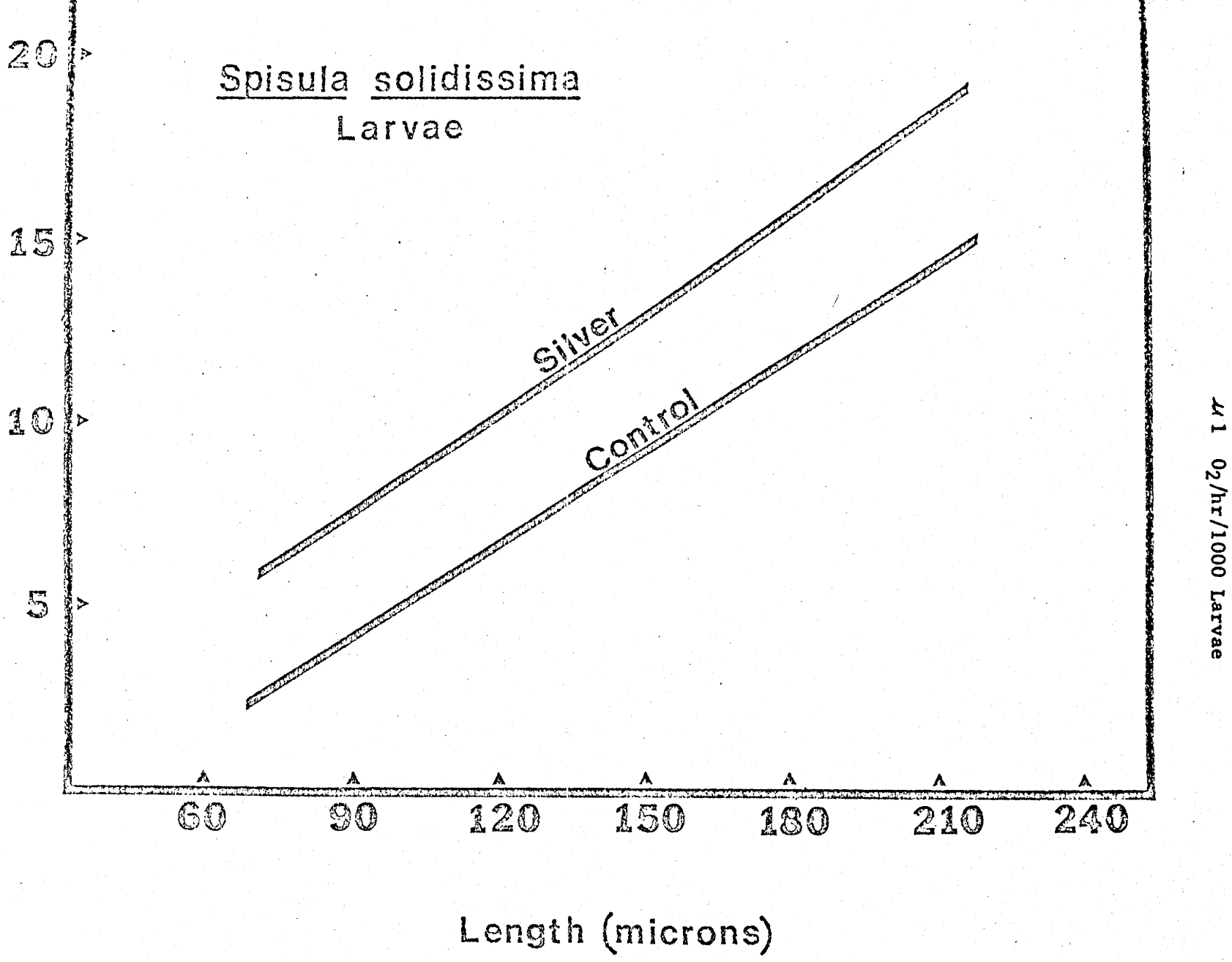


Figure 37. Spisula solidissima Larvae. Oxygen Consumption Regression Lines of Larvae Grown in Cultures Containing 50 ppb Ag and in Control Cultures.

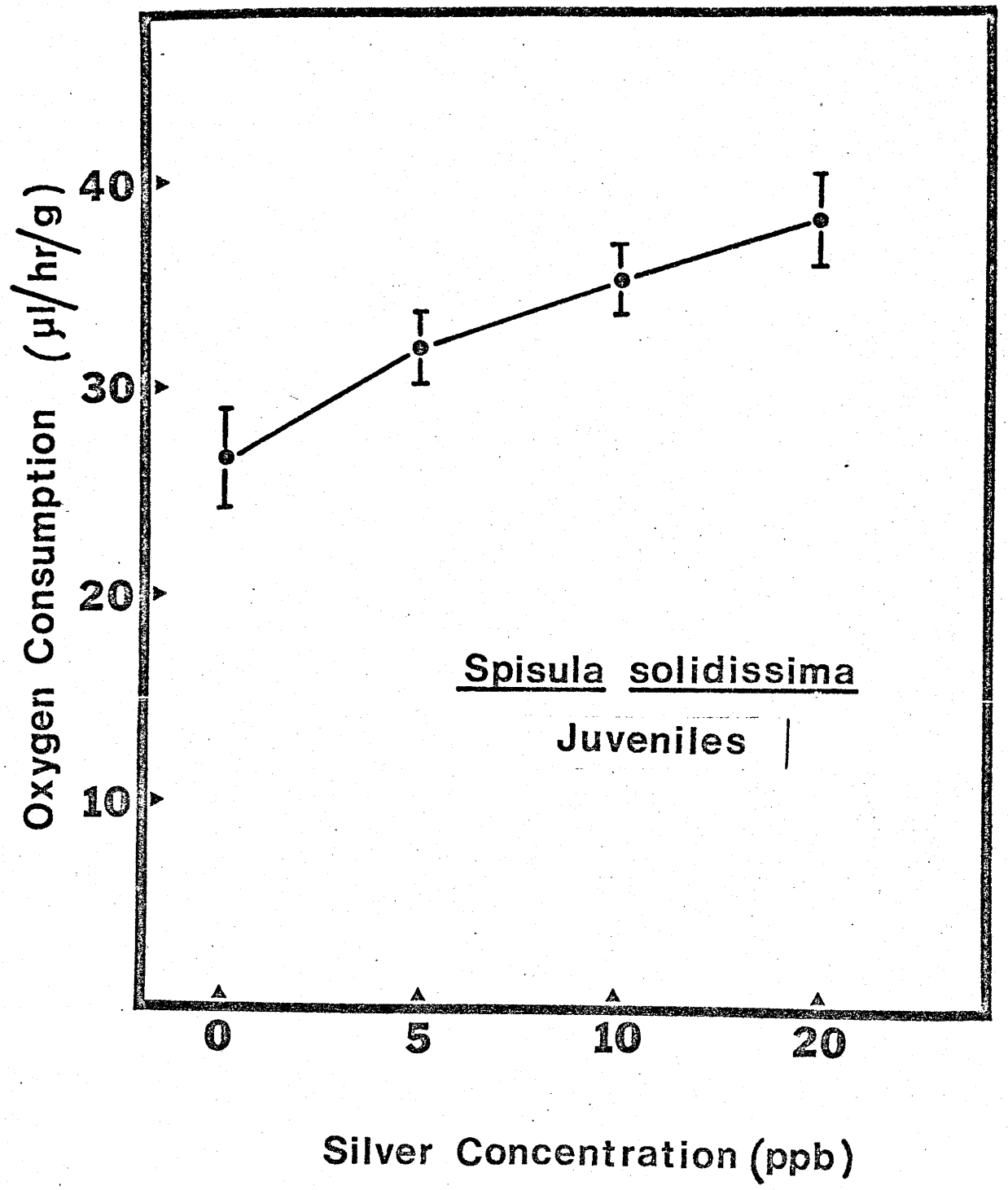


Figure 38. Spisula solidissima Juveniles. Effect of Silver on Oxygen Consumption. Each Point Represents the Mean Oxygen Consumption Value of 16 Clams. Bars: Standard Error.

by Duncan's multiple range test indicates that animals exposed to 10 and 20 ppb Ag respired at significantly higher rates than controls. There was no significant difference between the rates of controls and animals exposed to 5 ppb Ag.

Adult gill-tissue oxygen consumption values are shown in Figure 39. Once again, the mean oxygen consumption rate increased with increasing silver concentration. Duncan's multiple range test shows that gill tissue from animals exposed to 50 and 100 ppb Ag exhibit significantly higher oxygen consumption rates than do controls. There was no difference between the rates of controls and animals exposed to 10 ppb Ag.

Oxygen consumption was elevated in the presence of silver in all three life stages tested. This increased oxygen consumption suggests energy expenditure in response to silver-induced physiological alterations.

The respiratory data obtained here are consistent with the elevated oxygen consumption rates reported for four other silver-exposed bivalves but differ from the decreased consumption rates reported for a silver-exposed gastropod mollusk, Nassarius obsoletus. These two opposite effects of silver on bivalve and gastropod respiration, as well as the recent observations of other researchers, serve to emphasize that metal-induced physiological alterations may differ greatly. These alterations differ with the species, the life stage, and the metal used, as well as with such experimental conditions as temperature, salinity, and pH. Considerable caution, therefore, should be exercised in extrapolating the results of one study to other animals, other metals, and other conditions.

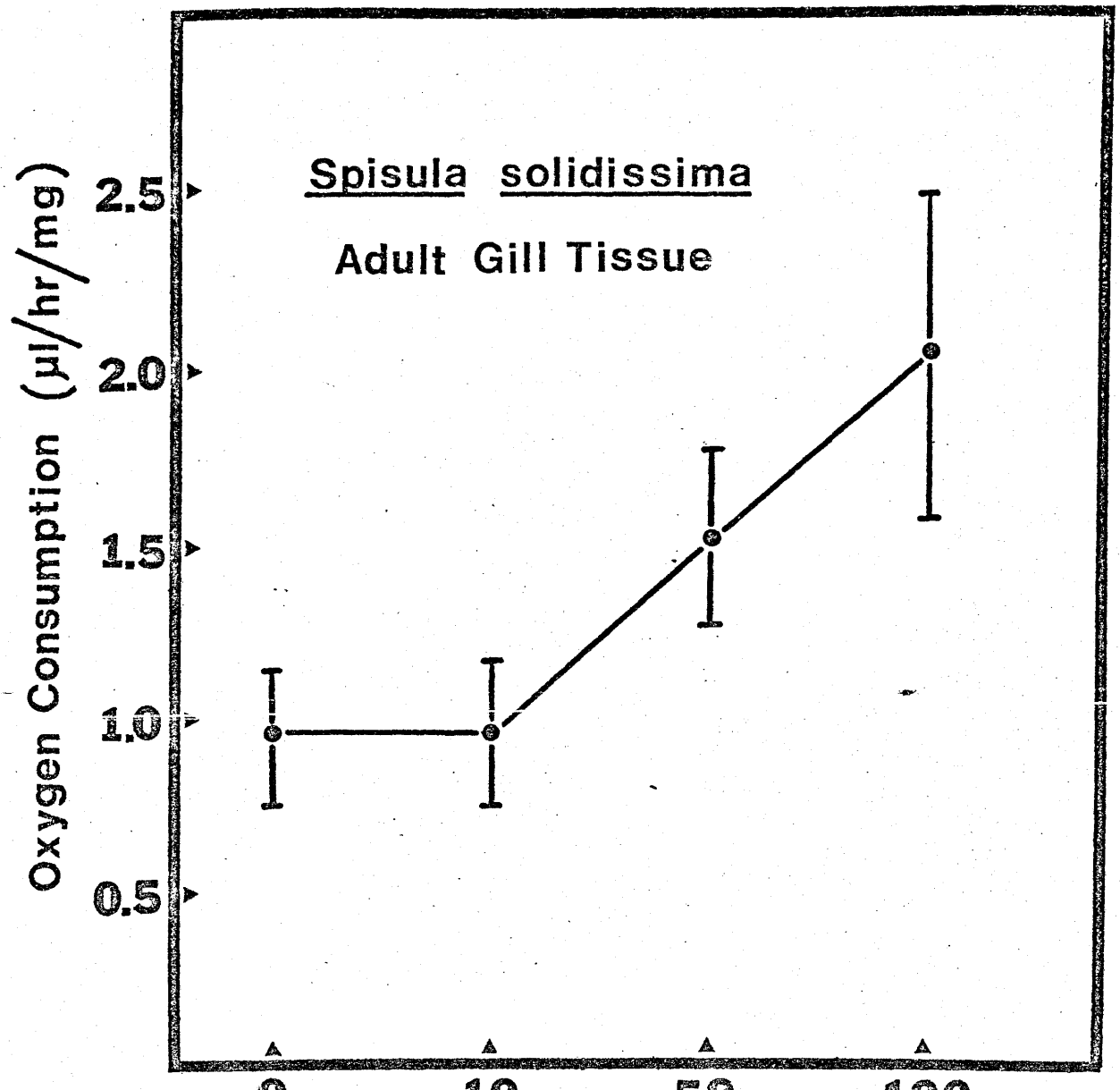


Figure 39. Spisula solidissima Adult Gill Tissue. Effect of Silver on Oxygen Consumption. Each Point Represents the Mean Oxygen Consumption Value of 16 Gill Tissues. Bars: Standard Error.

Silver Uptake Studies:

The results of the silver uptake studies are shown in Table 22. The concentrations of silver in the tissues increased with increasing silver concentrations up to 50 ppb. There was no difference in tissue concentration of silver between 50 and 100 ppb exposures. The gills concentrated significantly higher levels of silver than the bodies, an observation consistent with the findings of Thurberg et al. (1974), who examined gills and bodies of silver-exposed oysters (*C. virginica*) and hard clams (*M. mercenaria*). These results again demonstrate that although considerable individual variations occur, many bivalves rapidly accumulate high levels of silver in their body tissues.

Valve Movements:

Valve movements of 8 control clams and 8 clams given a 96-hour exposure to 50 ppb silver were recorded over 4-to 6-hour periods. Both exposed animals and controls adapted quickly to the recording apparatus and began a rather stable rhythmic pattern of movements within an hour. Exposed animals averaged 24.6 movements, or partial closures, per hour. This is significantly higher than the mean rate of 12.0 movements per hour for controls. Figure 40 is a portion of a recording from a control and an exposed clam run simultaneously.

Rhythmic shell movements in bivalves are closely related to respiration and filtration. Salánki (1965) obtained an increase in the rate of shell movements upon exposure of the freshwater mussel, *Anodonta cygnea*, to

Table 22. *Spisula solidissima* Silver Uptake. Concentration of Silver in Body and Gill Tissues after a 96-hr Exposure to Silver. Mean Concentrations were Determined from Three Exposure Studies with Four Clams Examined per Concentration per Study.

Exposure ppb Ag	Silver Concentration in ppm Wet Weight			
	Body Minus Gills		Gills	
	Mean	S. D.	Mean	S. D.
Control	0.08	0.03	< 0.63	-
10	1.01	0.84	5.73	3.19
50	2.23	0.90	8.72	2.44
100	2.00	1.01	8.52	3.39

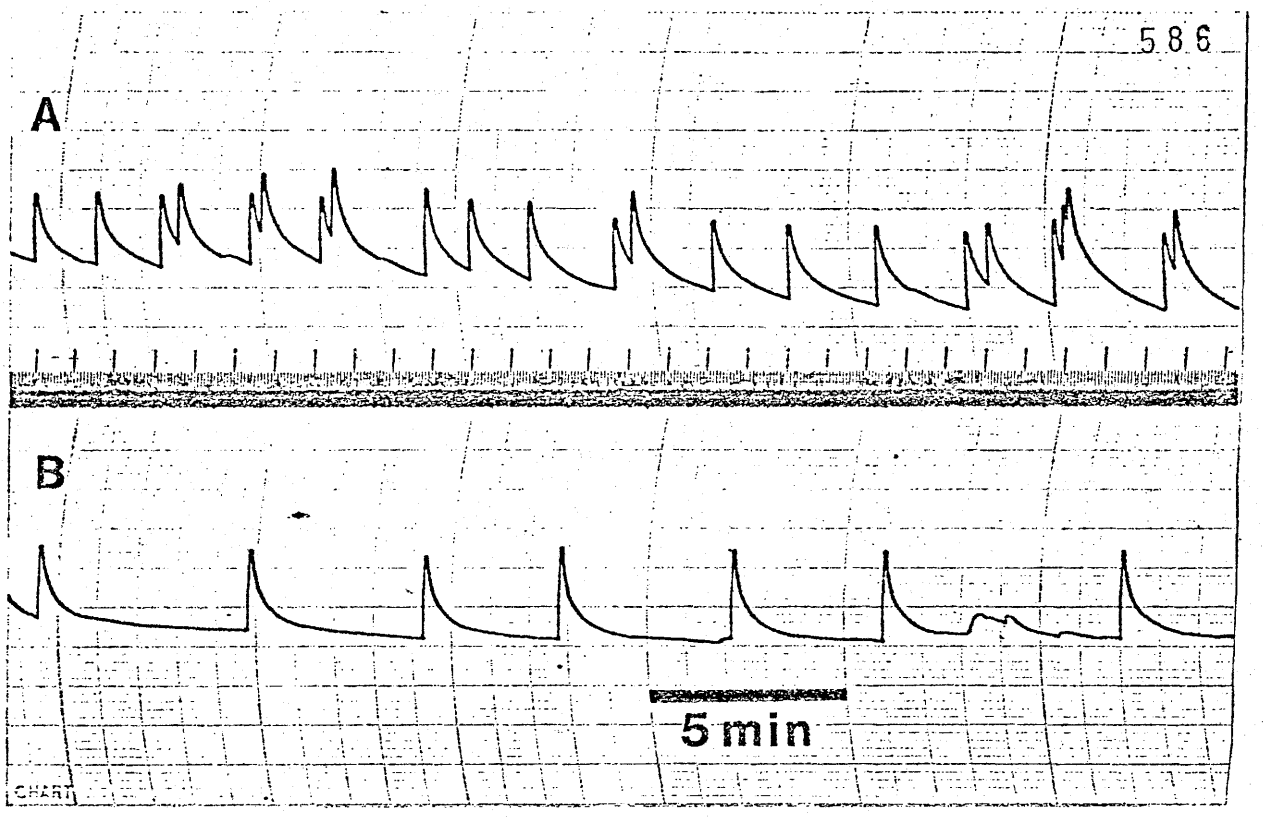


Figure 40. Spisula solidissima Valve Movement. A: Rhythmic Valve Movement of Clam Exposed to 50 ppb Ag. B: Same for Movement of Control Clam.

oxygen-deficient water. Exposure to cadmium produced effects similar to lack of oxygen. Similarity of effects from lack of oxygen and exposure to cadmium led Salánki to surmise that cadmium acted on the respiratory enzymes to produce an oxygen deficit in the exposed animals. The increased respiration noted in the present study also supports the concept of an oxygen deficit.

Heavy metals affect marine animals in a variety of ways. They disrupt enzymes systems, cause tissue and organ damage resulting in abnormal function and provoke behavioral changes. The action of silver on aquatic animals has received little attention, however, and its mechanism of action is poorly understood. The increased metabolic activity of surf clams exposed to silver demonstrates physiological disruption and an abnormal expenditure of energy. Further biochemical and physiological studies should be conducted to understand more fully the mechanisms of this alteration.

Striped Bass - Effects of Cadmium and Mercury

Striped bass, *Morone saxatilus*, were exposed to $HgCl_2$ at concentrations of 1, 5, and 10 ppb Hg for periods of 30, 60, 90 and 120 days and allowed to recover for 30 days following the 120-day exposure (see Physioecology Subtask report). Gill tissue was removed from these fish for oxygen consumption measurements. No significant difference was found between controls and fish exposed to 1 ppb Hg regardless of length of exposure. Bass exposed to 5 ppb Hg exhibited depressed respiration

after 30 days, returned to control levels by 60 days and maintained that level during longer exposures. Fish exposed to 10 ppb Hg had a significantly decreased respiration rate after 30 and 60 days exposure. After 90 days and after the recovery period the respiration rate returned to normal, although fish exposed for 120 days respired at a higher rate than controls. The data are summarized in Table 23.

In a similar study striped bass were exposed to $CdCl_2$ at concentrations of 0.5, 2.5, and 5.0 ppb Cd for periods of 30 and 90 days and allowed to recover for 30 days following the 90-day exposure. Measurements were made of gill tissue oxygen consumption. There was no significant difference between controls and animals exposed to 0.5 ppb Cd for any length of time. Animals exposed to 2.5 and 5.0 ppb Cd showed a significant drop in respiration compared to controls at 30 days. The respiration of animals tested after 90 days exposure to 2.5 and 5.0 ppb Cd had returned to control values. There was no significant difference between any exposed group and the controls after the 30-day recovery period. The average respiratory rates for all groups are presented in Table 24.

Cunners - Effects of Cadmium

The cunner, *Tautogolabrus adspersus*, was exposed to cadmium for 30 and 60 day periods (see Physioecology Subtask report) as a follow-up study to the recently published Center cooperative study using this fish. The results show at least a one-third decrease in gill tissue respiration in fish exposed for 30 or 60 days to 0.1 ppm Cd. These results are

Table 23. Respiratory Rates of Striped Bass Exposed to Mercury for Various Lengths of Time.

	Control	1 ppb Hg		5 ppb Hg		10 ppb Hg	
Exposure Time	$\mu l\ O_2$/mg/hr	$\mu l\ O_2$/mg/hr	% Control	$\mu l\ O_2$/mg/hr	% Control	$\mu l\ O_2$/mg/hr	% Control
30 days	1.74 ± 0.24	1.55 ± 0.21	89	1.10 ± 0.02	62	0.97 ± 0.04	54
60	0.86 ± 0.04	0.79 ± 0.04	92	0.85 ± 0.04	98	0.76 ± 0.04	88
90	0.77 ± 0.04	0.75 ± 0.03	97	0.73 ± 0.03	95	0.89 ± 0.05	115
120	0.68 ± 0.04	0.67 ± 0.03	99	0.79 ± 0.04	116	0.95 ± 0.06	140
120 + 30	0.82 ± 0.07	0.84 ± 0.06	102	0.93 ± 0.05	113	0.99 ± 0.04	120

Table 24. Respiratory Rates of Striped Bass Exposed to Cadmium for Various Lengths of Time.

	Control	0.5 ppb Cd		2.5 ppb Cd		5.0 ppb Cd	
Exposure Time	$\mu l\ O_2$/mg/hr	$\mu l\ O_2$/mg/hr	% Control	$\mu l\ O_2$/mg/hr	% Control	$\mu l\ O_2$/mg/hr	% Control
30 days	1.92 ± 0.11	1.59 ± 0.08	83	1.41 ± 0.31	73	1.38 ± 0.09	76
90	0.88 ± 0.08	0.81 ± 0.02	92	0.80 ± 0.03	91	0.75 ± 0.03	86
90 + 30	0.81 ± 0.06	0.83 ± 0.04	102	0.83 ± 0.04	102	0.87 ± 0.04	107

consistent with those obtained from fish in the above mentioned study exposed for a 96-hour period. Blood serum osmolality was not altered after 30 or 60 day exposures to 0.05 and 0.1 ppm Cd.

Lobsters - Effects of Cadmium and Mercury

Lobsters, *Homarus americanus*, were examined for changes in gill-tissue oxygen consumption and blood serum osmolality after exposure to either cadmium chloride or mercuric chloride. These animals were exposed for 30 and 60 days to concentrations of 3 and 6 ppb (see Physioecology Subtask report). At the end of each exposure period, gill-tissues were dissected from each animal and placed in 15-ml, Warburg-type glass flasks. Oxygen consumption measurements were made over a four-hour period in a Gilson Differential Respirometer. The effects of cadmium on oxygen consumption are shown in Figure 41. Cadmium induced elevations are apparent after 30-day exposures to both 3 and 6 ppb Cd. After 60 days no significant differences were noted, although a trend toward increased oxygen consumption may have been significant if more numbers were available. The 60-day test had 1/3 the number included in the 30-day experiments. No significant differences were noted in lobsters exposed for 30 or 60 days to 3 and 6 ppb mercury. Osmogulatory function was not altered in lobsters exposed to either cadmium or mercury.

Brett Respirometers

This year we obtained three Brett Cruising-Speed Respirometers with seven-inch diameter swimming chambers capable of holding swimming fish 12-15 inches in length. These units will allow us to monitor oxygen

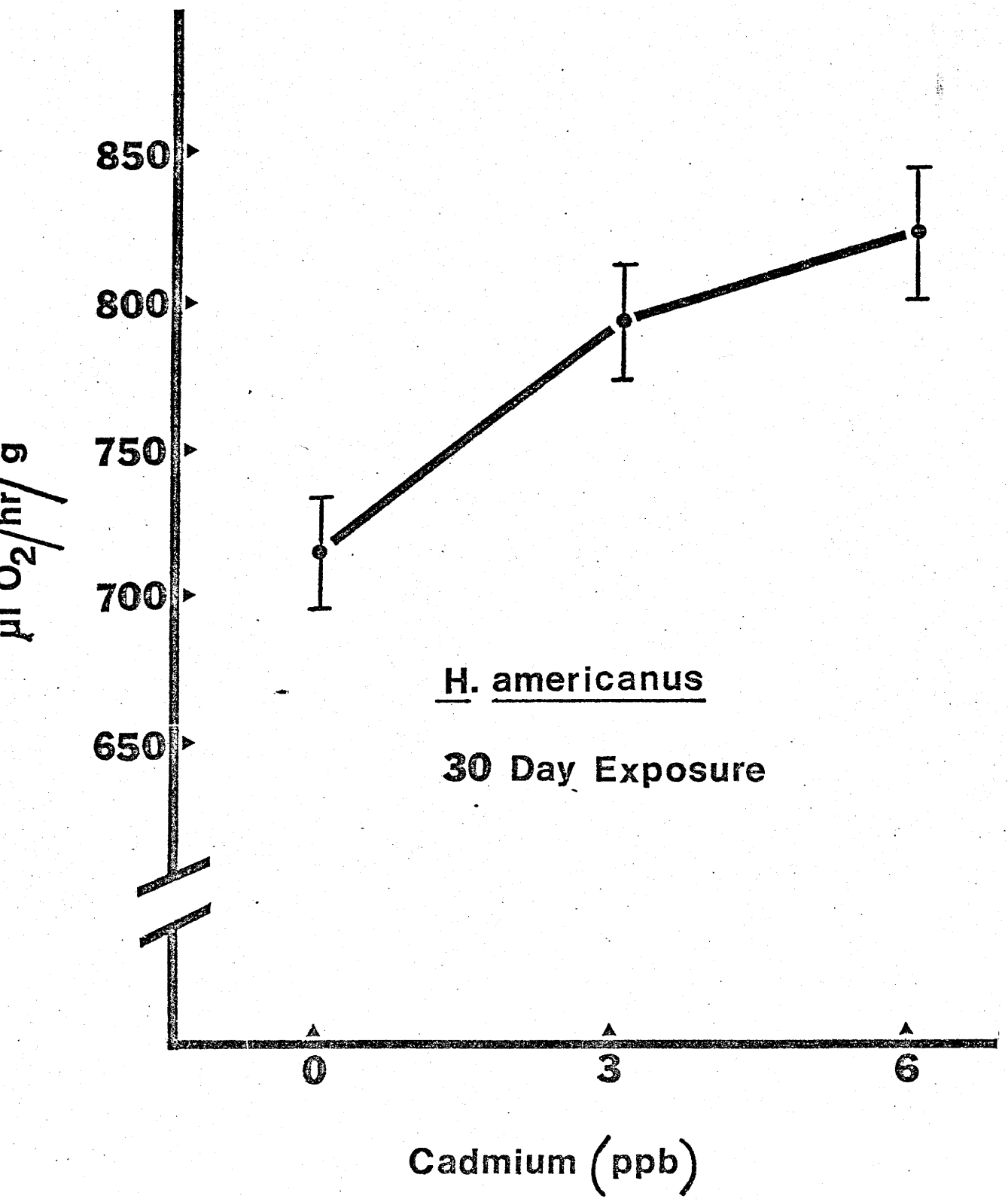


Figure 41. Respiratory Rate of Lobsters Exposed to Cadmium for 30 Days.

consumption of large-whole animals under static as well as swimming conditions, and will be used in our program of pollution studies to compare control and pollutant-stressed animals. We have installed the units in a recently constructed laboratory in the garage and have conducted a number of preliminary tests with striped bass and winter flounder. Studies with striped bass will start again in April of 1975 when fish become available. These studies will include electrode implants to monitor opercular movement and heart rate, in addition to oxygen consumption and swimming performance measurements. Two physiologists from the University of Massachusetts will also be conducting cooperative research with these respirometers next summer.

Flounder - Effects of Cadmium

Winter flounder, *Pseudopleuronectes americanus*, were exposed to cadmium for 60 days. At the end of this period measurements of gill-tissue oxygen consumption and blood hematology were completed. Gill tissue respiration was depressed after exposure to 5 and 10 ppb Cd; a finding consistent with bass and cunners exposed to this metal.

Blood samples collected from many of these same flounder were subjected to a variety of hematological tests. Red blood cells were counted using Henricks Solution. Plasma osmolalities were measured on heparinized blood. Plasma proteins were measured as percent dissolved solids using a Bausch and Lomb refractometer and hematocrits were

measured on heparinized samples. Hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemaglobin concentration will be analyzed when the standards are obtained. These standards have been on order for more than two months. The results of the hematological work thus far are summarized in Table 25.

Table 25. Summary of Hematological Studies of Winter Flounder Exposed to Cadmium.

	Cadmium Concentration								
	0		n	5 ppb		n	10 ppb		n
	Mean	S.E.		Mean	S.E.		Mean	S.E.	
Hematocrit (% packed red cells)	34.6	1.09	34	34.1	0.97	32	32.9	1.05	32
R.B.C. (10^6 cells/mm^3)	2.90	0.17	17	2.69	0.12	10	2.96	0.18	14
Plasma Protein (% disolved solids)	6.7	0.24	34	6.7	0.25	30	6.8	0.23	31
Plasma Osmolality (mOsm)	401	6.7	22	380	7.3	21	401	5.3	19

PHYSIOLOGICAL EFFECTS OF POLLUTANT STRESS - BIOCHEMICAL EFFECTS SUBTASK

E. Gould

Research continued this year on the biochemical response of marine animals to heavy metals in their environment. Three series of experiments were conducted to determine whether the nature of readily soluble salts of cadmium and silver influences the effect of the metal itself on the animal (it does). Several series of multidisciplinary experiments were performed, with animals exposed to sublethal amounts of metal for a month or more; in these, biochemical changes were more subtle, and we sought to discover whether sublethal metal challenge can induce change in the reactive capacities of enzyme systems (it can).

Specifically, testing was completed on an experimental series using the rock crab, Cancer irroratus, exposed to cadmium either as the chloride or as the nitrate, either for 4 days (acute-static) or for 30 days (chronic). A stress-indicator enzyme in heart preparations, aspartate aminotransferase (AAT), demonstrated that the "cadmium effect" was different for the two salts, a phenomenon we call the "anion effect." We used a coupled assay whose principle is the change in oxidation state of a pyridine nucleotide, with conditions made optimal for crustacean heart preparations. Wavelength was 340 nm, and unit of activity was taken as the change of 0.001 in absorbance per minute per mg protein.

For the acute-static experiments:

	AAT (in Rock Crab Heart)		
	$\bar{x}$ ±	S.E.	Level of Significance
Controls	1784.	204.	
1 ppm Cd as Cl	2963.	422.	P <.01
1 ppm Cd as (NO_3)	1510.	274.	N.S. P <.001

More mortalities occurred in crabs exposed to cadmium as chloride than in either the control group or the cadmium nitrate group.

A series of chronic experiments using sublethal amounts of cadmium corroborated the acute-static findings: AAT activity is elevated in the hearts of crabs exposed to cadmium as chloride, in contrast to a very slight depression with cadmium as nitrate. The elevation ($CdCl_2$) was significant at .001 level, whereas the cadmium nitrate produced data that were statistically different from the $CdCl_2$-exposed crabs (P <.001), but not from the controls.

The observations above were subsequently confirmed in a teleost, using two salts of silver. Acute-static studies were performed on the cunner, Tautogolabrus adspersus, exposed to silver, either as the nitrate or as the acetate, both easily soluble salts. Liver AAT was not significantly changed in fish exposed to silver nitrate (a slight depression) nor in fish exposed to the acetate (a very slight elevation), but the difference between the nitrate-exposed and the acetate-exposed fish was significant:

	AAT (in Cunner Liver)		
	$\bar{x}$ ±	S.E.	Level of Significance
Controls	523.	33.	
1 ppm Ag as (NO_3)	462.	24.	N.S.
1 ppm Ag as ($C_2H_3O_2$)	555.	29.	N.S. $P < .05$

Heart AAT showed the same pattern. In the livers of control fish, glucose-6-phosphate dehydrogenase (G6PdH), a magnesium-linked glycolytic enzyme involved in energy mobilization, did not differ significantly from either of the groups of exposed fish, nor was there an anion effect.

Biochemical changes brought about by exposure to sublethal amounts of heavy metals are not always apparent. We have found, however, that in several cases the *in vitro* addition of micromolar amounts of a metal salt (usually magnesium) to an enzyme assay medium will provide clues to altered enzymatic catalytic properties in the exposed animals. In a series of chronic exposures of the lobster, *Homarus americanus*, to 3 and to 6 ppb $CdCl_2$, there was no significant difference in heart AAT. In heart preparations containing high concentrations of magnesium (110 m*M*), however, AAT activity was depressed in control animals, and was less affected by the *in vitro* Mg^{2+} in the exposed animals. To a lesser extent, the same phenomenon was observed in lobsters exposed to sublethal amounts of mercury:

	AAT (in Lobster Heart)				
	Water Preps ($\Delta A \times 10^3$/min./mg prot.)		110 m$\underline{M}$ Mg^{2+} Preps (% original activity)		
	$\bar{x}$	± S.E.	$\bar{x}$	± S.E.	Level of Significance
Controls	1048.	43.	75.9	4.4	N.S.
3 ppb $CdCl_2$	1006.	45.	90.8	3.6	
6 ppb $CdCl_2$	1044.	69.	105.6	3.9	$P < .001$
Controls	1182.	57.	71.7	3.6	N.S.
3 ppb $HgCl_2$	1021.	21.	71.7	3.6	
6 ppb $HgCl_2$	1003.	79.	83.7	3.5	$P < .05$

These observations point to metal-induced changes at the subcellular level. Low concentrations of free magnesium ion have been found to lower the K_m of a purified enzyme (that is, Mg^{2+} enabled the enzyme to achieve half maximal velocity at substrate concentrations lower than is possible in the absence of Mg^{2+}); magnesium also potentiates many enzyme systems, and suppresses others. Several lobsters were examined for serum magnesium, and (surprisingly) showed no difference between control and exposed animals; the change, then, was not brought about by a stress- or metal-induced elevation of magnesium _in vivo_. The increased reactivity of AAT in metal-exposed lobsters to high concentrations of assay Mg^{2+} can therefore reasonably be attributed to heightened receptivity of the enzyme to substrate, a molecular alteration brought about by the animals' exposure to cadmium or mercury.

In chronic exposures of cunners to cadmium chloride, AAT was significantly depressed in the livers of exposed fish, more so in magnesium preparations (25 m$\underline{M}$). AAT of both heart and skeletal muscle remained unchanged. Liver G6PdH, however, was very significantly depressed ($P < .02$ at 0.5 ppb Cd, and $< .001$ at 1.0 ppb Cd).

A series of exposures of striped bass, Morone saxatilis, for 30, 60, and 90 days elicited no significant changes in liver AAT or G6PdH, either for $CdCl_2$ or $HgCl_2$. The only significant data were from fish held 30 days in clean seawater after 90 days' exposure to sublethal amounts of $CdCl_2$: liver AAT was depressed ($P < .001$) in cleared fish after 5 ppb Cd, and in Mg^{2+} preparations (25m$\underline{M}$), an increased catalytic activity was found in cleared fish after 2.5 ppb Cd ($P < .05$) and in cleared fish after 5 ppb Cd ($P < .01$). Liver G6PdH was depressed in cleared fish after 5 ppb Cd ($P < .001$). An analogous series of experiments with $HgCl_2$ produced no significant changes. This anadromous fish appears to adapt very successfully to sublethal amounts of metal ions in its environment; as a result, it is not a good test animal for physiological and biochemical research on heavy metals, although its rapid metabolic turnover makes it an ideal fish for immunological studies.

MUTAGENIC EFFECTS OF POLLUTANTS

A. C. Longwell, S. S. Stiles and J. B. Hughes

That some contaminants, as heavy metals and pesticides, can be mutagenic for a variety of non-marine forms has been known for years. The significance of this though for fish eggs in nature has not been appreciated, and implications for the commercial fisheries have not been considered.

General cytological disturbances in fertilized cleaving American oyster eggs of the sort that accompany more specific genetic effects were found for four different heavy metals - silver, cadmium, arsenic and manganese. These were often at levels below the LC_0 for two-day-old straight-hinge larvae (see Middle Atlantic Coastal Fisheries Center Informal Report No. 26, reporting period 1972-1973). Since this initial work, a four-phase program has been developed to appraise the likely mutagenic effects of some of the major marine contaminants. In a cyto-genetic approach chromosome aberrations, derangement of the chromosome division apparatus, and cyto-toxic effects which also often lead to genetic disturbances are scored. This is after either experimental chronic exposure of adults prior to, during or after gametogenesis, or following acute short-term exposure of spawned eggs to suspect chemicals. A second approach is the study of the chromosome figures during gametogenesis in adults given chronic low-dose exposures. Cytological abnormalities of the type leading to genetic aberrations are observable in gametogenesis to greater or lesser degrees in various

organisms. A third approach is the dominant lethal gene test in a genetically-oriented study of embryo and larval mortality. Usually, exposure is to the adults and their progeny studied, and is low-dose and chronic. However, short-term exposure of gametes can also be tested in this way. The fourth approach just recently adopted is field- instead of experimentally-oriented and consists of the cyto-genetic appraisal of chromosome abnormalities and mitotic disturbances in fish eggs taken from polluted water.

Experimental results to date have demonstrated a high sensitivity of the cyto-genetic test. Exposure of spawned, developing oyster eggs to silver and cadmium have been shown to be mutagenic. This is at concentrations well below those no longer causing any lethality of the first-stage larvae. Chronic exposure prior to or during gametogenesis had a more severe effect. Concentrations of metals giving genetic effects are low enough for results to have implications for the fisheries.

A procedure has been developed for processing neuston- and bongo-sampled fish eggs for cyto-genetic study. Limited examination of the blastodiscs of eggs from some sample stations in the New York Bight shows chromosome and mitotic abnormalities of the same sort that can be experimentally induced by recognized mutagens. These occur at a significant frequency and show station variation. This is the first time eggs taken directly from a fishery or any neuston components have been so studied. An entirely new approach to assaying the quality of the marine environment is opened.

POLLUTANTS AND PHYTOPLANKTON FOOD-CHAIN SPECIES

Ravenna Ukeles, William Rose and Jean Bishop

Microscopic algae have a fundamental role in the productivity of the marine environment since they form the basis of the oceanic and estuarine food web. It should, therefore, be obvious that marine contaminants affecting microscopic algae can indirectly affect the total ecology of shallow and deeper waters. This can occur through an inhibition of normal algal population increases, hence result in a decrease of the food supply of a fishery. Contaminants may also stimulate growth of certain algae resulting in destruction of an aquatic area through eutrophication. Microscopic algae also may store toxic material which, when ingested by commercially valuable species, becomes a method by which small quantities of contaminants become concentrated to toxic levels.

This investigation is conducting experimental work on marine contaminants that fall into three different categories: non-viable inorganic, non-viable organic and viable sources of contaminants. In addition, this investigation is maintaining a service for other investigations in Experimental Biology. Algal cultures are being maintained in carboy cultures under controlled conditions and in open tanks to provide a source of food for adult animals and larval stages of species that are being studied in other marine contaminant investigations. This amounts to a total harvest of about 80,000 liters

annually. A culture collection of about 90 strains is also being maintained to serve as a source of starters for food cultures, as a source of supply for outside investigators, and as a source of experimental material for this investigation.

A high concentration of bacteria, apt to be found in polluted estuaries, constitutes a source of viable pollutants. Not much information is available on bacteria-algal interrelationships. There are some isolated reports in which bacteria are considered stimulatory to algae, although in two reports bacteria were considered responsible for algal mortality. It seems as if the result of bacterial-algal interactions must be a species-specific one. In this investigation a study was completed (manuscript now in press) on the effects of three marine bacteria--*Vibrio anguillarum* #19264, *V. anguillarum* #19109 and *Escherichia coli*--on the growth of 10 phytoplankters from different classes of algae. Of particular interest was the observation that *V. anguillarum* did not inhibit algal growth although these bacteria and many variant strains have been described as the causative agent in diseases of many fish. A disc method was used to evaluate the algal growth response. Growth enhancement of all algae in the presence of *V. anguillarum* #19264 occurred on an enriched agar medium, while other bacteria yielded variable responses (Table 26). Numerous experiments demonstrated that the growth enhancement of algae could only occur in the presence of viable actively metabolizing bacteria and was not a result of stimulation from excreted bacterial metabolites. The tentative hypothesis was formulated that growth enhancement was related to the release of algal-stimulating substances through bacterial hydrolysis of the agar.

Table 26. Radial Zones of Growth Enhancement of Algae Surrounding Discs After Three Weeks.

Algae	*V. anguillarum* #19264			*V. anguillarum* #19109			*E. coli*		
	live	heated	dried	live	heated	dried	live	heated	dried
Chlorella autotrophica	2 mm	0	2 mm	1 mm	0	0*	0	0	0*
Chlorophyte clone U-710	1.5 mm	0	0*	1 mm	0	0*	0	0	0*
Nannochloris sp	1 mm	0	1 mm	1 mm	0	0	0.5 mm	0	0.5 mm
Stichococcus bacillaris	1 mm	0	1 mm	1 mm	0	0	0.5 mm	0	0.5 mm
Tetraselmis maculata	2 mm	0	1 mm	0.5 mm	0	0	0	0	0
Platymonas sp	1 mm	0	1 mm	1 mm	0	0	0.5 mm	0	0.5 mm
Pyramimonas sp	4 mm	0	4 mm	0	0	0	2 mm	0	2 mm
Dunaliella euchlora	2 mm	0	2 mm	0.5 mm	0	0.5 mm	2 mm	0	2 mm
Nitzschia sp	0.5 mm	0	0*	0	0	0*	0.5 mm	0	0.6*
Phaeodactylum tricornutum	2 mm	0	2 mm	2 mm	0	0	2 mm	0	2 mm

* These discs were dried overnight at room temperature as contrasted with other samples which were stored at 0°C overnight.

Note that all central discs, paper discs to which nothing was added and discs to which growth medium was added always yielded a 0 growth response.

A systematic study was completed on the effects of algal growth of a homologous series of acids, alcohols and sugars (manuscript in preparation). Industrial effluents, as well as domestic sewage, often contain these simple acids, alcohols or sugars in varied concentrations. Investigating these compounds under controlled conditions can indicate the nature of the growth effect on individual species. Of 15 monohydric and polyhydric alcohols, 3 were widely inhibitory - allyl, pentanol and hexanol. Ethanol stimulated growth in several chlorophytes and, in fact, supported growth in the dark. Glycerol was often stimulatory in high concentrations. The 15 sugars studied were generally without effect on growth with the exception of glyceraldehyde and 2-deoxyglucose which in some species inhibited growth. Response to the acids varied considerably - thioglycolic, acrylic, butyric and caprylic acids were inhibitory to more than 50% of the species tested. Lactic acid stimulated growth of only Phaeodactylum tricornutum (of 13 species), whereas acetic, glycolic and pyruvic acids often stimulated growth.

Investigations into three different types of contaminants that fall into the category of inorganic contaminants are in progress. These are as follows: a) experimentation on adaptation to increasing salt concentrations, b) studies on the effects of the alkali metals lithium, rubidium and cesium on phytoplankter growth, and c) studies on the effects of the element selenium on growth of phytoplankters. Physical changes in land drainage patterns often bring about dramatic changes

of saline content in inland waters. The mechanism by which organisms adapt to these changes has long been a subject of interest. A chrysomonad flagellate that has been in laboratory culture on a medium with low salt content was selected for studies on adaptation. Tolerance to increasing salt concentration in the medium was achieved by a very gradual acclimatization to each salt concentration. The concentration of certain metals, e.g., iron, appears to play a role. Studies on the alkali metals reported in MACFC Informal Report No. 26 are continuing. This information should be useful in evaluation of effluents in industrial areas since lithium is often used in industry and recently has been employed in pharmaceuticals. The element selenium was identified as a potent toxic substance for cattle many years ago but attention is now being focused on its role as a micronutrient. Work on selenium is being carried out since information is not available on its role in the nutrition of phytoplankters or in the aquatic environment. Toxic levels for this compound are being determined. Preliminary experiments have shown some species to be stimulated by micromolar amounts of this element.

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PATHOBIOLOGY INVESTIGATIONS

CRUSTACEAN PATHOBIOLOGY

Phyllis T. Johnson

Chronic Exposure of *Callinectes sapidus* to Cadmium: Histopathology

During 1974, two chronic tests were carried out with *Callinectes sapidus* (blue crab) exposed to cadmium. The first was for 30 days (12 August - 9 September, 1974), and the second for 60+ days (11 October - 10 December, 1974). Survivors of the 30-day test were dissected at Milford; those of the 60-day test were transported back to Oxford in cooled containers and dissected there. In both series there were 9 control animals, and 9 each exposed at 0.1, 0.5, and 1.0 ppm $CdCl_2$. Crabs were kept in 20-gallon aquaria, 3 crabs per aquarium.

Juvenile female and small male crabs were used in both tests. The 30-day series utilized crabs collected in Long Island Sound, near Milford, while the 60-day-test crabs were trap-collected in Chincoteague Bay and shipped to Milford in cooled containers on September 20, 1974. The latter series was acclimated for 17 days before cadmium exposure.

Mortality during the tests was considerable in both series (Table 27). Exposure for 30 days to the highest concentration of $CdCl_2$ used (1.0 ppm), is not tolerated well by *Callinectes*, with only 2 of 9 animals surviving. At 60 days, all crabs kept in 1.0 ppm $CdCl_2$ died,

Table 27. *C. sapidus*, Chronic Exposure to Cadmium Chloride Mortality

	30-day exposure	60-day exposure
Controls	7/9[1]	5/9
Test:		
0.1 ppm	4/9	2/9
0.5 ppm	6/9	8/9
1.0 ppm	7/9	9/9

[1]no. of deaths/no. exposed

and only 1 of 9 crabs exposed to 0.5 ppm survived. Although numbers are too small to allow more than an educated guess, both 30- and 60-day exposure at 0.1 ppm $CdCl_2$ may have been beneficial to the crabs, for there were more survivors from the 0.1 ppm groups than among control groups.

The only histopathology that could be attributed to cadmium alone was damage to the gills (Tables 28 and 29). The concentration threshold causing consistent damage was 0.5 ppm, and may have been the major stress factor causing death in crabs held at 0.5 and 1.0 ppm $CdCl_2$. The presence of disease organisms in both series introduced a second type of stress that may have operated in a synergistic fashion with cadmium. Paramoeba was present in survivors of the 30-day exposure, both in control and test animals, while a condition of unknown etiology affected 10 or 12 of the 60-day group, including all of the 4 surviving controls. Paramoeba was also present in 1 of the 60-day crabs and may have been a stress factor in that series.

Within the group of 30-day-exposed crabs, large irregular cytoplasmic inclusions occurred in the bladder epithelium of both control (1/2) and test (8/10) animals. Had this condition been confined to cadmium-exposed animals, it would be tempting to suppose that the effect was due to cadmium. The bladder acts as a reservoir for fluid (urine) secreted into it through the antennal-gland epithelia. In normal crabs, the bladder epithelium and the labryinthal epithelium of the antennal gland undergo similar changes in appearance. The antennal gland is

Table 28. *C. sapidus*, 30-day Exposure to Cadmium Chloride, Histopathology

	Gill Pathology	Bladder Crystals	Paramoeba
Control 1	0	+	0
Control 2	0	0	+++
Test 0.1 ppm 1	0	+	++
2	±	+	0
3	+	+	0
4	±	+	0
5	0	+	0
Test 0.5 ppm 1	++	0	0
2	+	0	0
3	+	+	0
Test 1.0 ppm 1	++	+	+
2	++	+	0

Table 29. *C. sapidus*, 60-day Exposure to Cadmium Chloride, Histopathology and Disease.

	Gill Pathology	Bladder Crystals	Paramoeba	Unknown Agent
Control 1	0	0	0	++
2	0	0	0	+
3	0	0	0	+
4	0	0	0	+
Test 0.1 ppm 1	+[1]	0	0	++
2	0	0	0	+
3	0	0	0	+
4	0	0	0	0
5	0	0	0	+
6	0	0	+	+
7	0	0	0	+
Test 0.5 ppm 1	+	0	0	0
Test 1.0 ppm -	-	-	-	-

[1]This animal had been badly injured during transit; lost most of its blood and was moribund at time of dissection.

active in ionic exchange but is not an osmoregulatory organ (in marine crustaceans so far studied). The nature of the inclusions in the bladder epithelium of the 30-day series has not been investigated histochemically. They are not removed by exposure to acid, and refractory to the stains I used. It is known that one metallic salt, mercuric chloride, is concentrated in the antennal gland by prawns, but its position within the gland has not been studied. A possible explanation of the presence of the inclusions may be the following: The crabs had been receiving a rich diet (clams), were very "fat", and the blood was loaded with PAS-positive materials. The diet might have led to faulty excretion of metabolites, and thus led to formation of the bladder inclusions. Mitigating against that hypothesis is the fact that crystals were not found in bladder epithelia of 60-day crabs, which had also been fed clams.

Gill pathology was evident in the so-called "salt glands" (specialized, thickened lamellar epithelium which occurs only in certain areas of the lamellae), which are deemed the area of salt regulation. The salt glands contain specialized mitochondria thought to be the active energy producers for pumping salts. Pathology also occurred to a varying degree throughout the nonspecialized lamellae. Commonly, there was enlargement of the hemal space, which was filled with blood, hemocytes, cell debris, etc. There was variable destruction of epithelial cells of the lamellae, with pycnosis and karyorrhexis; small areas of caseous degeneration; and occasionally beginning fibrosis,

and sloughing of the epithelium. Denuded cuticle occurred mainly in the salt-gland areas, suggesting that these specialized cells were more prone to damage by cadmium. Of considerable interest, at times in 30-day animals, and perhaps governed by the molt state of the crab involved, the lamellar cuticle was locally thickened or underlaid by an alcianophilic substance. This material is associated in normal animals with cuticular diposition in the premolt state. If chemical stressors other than cadmium can cause the same condition, it might serve as an indicator of chemical stress in the natural environment.

Other tissues of exposed crabs appeared normal in crabs not infected by Paramoeba or by the unknown agent.

The occurrence of Paramoeba in crabs collected from Long Island Sound (30-day exposure) extends the known range of that parasite. Previously, it has been reported in blue crabs from Chincoteague Bay south through Florida.

In conclusion, blue crabs can undergo chronic 60-day exposure to $CdCl_2$ at 0.1 ppm with little or no obvious histopathology. At 0.5 and 1.0 ppm, necrotic changes in epithelia of the gill lamellae indicate major disruption of gill function. Such disruption probably includes impairment of gas exchange as well as partial loss of osmotic control.

I am grateful to personnel at the Milford Laboratory, particularly Dr. Tony Calabrese and Mr. Jim Miller, for their indispensable aid in arranging for and in monitoring the tests.

PATHOBIOLOGY INVESTIGATIONS

IMMUNITY IN MARINE FISH

R. A. Robohm

Antibacterial Antibody Response in Cunners (*Tautogolabrus adspersus*) Following Short-Term, High-Dose Cadmium Exposure

We have previously showed that 96-hour exposure of cunners to 12 ppm cadmium significantly inhibits destruction of bacteria trapped within phagocytes of the liver and spleen, but does not affect antibody response to sheep red blood cells (SRBC). It is possible that the lack of antibody effect is related to the processing of the unusual SRBC antigen. We therefore set up an experiment to determine whether a similar affect would be seen after injection of a bacterial antigen.

Cunners were injected intraperitoneally (i.p.) with about 5×10^7 formalinized *Bacillus cereus* cells in Freund complete adjuvant and exposed for 96 hr to 0 or 12 ppm cadmium. Fish were then held at 17°C (in one experiment) or 14°C (in a second experiment) and sera were tested after 6 wk for agglutination titers against formalinized *B. cereus* cells.

Figure 42 shows that the reciprocal titer (geometric mean) for cadmium exposed fish was 64 compared with a titer of 106 for fish not exposed to cadmium. Although the difference between the two treatments appears large enough to indicate a definite cadmium suppression of antibody response, a "t" test on $\log_{10}$ transformed data shows this difference to be statistically non-significant. Because of extreme

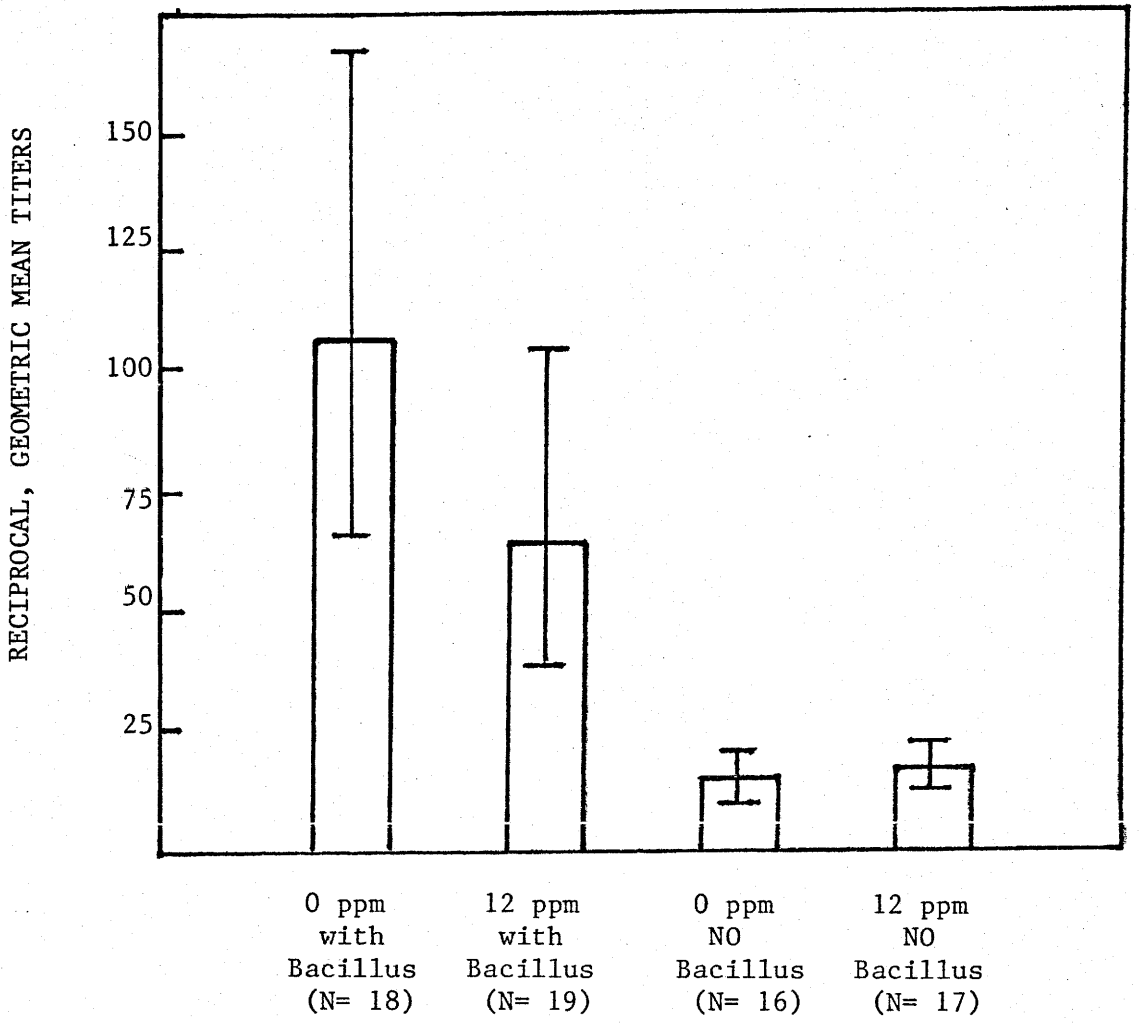

Figure 42. Antibody Titers in Cunners Six Weeks after Exposure to 0 or 12 ppm Cadmium for 96 Hours. Fish were Injected with Bacillus cereus in Freund Complete Adjuvant (or with Freund Adjuvant Alone) at the Time of Cadmium Exposure. Bracketed Lines Indicate Standard Error of the Geometric Mean.

variability of response among fish in the experimental groups, a doubling or tripling of fish numbers would be required to gain statistical significance. However, it is noteworthy that in the first experiment (in which fish were held at the higher temperature) five of nine cadmium exposed fish (that had also been injected with bacteria) died of a non-identified infection during the holding period. No other fish from the 0 ppm cadmium group nor from the control groups died of infection. This suggests decreased immunity in the cadmium-exposed, antigen-stressed fish.

Immune Responses in Striped Bass (*Morone saxatilus*) Following 60 - Day Low-Dose Cadmium Exposure

Antibody production and presence of lytic enzymes in phagocytic cells was examined in juvenile striped bass after 60-day exposure to 0, 5 and 10 ppb cadmium. In this experiment striped bass (6 to 7 inches) were exposed to cadmium in continuous flow tanks. After 30 days fish were injected i.p. with about $2x10^8$ formalinized *Aeromonas salmonicida* cells in Freund complete adjuvant. Water temperature during the experiment ranged from 14°C to 8°C; however, during antibody response, temperature was at 9 ± 1°C. Four days prior to exsanguination a portion of the cadmium treated and untreated control fish were injected i.p. with 1.2% sodium caseinate to stimulate accumulation of phagocytic peritoneal exudate cells (PEC). After four days the peritoneal cavities of these fish were washed with chilled, heparinized Hanks balanced salt solution containing 0.4% additional NaCl and 2.5% bovine serum albumin.

PEC were allowed to attach to the bottom of polystyrene tissue culture wells for 1 hour at room temperature. These cells were stained with cytochemical stains (alkaline phosphatase, acid phosphatase and lysozyme) and with Wright's stain and observed by oil immersion brightfield microscopy.

Figure 43 shows that there was little variation in antibody response between the 0, 5 and 10 ppb cadmium-treated fish. Analysis of variance for the data indicates no significant differences. Further, no significant differences were noted in the number of alkaline phosphatase granules within cells of cadmium-treated and non-treated fish (Fig. 44). Tests were not successful in demonstrating either acid phosphatase or lysozyme.

It may therefore be concluded that 60-day exposure to 10 ppb cadmium does not demonstrably affect two measures of immunity within striped bass held at 8 - 14°C. However, since low temperatures result in low metabolic rates in poikilotherms, it is possible that an effect not seen at 8 - 14°C would become evident at higher temperatures.

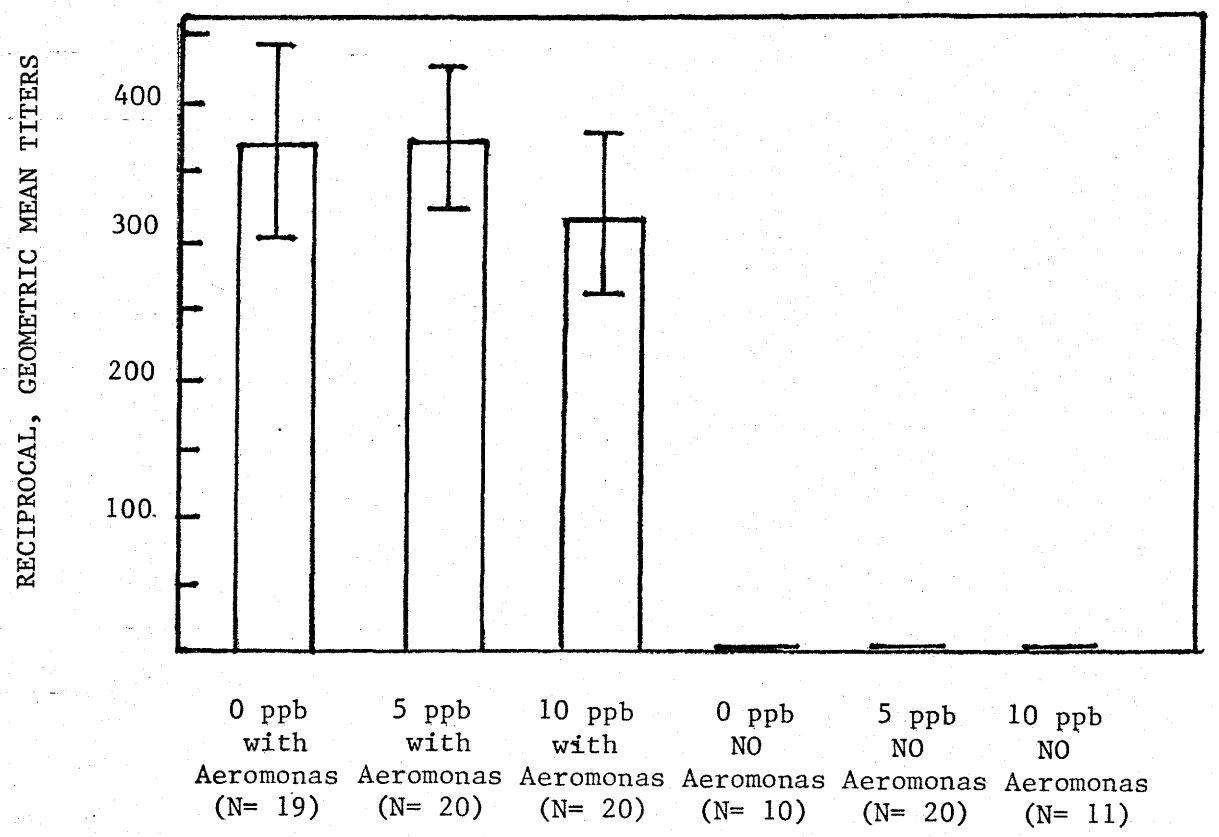

Figure 43. Antibody Titers in Striped Bass After Two Months, Continuous Exposure to 0, 5 or 10 ppb Cadmium; Fish were Injected with *Aeromonas salmonicica* in Freund Complete Adjuvant (or with Freund Adjuvant Alone) Half-way Through the Cadmium Exposure Period. Bracketed Lines Indicate Standard Error of the Geometric Mean.

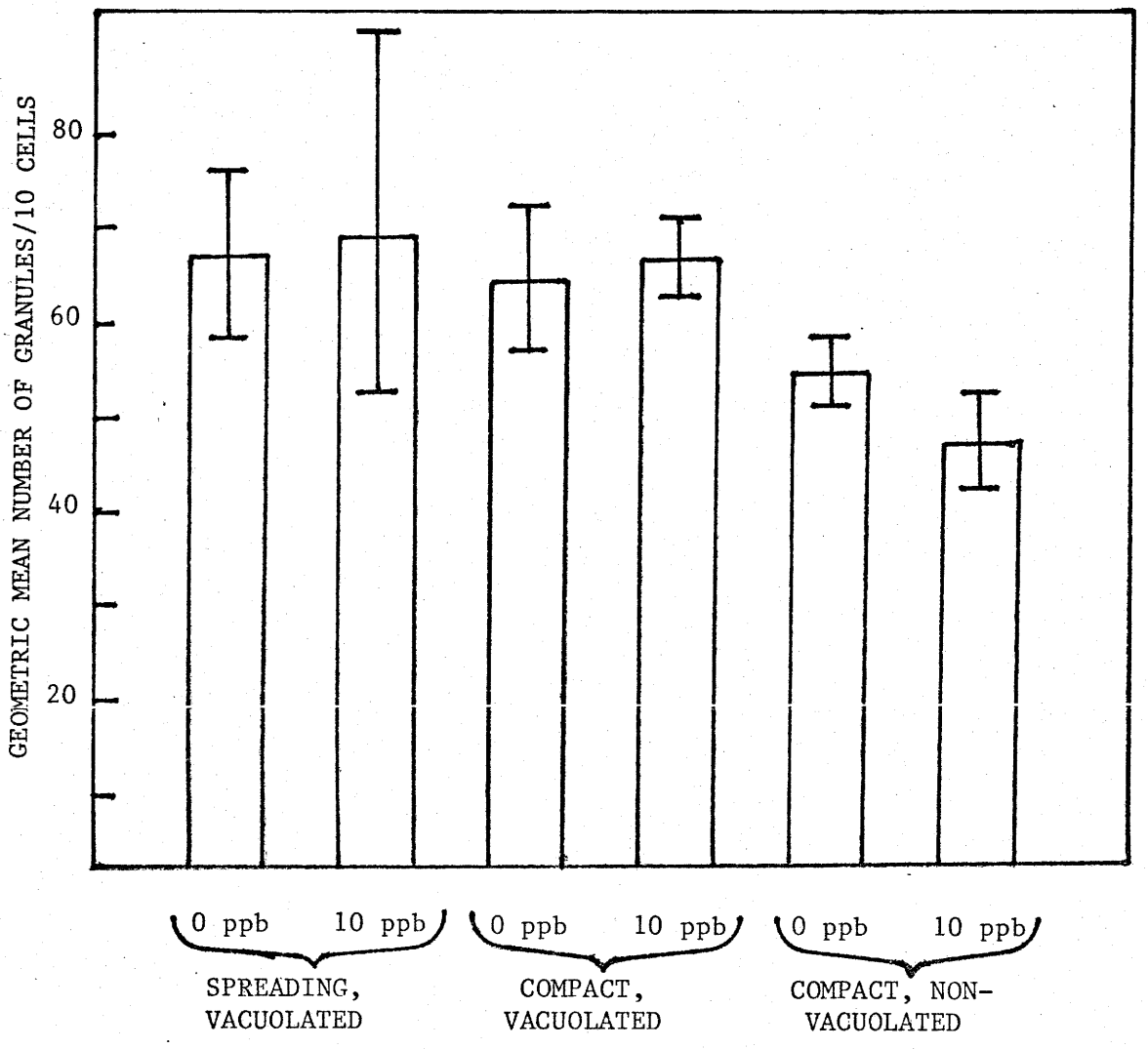

Figure 44. Alkaline Phosphatase Granules within Phagocytic Peritoneal Exudate Cells of Striped Bass After Two Months of Continuous Exposure to 0, 5 or 10 ppb Cadmium. Fish were Injected with Freund Complete Adjuvant Half-way Through the Cadmium Exposure Period and with 1.2% Sodium Caseinate 4 Days Prior to Cell Collection. Height of Bars Represents the Geometric Mean Number of Granules per 10 Cells from Each of 6 Fish. Bracketed Lines Indicate Standard Error of the Geometric Mean.

PATHOBIOLOGY INVESTIGATIONS PUBLICATIONS

Published

Newman, M. W. and S. A. MacLean (1974).

Physiological response of the cunner, Tautogolabrus adspersus, to cadmium. VI. Histopathology. In: Physiological Response of the Cunner, Tautogolabrus adspersus, to Cadmium, pp. 27-33. NOAA Tech. Rep. NMFS SSRF-681.