A PROPOSED FIVE YEAR STUDY OF BIOLOGICAL AND CHEMICAL BASELINES AND EFFECTS OF ENVIRONMENTAL CHANGES ON MARINE ORGANISMS IN THE NEW YORK BIGHT

U.S. DEPARTMENT OF COMMERCE
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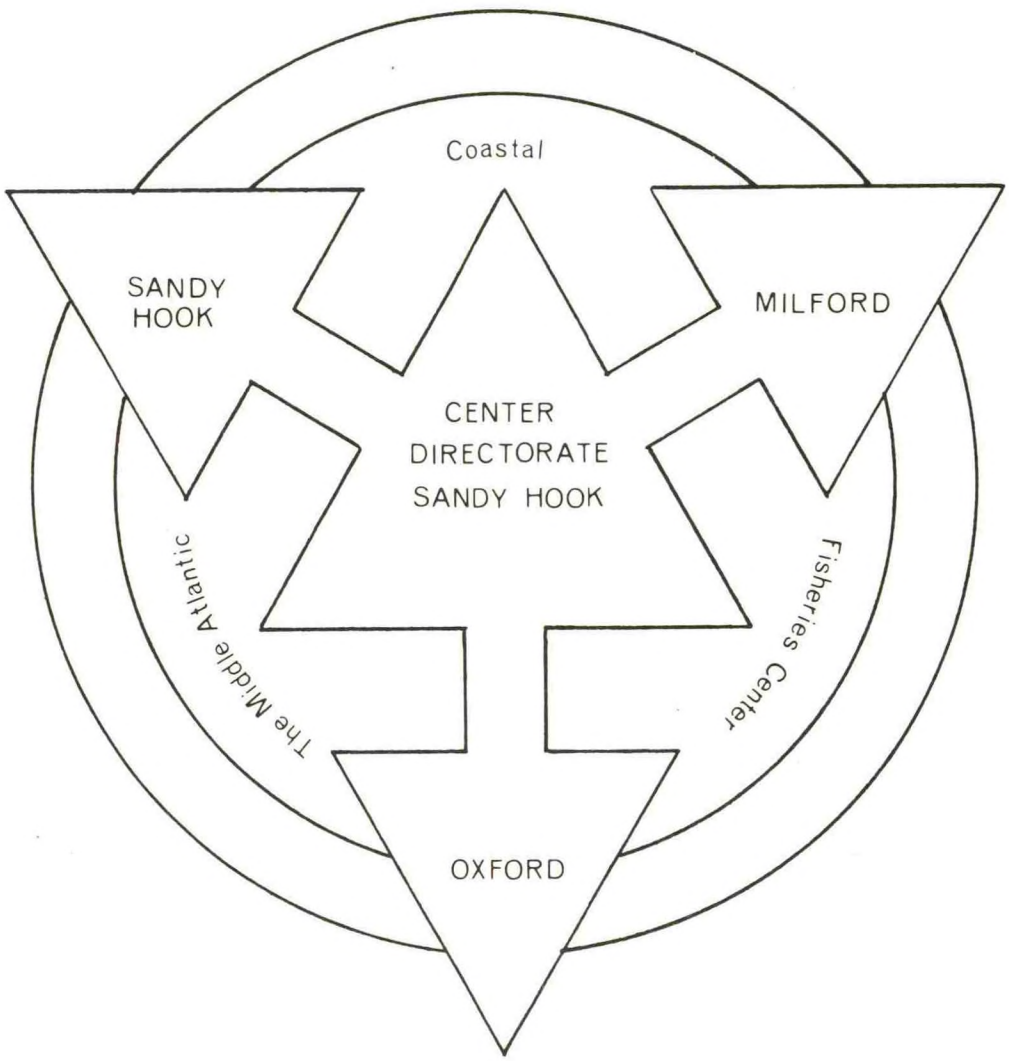
MIDDLE ATLANTIC COASTAL FISHERIES CENTER



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NOAA-NMFS

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INTRODUCTION

The most important multidisciplinary effort of the Middle Atlantic Coastal Fisheries Center at present is the New York Bight Contaminant Study. Our particular concern, and the basic reason for our involvement, is assessment of the possible effects of marine contamination on living resources and on the ecosystems which support them. Many of the component units of the Center have already been focused on this study -- ecology, experimental biology, microbiology, pathology, and chemistry -- and expected increases in funding will permit even more extensive studies.

Several important steps have already been taken. An extensive contract report to the Corps of Engineers on the effects of ocean dumping on the New York Bight area has been completed by the Sandy Hook staff. This document will serve as very important background work for most of our future efforts. Additionally, a Center coordinator for the New York Bight Study has been designated, and a series of bimonthly meetings with participants has been initiated. The coordinator recently headed a Center task force which assembled the second annual report of progress in marine contaminant research titled "A multilaboratory cooperative study of contaminants in the coastal environment and their effects on living marine resources." The coordinator also headed a task force which prepared the present document.

With the advent of an expanded NOAA project for the New York Bight in FY 1973, it seems important to outline in some detail the proposed contribution of this Center to the project. Much of the proposal is built on ongoing research; augmented efforts are of course contingent on increased funding under the NOAA/MESA Program. Major emphasis has been placed on the development of biological/chemical baselines, as well as on experimental studies of the effects of pollutants on marine organisms. These principal thrusts, when combined with data obtained by other NOAA elements and that obtained by colleges and universities in the area, should provide a substantial basis for assessing the impact of man-induced changes on living marine resources in the New York Bight area.

BIOLOGICAL BASELINES AND PROCESSES

Ecological baselines will define the dynamic interrelationships between the living biota and the non-living environment. Assessment will be made of the composition, abundance, and distribution for aquatic species and these factors will be correlated with nutrient levels, concentration of pollutants, and similar environmental parameters. When these baselines are fully understood, the sensitive sections of the ecosystem will be identified and can be protected from the deleterious effects of man's activities.

One of the major goals of the New York Bight Study will be to establish detailed characteristics of the entire ecosystem. This will provide a clear picture of the present state of the system, and will also help to determine the present and future rates of change. Such an extensive study must be accomplished over a considerable period of time since anomalous variations can be expected to occur. A 5-year study should provide ample opportunity to identify these variations and to correct the data base accordingly.

Environmental quality is often equated with the quality of living processes. The deleterious effects of ocean contaminants are primarily seen in limitation of marine populations, before environmental degradation results in the limitation of man's water-related activities. One measure of environmental quality is the abundance, distribution,

and well-being of the living organisms in the sea. Such measurement requires a detailed understanding of the numbers of animals at any point in time (i.e., standing crop or biomass), and dynamic processes which limit abundance and distribution, and the efficiency levels at which energy is transferred through the food web. If the deleterious effects of pollution and contamination are to be evaluated, those natural factors that limit marine populations must be understood. Degradation of the environment usually results in complex toxicological effects on man and marine organisms, and the physiological changes that result generally must be measured at the cellular level. In addition to the negative aspects of pollution, the natural processes of recovery must be defined, and methods to accelerate reclamation of marine habitat and resources must be developed. Many of these processes are known to be biological, but the mechanics of operation are largely unknown and need to be defined by controlled laboratory experiments as well as field observations.

Essential to assessment of environmental degradation is a sound baseline of species composition, abundance, and distribution in the New York Bight area. Biological surveys of fish and invertebrates can be accomplished through use of standard collection equipment (otter trawls, scallop and hydraulic dredges, bottom grabs complete with photographic equipment, plankton net) and is fully within the capabilities of NMFS. Additional collections will be obtained through

arrangement with the ongoing marine resources assessment, monitoring, and prediction study (MARMAP) and will be included as an integral part of the broader NOAA Plan in the Marine Environment. MARMAP will not only provide information for those living resources of the New York Bight but for migratory species or life stages of species that normally live outside of this area, and have influence on the biota at some point in time. Laboratories outside of the New York Bight region will provide specific fishery information as requested. The value of these baseline surveys will depend on accurate taxonomic identifications. Technical assistance will be used as available within the cooperating facilities, and, in addition, NMFS's National Center for Systematics will examine representative specimens from all collections for verification.

While population surveys are underway, the general biology of significant species will be investigated so that normal life-history processes can be understood. Population dynamics including reproduction, spawning, recruitment, and mortalities of selected species will be explored.

Benthic organisms are known to be excellent indicators of changing environmental characteristics, and the definition of benthic faunal communities within the New York Bight Study area is necessary to assess the dynamic processes in the physical and chemical environment. In addition to their value as indicator

organisms, the benthic fauna are a primary source of food for generally larger organisms at higher trophic levels. Therefore, the abundance and species composition of animals living on or in the bottom are often critical in the well being and productivity of food animals. NMFS's Sandy Hook Marine Laboratory is excellently located and has the required staff expertise to undertake studies of benthic communities in the New York Bight. Such studies are already underway on a limited basis in the Bight area, and will be expanded to meet the needs of the environmental quality study.

In the New York Bight, there have been few studies on productivity and bioenergetics. Although some recent preliminary effort has been directed toward the measurement of energy conversion rates for selected marine gamefish, a much more ambitious study will be required to understand the various efficiencies at trophic levels within the biotic components of the ecosystem. A more complete understanding of bioenergetics is essential to assessment of environmental quality and those factors that may limit or enhance living marine resources. A study will be developed to define those energy pathways.

Phytoplankters are the lowest elements in the energy transfer chain, converting fundamental inorganic elements into organic components, and are the major source of the world's oxygen supply. Pollutants may be directly toxic to some or all species of phytoplankton,

or may so alter the normal physiological processes that productivity is greatly reduced. Studies will be undertaken to examine the correlation between nutrient levels, contaminants, and phytoplankton composition and abundance in the New York Bight region to assess negative aspects of environmental degradation. Additionally, those phytoplankton species found to be excellent indicator organisms will be used in bioassays for contaminants.

The NMFS contaminant study, now underway, will be a vital aspect of the New York Bight Study. This study is essentially an intensive survey of pollutants in marine organisms and surrounding environment. The contaminant study is concentrating the analytical capability of NMFS laboratories to determine levels of heavy metals, pesticides, plasticizers, radionuclides, and petroleum waste products. In addition to three selected target species (a fish, crab, and clam), the contaminant study is establishing levels throughout the entire food web (i.e., microflora and fauna, benthic organisms, primary consumers, and carnivores at several trophic levels) and in water and sediments of the New York Bight region.

Intensive examination is now being made of two sewage sludge disposal sites (off New York Harbor and Delaware Bay) where high levels of contaminants are known to occur and a dredge spoil disposal site in Long Island Sound. Contaminant assays are made on whole organisms and on distinct organs or tissues. The identical materials are also examined for disease or pathological conditions

that might be associated with contaminants.

Specimens for heavy metal and pathological examination are routinely collected on the MARMAP groundfish surveys, which include the entire Continental Shelf from Nova Scotia to Cape Hatteras. Port agents are also obtaining specimens of commercial fish, as landed at the major ports, for heavy metal analysis. In the northeast, many of these commercial catches are made within the New York Bight region and the analyses will contribute to our understanding of contaminant levels within the Bight system. Catches from outside the Bight can be used for comparative control levels.

The present coastal contaminant study will be expanded under the environmental quality program. The entire area of the New York Bight including all the wastes disposal sites will be examined for toxic levels and the effects of contaminants on aquatic populations.

EFFECTS OF STRESS ON AQUATIC ECOSYSTEMS

Environmental stress alters the ecosystem and limits the abundance and distribution of living aquatic resources. Special attention in the New York Bight Study will be given to the effects of pollutants on physical, chemical, and biological features through observations in natural marine ecosystems and through laboratory experiments. The effects of ocean dumping in relation to disease in aquatic fauna will receive special emphasis. Armed with knowledge of the factors that cause stress and the consequences of environmental stress for marine resources, proper management activities can be recommended to attain the highest levels of environmental quality.

Some contaminants or physical pollutants kill marine organisms outright. The widely publicized oil spills, dispersal of certain industrial wastes, and thermal loading of the environment by electric power stations are commonly known examples. To provide a basis for environmental management, we must establish the precise levels of pollutants that can cause mortalities and the differential responses of marine organisms, including various stages in their life history. But perhaps of even more importance are the long term effects of exposure to sub-lethal (stress) levels. Such exposure may limit development, growth, reproduction, metabolism, or other physiological processes.

The laboratory experiments and faunal surveys, when correlated with contaminant levels, will show that some animals are extremely sensitive to minute amounts of pollutants, or that certain animals or communities will flourish where specific contaminants are available at trace levels. Armed with this knowledge, the species identified by the studies will be useful as indicator organisms. Thus, a ubiquitous species may be absent wherever low levels of a specific contaminant are present. Conversely, indicator organisms may be used in laboratory bioassays where survival has a direct relation to contaminant concentrations.

Environmental research at the NMFS Biological Laboratory, Milford, Connecticut, is concerned with the effects of contaminants on survival and growth of marine organisms, and with the occurrence and distribution of contaminants in aquatic populations. The Laboratory is also developing rearing methods for estuarine and oceanic species of fish and shellfish for physiological studies. Special attention is being given to the effect of various physical, chemical and biological factors found in the marine environment on survival, growth, and reproduction. Assessment of physiological changes in several species of mollusks common to the New York Bight region is now underway. Eggs and larvae are being challenged by 11 heavy metal ions. These laboratory experiments will be expanded, as part of the New York Bight Project, to include important crustacean and fish

species, and the organisms will be challenged with a greater assortment of contaminant materials.

NMFS's Biological Laboratory at Oxford, Md., conducts studies to determine pathological and disease conditions in fish and shellfish under natural and experimental conditions. One of the studies now underway in a cooperative effort with other NMFS facilities is to critically examine the effects of ocean dumping in the New York Bight in relation to disease problems. Their particular emphasis in the contaminant program is on environmental conditions (natural or man-made) that may enhance microbiotic, viral, or parasitic entities and the resulting mortalities in marine populations. They are also concerned with the histological changes (at the tissue or cellular level) that may result from contaminants in the environment. Part of their present research involves histological examination for tissue and cellular damage from exposure to natural and laboratory levels of contaminants.

The research at the Milford and Oxford Laboratories will increase as the coastal contaminant study is expanded to include a greater geographical area and more waste disposal sites.

OBJECTIVES OF THE FIVE YEAR PROGRAM

1. To determine the composition, abundance and distribution of aquatic species in the New York Bight area and to correlate these factors with nutrient levels, concentration of pollutants and similar environmental parameters.

2. To determine precise levels of pollutants that can cause mortalities and the differential responses of marine organisms, including various stages in their life history.

3. To determine species of organisms which would be "indicator organisms" as means of assessing changes in environmental characteristics which would then enable us to determine "alert conditions" for these changes.

OBJECTIVE NO. 1

To determine the composition, abundance and distribution of aquatic species in the New York Bight area and to correlate these factors with nutrient levels, concentration of pollutants and similar environmental parameters it is necessary to conduct the following studies:

1. Ecological baseline studies.
2. Microbiological baseline studies.
3. Chemical baseline studies.

ECOLOGICAL BASELINE STUDIES

The initial phase of this study in FY 1973 will be an intensive survey of the estuaries and nearshore area within 20 miles of New York Harbor to inventory the biological and chemical conditions of this highly polluted region. Within this area are examples of almost all types of man's activities that affect the Bight; i. e., domestic sewage, industrial wastes, ocean outfall lines, ocean disposal of sewage sludge and dredge spoils, bulkheading and siltation. The initial inventory will include a sampling program for benthos, malacostracans, fish and plankton.

A one time sampling program for benthic infauna with a Smith-McIntyre grab sampler will be initiated and completed in FY 1973. Samples will be taken on a one mile grid pattern with additional collections in areas of rapidly changing environmental factors. This will result in approximately 800 samples which will be screened to the 1.0 mm level, with aliquots saved for meiofauna (organisms between 1.0 mm and .062 mm) studies.

The processing of the samples, which will begin in FY 1973 and extend into FY 1974, will result in species lists, species distribution maps, community structure, diversity indices, biomass estimates, comparative data from impacted and non-impacted areas, community phenomena associated with different impact areas and animals for heavy metal, pesticide and PCB analyses.

At each benthic infauna station sediment cores of 4 to 6 inches in length will be taken and analyzed for heavy metals, pesticides, PCB's organic content and sediment grain size. These analyses will result in distribution maps of the various pollutants, sediment grain size, correlation of specific animals with various pollutant levels and grain sizes, extent of pollutnat spread in relation to its sources, estimates of the accumulative phenomenon of pollutants in sediments, data for pollutant flow models and baseline information for comparative studies after pollution abatement or activity changes (i.e., stopped ocean disposal, relocation of disposal sites, new methods of disposal, increased or decreased dredging activities, current changes caused by various man-made structures or topographic alterations) have occurred. The collection will be accomplished in FY 1973, the analysis completed in FY 1974 and data outputs provided in FY 1975.

Seasonal collections for benthic infauna and cores will be done at 10% of the stations. The stations will be selected on the basis of their being representative of a particular biocoenosis and ecotome, i.e., within an impact area, in a control area or in a marginal area. This will provide data on seasonal and time variation in community structure and pollutant loads. This sampling level will be carried out through FY 1975 in all study regions.

Scallop dredge tows will be made for five minutes in the areas between Smith-McIntyre sampling sites. These samples will be sorted for benthic macrofauna and used as semi-quantitative data for the larger and rarer organisms not sampled by the Smith-McIntyre grab. Other than a retention level of 10 mm instead of 1 mm and 0.62 mm, the samples will be treated and used as indicated in the section on Smith-McIntyre sampling. This work will be accomplished when the Smith-McIntyre and core sampling is done.

Benthic target species to be monitored for heavy metals and pesticides should be hard clams, Mercenaria mercenaria, soft clams, Mya arenaria, sand worms, Nereis sp., and any other species that proves to be numerous and an important food item.

Fish will be collected by use of an otter trawl. Fifteen and thirty minute tows with a standard 30/60 trawl will be made monthly at 20 stations located in selected areas of the estuaries and nearshore regions. These stations will be in areas that represent the different environmental conditions that occur in the Bight area. Fish will be identified, measured, weighed and examined for gross pathology on board ship. Selected species (particularly winter flounder, Pseudopleuronectes americanus; yellowtail flounder, Limanda ferruginea; silver hake, Merluccius bilinearis; red hake, Urophycis chuss; white perch, Morone americanus; and striped bass, Morone saxatilis) will be saved for stomach analyses and pollutant content. These samples will result in information on species distribution, species

diversity, community relationships, relationship of animal distribution to environmental phenomena, food chains and the distribution of pollutants in animals. Sampling will be initiated in FY 1973 and continued on a reduced level in FY 1974. Data analysis will be completed in FY 1974 and model analysis begun.

Malacostracan collections will be made in conjunction with the otter trawl collections. A one-half meter and one meter epibenthic sled fitted with a #0 mesh net will be mounted on the sweep of the otter trawl. The samples will be split and one-half frozen for contaminant analyses and one-half preserved for faunal enumeration and identification in the laboratory. The samples will be used for species lists, species distribution, community structure and relationships, relationship of animals present to fish stomach content, biomass estimates, contaminant levels and food chain pathways. The program schedule will be the same as the fish sampling program.

Key malacostracans to be monitored for pollutant levels are sand shrimp, Crangon sp.; opossum shrimp, Neomysis americana; grass shrimp, Palaemonetes spp.; blue crabs, Calinectes sapidus; hard crabs, Cancer spp.; lobsters, Homarus americanus; and amphipods, Gammarus spp. Other species that prove to be important members of the food chain will be included.

The various species of marine organisms collected in these Ecological Baseline Studies will be examined for the presence and

levels of heavy metals, chlorinated hydrocarbons (PCB's, DDT's dieldrin, aldrin and others as feasible), hydrocarbons (petroleum waste products) and radionuclides (see Chemical Baseline Studies section).

On-going programs at the Sandy Hook Laboratory are investigating the phytoplankton of this region. Zooplankton studies have been conducted in the nearshore Bight region, Raritan Bay, Sandy Hook Bay and Hudson River Estuary. These studies will be further examined for the presence of reliable data on species lists, community relations and diversity indices, seasonal patterns and standing crop estimates. Where research is found to be needed, programs should be initiated by the Middle Atlantic Coastal Fisheries Center through cooperative studies with universities such as SUNY-Stony Brook, or by coordination with the MARMAP project. The MARMAP project will be able to provide the needed data from shelf areas, but the extent to which they will enter the estuaries is not known.

Plankton collections will also be made simultaneously with the otter trawl and malacostracan collections. Bongo nets fitted with #2 and #10 mesh net will be hauled obliquely during the otter trawl tow. The plankton samples will be split and one-half frozen for contaminant analyses and one-half preserved for species enumeration. This will permit identification of contaminant levels in the larger adult copepods

(those collected in the #2 mesh net) and of smaller plankters, including long chain diatoms and larger dinoflagellates taken in the #10 mesh net.

Collected at each monthly visited biological sampling station will be temperature, salinity, conductivity, pH, turbidity, chlorophyll and nutrient data from throughout the water column. Additional hydrographic and chemical collections will be made in coordination with the chemical program in order to fill their objectives. These data will be continually fed to the Environmental Data Service's data storage system along with the biological data as it becomes available.

Studies of the Hudson Shelf Valley and the North and South Coastal Zones will be instituted in FY 1974. The basic sampling program will be the same as for the estuaries and nearshore region except that the efforts will be reduced in the offshore region of the Canyon and the North and South Coastal Zones. Benthic sampling will be on a five-mile grid pattern in these areas. Plankton and fish population data will be drawn from the MARMAP program. The benthic and malacostracan collections will be initiated in FY 1974 and sample analysis completed in FY 1975.

Benthic sampling will be handled in segments by Sandy Hook Laboratory and outside contractors such as the Woods Hole Oceanographic Institute and the Marine Biological Laboratory at

Woods Hole. Sandy Hook Laboratory, which has an on-going program in the Hudson Shelf Valley, can extend their sampling program to cover more area, while WHOI has made some collections in the North and South Coastal Zones. The applicability of the existing samples and their state of processing will significantly change the effort required in the coastal zone areas.

Malacostracan samples and fish samples for contaminant levels can be obtained by piggybacking on MARMAP groundfish surveys. The groundfish program, which has been in operation for over ten years, will serve as an excellent program for monitoring the fish populations in the coastal zones. Fish and plankton data drawn from the MARMAP program will be available on a continuing basis.

Lower scale field collecting programs of the five major groups will be initiated in FY 1975 to provide continued time related data on community structure and dynamics and contaminant dynamics. These programs will be aimed at testing various models and monitoring systems for predicting environmental disturbances and community stress. The field programs will be phased out as model and monitoring systems become proven.

Sampling in the estuaries and nearshore regions will require a shallow draft vessel in the 40 to 60 foot class capable of handling an otter trawl, plankton nets, a Smith-McIntyre sampler, a bathythermograph, an STD, water bottles and electronic

hydrographic measuring and recording instruments. The vessel will be required for 60 sea days in FY 1973 and 24 sea days in FY 1974 and FY 1975. Time required for FY 1976 and FY 1977 are not included, but should be about 24 sea days each. A navigation system, such as RADUS, will be required to permit precise station location and resampling. The system must be portable, unless a single vessel is chosen for the work. It is anticipated that National Ocean Survey could provide the necessary system.

The field sampling gear are standard off the shelf items except for the Smith-McIntyre bottom sampler, which can be ordered from England or custom-made at local shops. Three samplers are currently being used in the programs at Sandy Hook Laboratory.

Field collections scheduled for FY 1974 and later will require a vessel capable of high seas work. The vessel must be able to handle all of the previously mentioned gear and have room to accomodate additional researchers concerned with the physical and chemical components of the New York Bight Study. The vessel will be required for 50 sea days in FY 1974 with one-half of the days spread seasonally. The same sampling equipment will be used on both vessels. The large vessel, if schedule time permits, can continue the low level of sampling in the nearshore area along with the Hudson Shelf Valley and North and South coastal zones in FY'S 1974-76.

Personnel for the collections of the samples will be provided by SHL or graduate students from contracted colleges under the supervision of SHL personnel. Total man hours (exclusive of ship crew) required are equal to four times the boat time. For FY 1973 this equals 5760 man hours. FY 1974 collections will require 5000 man hours and FY 1975 and 1976 collections, 1000 man hours.

The laboratory processing of the samples will be started in FY 1973 and completed for all areas in FY 1976. Sample sorting will be handled by contract to local universities and consulting groups. Where possible, the sorting centers should be at Sandy Hook Laboratory or close by so that quality control can be maintained at a high level. Facilities are available at Sandy Hook and local institutions, such as Lehigh University, Monmouth College, Brookdale College, University of New York and a consortium made up of New Jersey state colleges and universities.

Processing will be done in two phases. The initial phase will separate the animals from the non-living debris and into major taxonomic groups, such as crustacea, polychaeta and mollusca. This type of sorting can be done by a person with very little training and professional experience. High school graduates and college underclassmen will supply the necessary manpower for the process. The second phase will involve sending the major taxa to university

and college personnel who have an interest and expertise in certain taxonomic groups. They will then control the species identification and enumeration of the samples. Final analysis of the data and reports concerning the whole biological system will be the responsibility of the SHL Ecosystems Investigations.

Agencies, such as the Environmental Protection Agency and the Public Health Service, are now cooperating with the Sandy Hook Laboratory and their cooperation in studies suited to their expertise should be continued. The EPA will be able to add valuable information on the amount and source of pollutants in the study region, while the PHS will actively cooperate in testing for the presence of micro-organisms hazardous to public health.

Contractors for the processing of biological samples will be universities and colleges and private industry for chemical analysis, model building and monitoring devices. Other agencies such as EPA, National Ocean Survey and Environmental Data Service will be called upon to advise and assist on the latter two subjects.

MICROBIOLOGICAL BASELINE STUDIES

The microbiological baseline investigations for the proposed study will be an extension and a broadening of the current microbiological study underway at the NMFS's Milford Laboratory, on the New York Bight (which includes Long Island Sound) area. Because of lack of information the microbiological picture of the proposed area is at best ill defined. (The microbiological picture represents the sum total of the many diverse species of molds, bacteria, viruses and yeasts that may be present in the various elements of the ecosystem). The adaptibility of microorganisms results in the selection of predominant types depending on the specific cultural factors present in any one environment. When equilibrium conditions are established specific microflora will be established. Even though environmental conditions are dynamic in any one ecological niche, equilibrium conditions can exist. Man's activities can change the aquatic environment and thereby have a diverse effect on the established microbial flora. Increased discharges of organic nutrients and chemicals into the aquatic environs as well as elevation of water temperatures can modify the microbial flora, both quantitatively and qualitatively.

Of immediate concern is to determine whether man's activities have selective effects on the establishment of microorganisms

which can be related to diseases processes on the living marine resources and their utilization by man and man's activities in the aquatic environment. Certain species of bacteria belonging to the genus Clostridium, Vibrio, Pasteurella and Pseudomonas, have been possibily identified in this regard. Whether additional heretofore unknown and nondetected species of microorganisms exist in aquatic environments warrants further study.

In addition, certain microorganisms are involved in conversion mechanisms, i.e., methylation of mercury, hydrocarbon utilization, pesticide degradation, etc. The identification and an understanding of the physiological activities of these groups of microorganisms would result in a critical evaluation of natural processes of microbial degradation which may lead to pollution increases or abatement.

The current study involves the determination of distribution patterns of fecal coliforms in order to assess the degree of microbial contamination of certain areas. In addition, detection of other organisms is being attempted in order to determine their relationship to pollution factors.

In order to establish baseline information on the distribution of microbial types and numbers, the New York Bight area will be sub-divided into a grid of sampling stations (and will be coordinated with those for benthic sampling) in order to proceed with a

systematic and identifiable system of environmental sampling. Areas to be selected will be those areas which are known to contain pollutants, (which will be identified by other on-going programs) as well as areas in the same general vicinity, which are relatively free of pollutants (control sites), for comparative purposes. During the course of the 5-year study, off-shore ocean sampling will also be included for comparative purposes to the inshore areas. The basic rationale for the sampling design of the environment will be to establish a normal or undistributed microbial picture for any ecological niche and compare it with that which has been changed by the activities of man.

At each sampling station elements to be sampled will include bottom sediment, the water column, plankton and, where applicable, various species of marine organisms. The so designed protocols will establish whether concentrating mechanisms exist by the various elements for the microbial types being investigated.

The number of stations sampled will be dictated by the restraints imposed by personnel and budget restrictions. However, the initial sampling stations should be of sufficient number and distribution in order that predictions from the 5-year study can be made for future work.

Overriding the need for decisions on the frequency and number of samples to be examined is the availability of specific

techniques for the rapid detection and identification of microbial types. It is envisioned that a major portion of the first 3 years of effort will be in the development and evaluation of microbial techniques for the rapid and specific detecting and identification of specific microbial types.

Facilities for such studies are quite adequate at the NMFS's Milford Laboratory, but it is anticipated that university contracts would expand our capabilities. Sampling for microbiological baseline studies will be integrated initially with on-going sampling programs within the NMFS's Middle Atlantic Coastal Fisheries Center. As the study progresses in FY 1975, additional vessel time for specific microbiological studies would be needed.

Basic microbiological equipment is currently available in-house through NMFS. However, emphasis will be placed on the evaluation and development of equipment for the detection and characterization of microorganisms. These will include aseptic sampling procedures and methodology for monitoring microbial numbers, as well as instrumentation, to "finger print" bacterial types in regards to serological and biochemical activities of the organisms.

The Milford Laboratory will be responsible for the sampling protocols and microbiological work since it is a continuing program and needs no disruption. Specific contracts for the development, evaluation and study of microbial methods to be performed by

university grants or contractual services to private laboratories should be awarded.

Estimated funding for FY 1973 for in-house and contract work is $60,000 and for FY 1974 is $170,000. The estimated cost of this 5-year study is $680,000. Budget figures for FY 1973 include $20,000 for contract work to develop techniques for the rapid and specific identification of microbial types. This contract is vital in order to increase the frequency and magnitude of environmental sampling in ensuing years. The budget figure for FY 1974 includes $50,000 to purchase specialized instrumentation for identification of microbial types as well as $40,000 for contractual work on microbial methodology.

CHEMICAL BASELINE STUDIES OF MARINE ORGANISMS

In order to establish baseline data and provide a program to monitor changes in levels of chemical contaminants in marine organisms of the New York Bight, it will be necessary to conduct an intensive survey of the chemical contaminant levels in benthos, plankton, shellfish, and finfish of the New York Bight (see Ecological Baseline Studies section) in order to: (1) Establish baseline data on chemical contaminants in marine organisms of the New York Bight, (2) Select key organisms (and/or tissues) for monitoring contaminant levels, and (3) Provide information that can help describe the fate of pollutants in the New York Bight. It will also be necessary to establish statistically valid chemical contaminant levels in the key organisms (and/or tissues) for the 5 major zones of the New York Bight so that these organisms can be used in a monitoring program to assess future changes in chemical contaminant levels of the New York Bight.

The following chemical contaminants will be part of this survey in the initial phases -- metals, chlorinated hydrocarbons (PCB's, DDT's,dieldrin, aldrin, and others as feasible), hydrocarbons (petroleum waste products) and radionuclides.

Heavy metal analyses are well underway in the NMFS contaminant study. More in depth collections have to be made in order to complete

the survey of benthos and plankton in particular and also for certain shellfish and fish.

The chlorinated hydrocarbons and hydrocarbons have to be surveyed in all four classes of organisms. Considerable data has been accumulated on certain of these chemicals (DDT in particular) in marine organisms. The data that applies to the New York Bight has to be compiled and studied to determine whether sufficient information is available to allow selection of the key organisms that will be studied. Archive data from Environmental Data Service and cooperation from other Federal agencies such as Food and Drug Administration and the Environmental Protection Agency should allow the compilation of this data.

Radionuclides are being examined in certain selected samples of the New York Bight under the ongoing NMFS contaminant study. The Atlantic Estuarine Fisheries Center of NMFS has unique capabilities for the analysis of gamma-emitting radioisotopes, and the analytical instrumentation is not saturated to capacity by the requirements of the standing research objectives of the Center's Ecology Division. Sediment samples from New York Bight are currently being analyzed on an intermittent, available-time basis which is not at all suitable for regular routine analyses of large numbers of samples. In addition to sediment analyses, benthic animals or demersal macrofauna from various areas in New York

Bight (especially clams, but including also crabs, flounders, etc.) should be looked at, but the large sample size requirements (about 1500 grams wet weight, minimum) have precluded the collection of sufficient samples for meaningful characterization of the dump site with respect to radioisotopic distribution and abundance.

The Atlantic Estuarine Fisheries Center is equipped to correlate studies of radioactivity with analyses of stable element and pesticide distributions and experimental determination of toxicity of sediments to representative estuarine/marine organisms, but present personnel ceilings have prevented development of these programs.

In order to resolve this man power shortage, it is intended that contract work be made available to nearby universities for technical services to be performed in the Center's laboratories by interested faculty and graduate students. The standing relationship with North Carolina State University (i.e., adjunct faculty status of several staff members) should provide a basis for expanded cooperative effort, especially if contract funds can be made available for support of university personnel working at the Atlantic Estuarine Fisheries Center. Preliminary conversations with campus faculty indicate interest and enthusiasm for an expanded cooperative venture, but lack of available funds has always suppressed motivation and realization of the objective. Details of the program proposal will still depend upon availability of suitable personnel,

but it is intended that, beginning in FY 1973 and continuing through FY 1976 several graduate students can be occupied for about 2 years on each specific research problem in the following general areas:

1. Distribution, abundance and biologic availability of radionuclides and trace elements in New York Bight sediments and organisms.

2. Toxicity of sediment extracts and suspended matter to representative marine species.

3. Distribution, abundance and biologic availability of organic pesticides in sediments and organisms from New York Bight.

During FY 1973 this work should reveal whether or not abnormal levels of radionuclides are likely to be found in the New York Bight. Expansion of this work may be required beginning in FY 1974 based on the findings during FY 1973.

Key organisms (and/or tissues) have to be selected from the results of the survey of chemical contaminants in the four classes of organisms described. It is possible that the key organisms selected in this study may differ from the organisms that are found to be indicators of environmental stress based on physiological studies. However, these organisms can be built into the study at a later date if the information is not available at the time these key organisms are selected.

Once the key organisms are selected, sufficient sampling of these organisms must be done in order to establish statistically valid contaminant levels in these organisms for the 5 major zones of the New York Bight (Long Island Sound, North Coastal, Hudson Canyon, South Coastal and Delaware Bay). These statistically valid contaminant levels would be established as follows: enough individual animals or number of sets of composites of individuals for each key organism (and/or tissue) would have to be analyzed for each contaminant in order to determine the minimum number of organisms that have to be collected to monitor changes in the contaminant levels as a function of time or other parameters. Once this has been established, the key organisms (and/or tissues) would be analyzed for contaminants on a seasonal basis for at least a two year period for the five major zones of the New York Bight in order to establish a valid monitoring device for future years.

Representative organisms will be collected from on-going NMFS activities, such as MARMAP, and from cruises that will be established as a result of the MESA Program. It is possible that special ship time on a scheduled basis from NOAA will be required beginning in FY 1975 in order to meet these requirements. This ship time probably can be projected by the middle or end of FY 1973 (See Table 1 for sampling scheme).

Table 1. Sampling scheme for collection of organisms for chemical contaminant survey in the New York Bight

Organisms	Seasons	Organ Types	Geographic Locations
Fish	Spring Summer Fall	Muscle Liver Kidney Gonad	5 major zones of New York Bight
Shellfish	Spring Summer Fall	Muscle Digestive Diverticulum Gonad [1]	5 major zones of New York Bight
Plankton [2]	Spring Summer Fall	----------	Sample grid based on MARMAP and biological baseline grid
Benthos	Every two months	Whole or Muscle only	Sample grid based on MARMAP and biological baseline grid

[1] Gonads are difficult to dissect from most shellfish, but would be included for certain ones.

[2] Some collections will be separated by species types for analyses

General chemical type laboratories with proper ventilating equipment are required for the analyses of many of these contaminants. Some of the instrumentation required in this work, however, does demand laboratory rooms with precise humidity and temperature control. Some of these instruments are those needed for neutron activation analyses, which is an excellent method for analyzing certain metals and other ions, and those needed for the exact identification of the pesticide type compounds. Radionuclides analyses require very specialized facilities and personnel in order to ensure safety of the analysts and to insure validity of data.

Adequate facilities are available for analyses of most of the 11 metal ions that are to be analyzed. Neutron activation is used for some of the metal ion analyses on a contract basis (University of Michigan). Proper instrumentation for neutron activation analyses are owned by NMFS and are on loan to the contracter at present. The facility to house this instrumentation for in-house work is scheduled to be available by FY 1974. The facility to conduct analyses of chlorinated hydrocarbons and hydrocarbons is in the process of being completed at the Milford Laboratory. Several rooms in an old laboratory building are being refurbished and equipped with proper laboratory furniture that will allow "pesticide" type analyses.

Universities, state and other Federal agencies have the facilities required to conduct most of these analyses. Only a very few would

have the proper facilities for neutron activation and radionuclide analyses. Possibilities for such contract work are: Gulf Breeze Laboratory (Environmental Protection Agency) - pesticides; Atlantic Estuarine Fisheries Center - radionuclides; University of Rhode Island - metal analyses by neutron activation.

Sufficient personnel are present at the Milford Laboratory to perform most of the metal analyses required in this study, however, to conduct analyses of hydrocarbons and chlorinated hydrocarbons it would be necessary to either hire additional personnel or to contract the work out. New personnel would be required by FY 1974.

Most of the instrumentation required for metal analyses is available at the Milford Laboratory. New instruments required for radionuclide work include pulse height analyzers, sodium iodide crystal gamma-ray detectors and the proper lead shielding. For identification of pesticide type compounds, mass and infrared spectrophotometers are essential. These instruments are very costly and require specialized personnel to operate them and interpret the data generated.

To examine about 1,000 to 1,500 samples encompassing representative organisms and/or tissues from each of the four taxonomic groups of organisms and sufficient geographical locations within the New York Bight for metals, chlorinated hydrocarbons (including PCB's) and hydrocarbons in FY 1973, the total cost would

be $100,000 for both in-house and contract work. For completion of the chemical baseline survey in FY 1974 the cost would be $250,000. The estimated cost over 5 years would be $850,000.

The data collected in this work would result in baseline data on chemical contaminants in the New York Bight. It is also an important part of the overall approach to describe the fate of pollutants in the New York Bight. The package of information left at the end of 5 years would provide a monitoring device to follow changes in chemical contaminants in marine organisms of the New York Bight.

OBJECTIVE NO. 2

To determine the physiological changes resulting from controlled exposure of known levels of marine contaminants to various stages in the life history of selected marine organisms it is necessary to conduct " in vivo " studies of physiological stress.

Studies of physiological stress require the following phases:

1. Development of rearing techniques for estuarine and oceanic species of shellfish, fish and crustaceans.
2. Exposure (Bioassay) of marine organisms, including various stages in their life history, to precise levels of pollutants.
3. Studies to determine changes in physiological processes after exposure.
4. Studies in biochemistry to investigate key enzyme systems of marine organisms and changes in these systems after exposure.
5. Immune response studies will determine whether exposure of marine organisms to pollutants will reduce antibody response.
6. Histopathological abnormalities of organisms exposed to pollutants will need to be examined.
7. Genetic changes of marine organisms exposed to pollutants will be examined at the cellular level.

8. Chemical analyses of test water in which the organisms are held and on the animals themselves to determine uptake of pollutants is required.

Phase No. 1. - Development of Rearing Techniques

The Laboratory for Experimental Biology at Milford, Connecticut, has an on-going project to develop the techniques necessary for rearing marine shellfish at all times throughout the year. For conducting bioassay studies on the embryonic and larval stages of shellfish, it is necessary that these life stages be available on a continual basis during all seasons of the year so as to provide the researcher the necessary material for such studies.

The techniques for rearing embryos and larvae of the American oyster, Crassostrea virginica, and the hard shell clam, Mercenaria mercenaria, have been well worked out over the years at this facility. Techniques will be developed in FY 1973 and 1974 as part of on-going research to rear species of shellfish located in the New York Bight area, such as the surf clam, Spisula solidissima, the Arctic or mahogany clam, Arctica islandica, and the deep sea scallop, Placopecten magellanicus. However, in FY 1974-1977 techniques will be developed for rearing other invertebrate organisms, such as the rock crab, Cancer irroratus, and vertebrate organisms such as,the windowpane flounder, Scophthalmus aquosus. Once techniques are developed for rearing the various life stages of these organisms in the laboratory, it will be possible to expose them to various environmental pollutants, both individually and in combination, to determine levels at which mortality occurs and levels at which signs of physiological stress are observed.

NOAA's Sea Grant Program is sponsoring various mariculture projects in which techniques are being developed for rearing several commercially important species of marine organisms, such as the lobster and pompano. However, the rock crab and windowpane flounder, which are of no commercial value but are prominant in the New York Bight area, are not being studied. Thus, it is important that the Milford Laboratory initiate such studies in FY 1974.

The Duke University Marine Laboratory has the necessary expertise for rearing various crustacean organisms, such as the wharf crab, Serarma cinereum and the blue crab, Callinectes sapidus. Since these organisms are present in the high impact area of the New York Bight, it is anticipated that in FY 1973-75 Duke University will be contracted (see Phase No. 2) to conduct studies on the effect of heavy metals and pesticides on these ecologically important forms.

It is anticipated that no new funding would be required for in-house studies in FY 1973, but in ensuing fiscal years it would be necessary to hire additional personnel and purchase additional equipment. The equipment required would be mainly expendable items, such as plasticware and glassware, at a cost of $3,000. A major purchase of necessity would be a temperature control bath in which studies on the effect of temperature on the embryonic and larval forms of the above organisms could be conducted. The cost of this item would be $4,500. The total estimated cost of this project through FY 1977 for

both in-house and contract work would be $165,000.

Work outputs derived from these studies would be channeled to those individuals conducting bioassay/exposure studies (see Phase No. 2.). Not only would these studies provide information necessary for exposure studies, but the information developed would be most useful in mariculture projects, such as those sponsored by Sea Grant, and the MARMAP Program. Techniques developed for rearing New York Bight shellfish will be made available in FY 1973 and 1974 to the above agencies and will be published in the scientific literature. Information on rearing the rock crab and windowpane flounder will become available to the above in FY 1975 and 1976.

Phase No. 2. - Bioassay/Exposure

Environmental stress alters the ecosystem and limits the abundance of living aquatic resources. Special attention in the New York Bight Study will be given to the effects of pollutants on marine organisms through " in vivo " laboratory experiments. Armed with knowledge of the factors that cause stress and the consequences of environmental stress on marine resources, proper management activities can be recommended to attain the highest levels of environmental quality.

Some contaminants or physical pollutants kill marine organisms out-right and the widely publicized oil spills, disposal of certain industrial wastes and thermal loading of the environment by electric power plants are commonly known examples. To provide a basis for environmental management, we must establish the precise levels of pollutants that can cause mortalities and the differential responses of marine organisms, including various stages in their life history.

The NMFS Laboratory for Experimental Biology, Milford, Connecticut, is concerned with the effects of pollutants on survival and growth of marine organisms, particularly embryonic and larval shellfish. Assessment of physiological changes in several species of shellfish common to the New York Bight region is now underway. Embryos and larvae of the American oyster, Crassostrea virginica, and the hard shell clam, Mercenaria mercenaria are being challenged

to 11 heavy metal ions in acute tests. The adult shellfish are spawned on a weekly basis and the resulting embryos and larvae are then exposed to the above metals to determine concentrations which are non-lethal, 50% lethal and 100% lethal (LD_0, LD_{50} and LD_{100}). The setting up of these experiments and sampling to determine the effect of metal ions is a time consuming and tedious process, but the results are rewarding in that the embryonic and larval stages are generally more sensitive to changes of environmental quality than the adult animals. For establishing water quality standards it is necessary to know the levels of pollutants that affect the most sensitive stage in the life history of an aquatic organism.

As techniques for rearing the surf clam, Arctic clam and deep sea scallop under controlled conditions in the laboratory are made available by the Rearing Program, the embryonic and larval stages of these organisms can be challenged to heavy metals, pesticides and PCB's. It is anticipated that this work will be initiated in FY 1974.

These laboratory experiments will be expanded in FY 1973 to include important crustacean and fish species, such as the rock crab, Cancer irroratus, the green crab, Carcinus maenas, the cunner, Tautogolabrus adspersus and the windowpane flounder, Scophthalmus aquosus. These organisms will be challenged with a

greater assortment of contaminant materials, such as pesticides, detergents and PCB's.

In FY 1973 adult rock crabs, green crabs and cunners will be exposed to copper, cadmium and mercury in acute tests. These metals will be used singly and in combination to determine whether these metals act synergistically or antagonistically. It will not be possible to work with the embryonic or larval forms of these organisms until rearing methods are well established in FY 1974 and 1975. The embryonic and larval stages will then be exposed to environmental pollutants in FY 1975-1977.

The adult crustaceans and finfish will be challenged to cadmium, copper and mercury in 48 to 96 hour tests in 20-gallon glass aquaria on a bi-weekly basis. These short term bioassay tests will not only provide information regarding levels at which pollutants cause mortality, but will provide test organisms which have been exposed to various marine pollutants to other researchers within the Middle Atlantic Coastal Fisheries Center, NMFS, as part of a multi-disciplined cooperative study (see Phases 3, 4, 5, 6, 7 and 8).

Excellent facilities for such tests are available at the Milford Laboratory. To examine the effect of contaminants on the above mentioned organisms is within our present in-house capability. However, to do a more through job of examining the effect of more contaminants on more marine organisms and their various life stages,

it would be necessary to contract such work out to various private organizations and universities. As mentioned previously (see Phase No. 1.),Duke University Marine Laboratory will be contracted to challenge larval forms of several species of crabs to various heavy metals and pesticides in FY 1973-1975.

Perhaps of even more importance than the acute, short-term effects of exposure are the long-term, chronic exposures of sublethal stress levels of pollutants. Such exposure may limit development growth, reproduction, metabolism, or other physiological responses. These tests, through necessity, would have to be in continual operation for one to two years, depending on the life history of the organism being tested. The rock crab, the surf clam and the windowpane flounder will be continuously exposed to sublethal levels of a heavy metal, a pesticide and PCB, starting in FY 1974. Specimens will be periodically sampled for histopathological anomalies and changes in enzymological process, osmoregulatory ability, antibody response and measurement of growth (see Phases 3, 4, 5 and 6). These organisms will either be induced to spawn or will spawn naturally and the reproductive potential will be determined. The embryonic forms will then be reared through the larval and juvenile stages to determine whether the above physiological conditions were altered or not. Any genetic damage caused by these sublethal levels of pollutants will be assessed by examining chromosome linkage in the embryos of these

organisms (see Phase 7).

To conduct these chronic exposure studies, it would be necessary to purchase the proper equipment and develop the test systems. Such systems will be made available in FY 1974. Setting up this system would require the purchase of holding tanks, proportional diluters for dispensing pollutants, chemicals and a disposal system for waste treating the effluent from the test tanks. The cost of these items would be $35,000.

In order to initiate studies involving several species of marine organisms, we will contract some of these studies to private organizations and universities in FY 1973. The cost of the above contracting services would be $40,000 in FY 1973 and $50,000 per year through FY 1974-1977. The estimated cost for both in-house and contract work is $490,000.

The work outputs derived from these studies would be made available to the Environmental Protection Agency for developing water quality criteria, which, in turn, will be used for developing water quality standards for our aquatic ecosystems. The study would also provide an informational base useful in coastal zone management and future planning functions, as well as to provide information necessary to assess the environmental impact of proposed coastal zone developments.

Studies on the effect of eleven heavy metals on embryos and larvae of oysters and clams will be completed in FY 1973 and the information will be made available to the Environmental Protection Agency and will

be disseminated through scientific journals. It is also expected that studies of the effect of cadmium, copper and mercury on adult rock crabs, green crabs and cunners will be completed in late FY 1973 and will be made available as above.

Phase No. 3 - Physiological Processes

The exposure of marine organisms to pollutants may not only kill them outright, but may cause changes in physiological processes which decrease their chances for survival and reproduction. It is necessary, therefore, to conduct studies which determine the effect of pollutants on the physiological processes of marine organisms. Our present in-house research involves studies to determine changes in osmoregulation of the rock crab, Cancer irroratus, and green crab, Carcinus maenas, when exposed to copper and cadmium and changes in oxygen consumption rates of gill tissues from these organisms after exposure. In FY 1973 this research will be expanded to other metals, as well as to study changes in the osmoregulation of the cunner, Tautogolabrus adspersus. These studies will be completed in FY 1974. Studies will also be made in FY 1973-1975 to determine changes in osmoregulation of small marine organisms such as the grass shrimp and juvenile mollusks. Respiratory studies with stressed animals will be expanded to include polarographic measurements of large animals as well as an expansion of present micro-respirometry techniques. Respiration rates of crabs, fish and mollusks will be determined " in vivo " during exposure to heavy metals. This capability will be expanded to include larval fish and crustaceans, should rearing techniques for these species be developed within the Center. The estimated cost of the proper instrumentation required

to conduct these studies would be $11,800.

To study more fully the physiological effects of pollutants on marine organisms, studies of neuromuscular (including cardiac) physiology of stressed organisms will be initiated during FY 1974 - 1975. The organisms selected for such studies would be the surf clam, rock crab and windowpane flounder, since they represent the three major groups in the New York Bight area and are already being collected as the target species for an on-going dump site study in this area. The instrumentation needed for such studies can be adapted to record blood pressure, blood oxygen levels, bioelectric potentials, body temperature, pH and pCO_2 levels, as well as muscle forces displacement. The instrument required is a polygraph write-out recorder and its estimated price is $5,000.

In studies of this type, it is also desirable to trace movements and retention of contaminants within an organism to evaluate more fully the specific effects. By using isotopic tracers it is possible to determine the pathways by which contaminants are accumulated in marine organisms, thus, we would understand more fully the cause of death in marine organisms or the reason for stress. Studies using isotopic tracers will be started in FY 1975 and continued through FY 1977. The instrumentation needed for such studies is a liquid scintillation counter at a cost of $12,000.

With the limitations on new positions in-house and the amount of work to be conducted in such studies, it would be possible to develop cooperative arrangements with local universities in the vicinity of the Milford and Sandy Hook Laboratories or to contract this work out to these universities. The estimated cost for such contract work would be $125,000 over five years. The total cost of in-house work and contract work for FY 1973 - FY 1977 would be $280,000.

The output from this unit will be in the form of quarterly reports and published manuscripts. The data from initial osmoregulatory studies will be in publishable form by November 1972 and a second report on these studies by January 1973.

Phase No. 4 - Biochemistry/Enzymology

In FY 1973 a long-range study will be initiated to determine " barometer " enzyme systems in selected marine organisms, both normal and subjected to pollutants, and to coordinate findings with concurrent studies in bioassay, physiology, immunology, histology and genetic stress (see Phases 2, 3, 5, 6 and 7).

The purpose of this project is to discover significant enzymological changes in the tissues of marine animals during exposure to known amounts of selected pollutants at known rates, and to interpret any such changes from the standpoint of biochemical adaptation or malfunction in response to the pollutant. By determining the degree and rate of such change in the light of concurrent work in related disciplines, it should be possible to monitor and even to predict the probable success or failure of the species to survive under known conditions of pollution.

FY 1973 will be the requisite exploratory period, during which tissues of the target animals (crustaceans, fish and shellfish) will be examined and protocols developed for key enzyme systems (for instance, NADP-linked oxidoreductases that generate NADP for lipogenesis and for steroid synthesis) and for those enzymes that exist in more than a single form (polymorphs). Normal patterns, both enzymographic* and spectrokinetic*, will be established for selected

* Enzymography = visualization of polymorphic enzyme systems via gel electrophoresis followed by staining with a medium that is specific

for a particular enzyme activity and that incorporates precipitable and chromogenic proton acceptors. Spectrokinetics = spectrophotometric measurement of an enzyme's activity under programmed assay conditions

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enzymes in the target species. In vitro work with pollutant metals, to determine their direct effect upon enzyme reactivities, will also be carried out during FY 1973, and acute-static in vivo work will begin. FY 1974 will continue work begun in FY 1973 and will concentrate upon acute-static in vivo studies, beginning with exposure of the target animals to a range of concentrations of selected heavy metals, and progressing to a study of synergistic effects. Main objects of scrutiny will be the polymorphic enzymes, whose enzymographic profiles may well alter as the animal adapts to its changing environment by changing the proportional concentrations of isoenzymes catalyzing the same reaction but differing in their optimal requirements. Altered enzymatic reactivities to imposed stress in vitro (for instance, a mild heating of the tissue, or addition of negative allosteric effectors to the assay medium) will also be examined. It would be useful both here and in the later chronic studies to have a bacteriological capability, which could explore the relative susceptibility of the test animals to specific bacterial infections (see Phase No. 5).

By FY 1975 it will be possible to perform chronic-exposure studies that would more nearly reflect environmental conditions as they are or might be. By this time, specific enzyme systems will

have been selected for study, their protocols (enzymographic and spectrokinetic) worked out, and normal and acute-toxic patterns established. Chronic-exposure studies will furnish the data necessary to fill in that range of pollutant concentrations where living animals are still attempting to adapt and survive.

In FY 1973-74 the equipment and supplies needed to conduct enzymographic and spectrokinetic studies are software ($4,500), biochemicals and chemicals ($1,000.), double-beam ratio recording spectrophotometer ($10,000), pH meter ($600), semi-micro analytical balance ($1,000), electrophoretic apparatus ($2,000) and a preparative ultracentrifuge ($13,000).

With the restrictions imposed upon hiring in-house, it is obvious that all of this work can not be handled by present in-house capabilities. It is intended, therefore, that some phases of the biochemistry-enzymology studies be contracted to competent outside personnel. Possible contractees are the University of Connecticut, Clapp Memorial Laboratory at Duxbury, Massachusetts, and the University of Bridgeport.

Initial phases of these studies will be started in FY 1973 and be continued in FY 1974, especially acute-static " in vivo " studies. Chronic exposure studies can be initiated in FY 1974 through contract and in-house work and continued through FY 1977. The estimated cost over 5 years would be $130,000 for in-house studies and $145,000

for outside contract work.

By detecting and measuring significant changes of enzymatic properties in light of concurrent work in related disciplines, it would be possible to monitor and even predict the probable success or failure of marine organisms to survive under known conditions of pollution. This information would, in turn, be most valuable to the Environmental Protection Agency, the U. S. Army Corps of Engineers and various planning agencies whose function it is to protect aquatic resources.

Phase No. 5 - Microbiology/Antibody Response

Although marine fish, crustaceans and shellfish have been found with high levels of chlorinated hydrocarbon and heavy metal contamination, there has been no direct evidence up until now linking these levels with death of the animals. One important link can be through the immune system of the animal. Individual elements of immunity such as antibody response, ability to rally phagocytic cells, antigen clearance rates and ability to resist large challenge doses of bacteria need to be examined in marine animals that have been exposed to heavy metals, chlorinated hydrocarbons and other pollutants.

Work currently in progress at the NMFS's Milford Laboratory concerns antibody response in a finfish, the cunner, Tautogolabrus adspersus, exposed to cadmium and projected work with several other heavy metals. With increased support, immunity in the following animals will be examined: windowpane flounder, Scophthalmus aquosus, rock crab, Cancer irroratus, green crab, Carcinus maenas, and surf clam, Spisula solidissima. Immune responses in these animals will be examined in the presence of cadmium, copper, mercury and at least two chlorinated hydrocarbons (at least one pesticide and one PCB). These studies will be initiated in FY 1973 in laboratory systems where controlled levels of pollutants will be metered to the animals in both acute and chronic exposure patterns (see Phase No. 2). Simultaneously with pollutant exposure the

animals will be injected with an antigen. Initially, in order to observe effects on the primary immune response, the animals will be injected with bovine serum albumin (BSA) or similar antigen to which the animal has had no prior exposure. Antibody response in finfish will be determined by radioimmunoassay. Antigen clearance rates in shellfish and crustaceans will be examined by injecting tritium labeled BSA followed by time lapse assay of the hemolymph using liquid scintillation spectrometry. Uptake of antigen by phagocytic cells will be examined by isolation of phagocytes and measurement of the quantity of tritiated BSA per phagocyte using liquid scintillation spectrometry.

After determining pollutant levels that have an effect on some aspect of primary immunity, animals will be exposed to the pollutant at the effective dose level and simultaneously challenged with selected species of environmental bacteria. Gross signs of physical deterioration or death of the animal versus lack of these symptoms in control animals (not exposed to pollutants) will be taken as prima facie evidence of pollutant effect.

These studies are important for two reasons: (1) We already know that many chemicals dumped into water systems are taken up by aquatic animals. It is likely that some of these will suppress the immune system and make the animal susceptible to bacteria that under normal conditions are harmless. This phenomenon is already well known in medicine; chemicals given to organ transplant patients (in order to

reduce immune rejection of the organ) often cause the patient to die of infection from a normal microbial resident of the patient's body. Any pollutants causing similar effects in marine animals should be identified and steps taken to reduce their levels. (2) A sensitive assay for pollutant effects may be developed using the immune response systems as a measure of toxicity. This is explained by the following: The primary immune response in animals requires the production of large numbers of new cells; any chemical affecting one of the pathways during protein synthesis is likely to limit the production of these cells. Therefore, inhibition of cells producing anitbody in fish and, possibly, inhibition of phagocytic cell production in crustaceans or shellfish, can prove to be a simple measurable indicator of potential harm to aquatic animals.

On-going studies at the NMFS's Milford Laboratory can provide the necessary expertise for the aforementioned studies. In addition to a yearly fixed cost for supplies, an expenditure of $12,500 is needed in FY 1973 for a liquid scintillation spectrometer. Currently, antibody response is measured by hemagglutination testing; antigen uptake and clearance is estimated by visual observation of carbon particle uptake. These methods are slower, more difficult and somewhat less reliable than those possible by liquid scintillation spectrometry. Approximately $4,000 of cost is project in FY 1974 for

autoclavable closed system tanks and accessory equipment to be used in exposure of marine animals to various species of bacteria.

With respect to outside contract work, it is difficult to find expertise in immunology-microbiology where there is also access to fish holding systems. Immunologists are traditionally affiliated with medical institutions and fishery biologists have not focused on immunity. Since NMFS's Milford Laboratory has both holding systems and expertise, it is suggested that a contract arrangement may be feasible with a biology or microbiology department of one of the nearby universities (Yale University, Fairfield University, University of Bridgeport or University of Southern Connecticut) whereby one (or more) graduate student could work at the Milford Laboratory on the stated problem.

It is anticipated that no university contract work can be awarded in FY 1973, but in FY 1974-1977 contract work for graduate student support can be awarded at a cost of $20,000 per year. The estimated cost of this 5-year program for both in-house and contract work is $182,000.

Projected time periods for completion of work parts are the following: FY 1973 through FY 1975 determination of primary immune response in animals exposed to pollutants; in 1973 emphasis will be placed on establishing and refining the measurement systems - focusing first on one animal and one pollutant. FY 1975 through

FY 1977: exposure of marine animals to a broad series of bacterial species in the presence of pollutants; also refinement of pollutant dose-effect relationships to establish an assay system based on immune response.

This work should result in information that will provide a focus on certain types of pollutants in the aquatic ecosystem. Cooperative efforts between NOAA, the Environmental Protection Agency and state and local pollution abatement agencies can then be directed toward reduction of specific contaminants. This information should also be widely disseminated through publication in appropriate technical journals.

Phase No. 6 - Histopathology

Utilizing tissues from " in vivo " studies (see Phase No. 2) it is essential that the pathological conditions, both gross and microscopic, in cells, tissues and organs of marine organisms under natural or experimental conditions that may be caused by infectious agents or as a result of exposure (long term or short term) to environmental pollutants be examined. Special attention should be given to tissues known to be affected by specific pollutants. Standard histochemical, cytological, immunological and biochemical procedures will be used to describe and relate observed pathological changes to effects of certain pollutants.

Analyses should be made on organs, tissues, cells, and sera from selected invertebrate and vertebrate marine organisms from the New York Bight area to establish " normal " or baseline morphology and physiology. Animals can then be challenged with known toxic agents (see Phase No. 2) to determine sites or localization of infection, types and severity of host response, micropathogen development, mechanisms of disease resistance, and disruption of tissue and cellular integrity. Histological, bacteriological, tissue culture, immunological and biochemical methods will be used to develop techniques for the rapid and precise diagnosis of disease and pathological changes and to isolate, grow, characterize and identify infectious agents (viruses, bacteria, fungi, protozoa, metazoa)

present in moribund animals.

The NMFS's Laboratory for Ecology and Pathology of Marine Organisms has excellent capabilities for such studies. Because of the heavy research load already associated with this Laboratory, it is anticipated that some phases of these studies can be contracted out to various universities.

The recent acquisition of an electron microscope at the Oxford Laboratory will permit the identification, description of life cycle stages and taxonomy of micropathogens, and ultrastructure studies of cytology in host animals.

In FY 1973 it will require the addition of one position for in-house studies to expand on-going research. The cost of equipment and staffing for in-house work in FY 1973 is estimated at $25,000. In FY 1974 it will be necessary to add two positions to on-going, in-house studies with the additional purchase of specialized virological equipment and a preparative ultracentrifuge. The cost of in-house studies in FY 1974 is estimated at $62,000. The total cost of in-house studies from FY 1973-1977 would be $210.000.

With personnel restrictions imposed on Federal Service, it will be necessary to contract some of this work out. Possible contract outlets are universities and private research foundations. Cost of contracting the work should be approximately $30,000/year for the 5-year period. Both in-house and contract work will be phased in

during FY 1973 and will continue through FY 1977 and the estimated cost over 5 years is $355,000.

By determining pathological changes in light of concurrent studies in related disciplines (see Phases 2, 3, 4, 5 and 7), it would be possible to monitor and even predict the probable success or failure of marine organisms to survive under known conditions of pollution. This information would then be most useful to those agencies whose responsibilities are to protect the marine environment.

Phase No. 7 - Genetic Stress

Not to be overlooked in studies on the effects of pollutants on aquatic organisms is the genetic damage which can be caused by sub-lethal levels of a pollutant. By now very large numbers of reports have shown terrestrial plants and animals to be genetically affected by any number of environmental pollutants. Genetically damaging, though sub-lethal, concentrations of a pollutant have been known to : (1) cause undesirable shifts in the gene frequencies of a population; increase the load of lethal genes; cause chromosome aberrations and abnormalities in the distribution of chromosomes in gamete formation and embryonic cleavage; affect sterilization or result in semi-sterility in large numbers of animals which otherwise would have been good breeders. Since most mutagens are also carcinogens, genetically damaging levels of a pollutant can further result in an increased incidence of tumors in natural or cultivated populations.

These genetic effects are immediately always less striking than large-scale sudden mortalities. However, they can ultimately be more damaging because the insidious nature of the damage makes chance detection unlikely until such extensive damage has occurred that it would require many generations for repair to occur.

Genetic damage to marine organisms by marine contaminants has not yet been much considered. The question though has been

briefly discussed in some manuscripts originating from the Milford Laboratory for Experimental Biology. It is inconceivable that marine animals could be resistant to genetic change and damage. Sooner or later deleterious changes in the genetic material of these species, commercial or otherwise, will affect recruitment of the marine organisms into the population.

Aquatic animals which spawn their gametes freely into the water, where the egg is fertilized and cleaves, must be particularly vulnerable to gross genetic changes in the egg. This is so since they are completely lacking in the sort of protection that internally fertilized eggs of mammals and of the higher plants have.

Genetics of marine organisms is only now beginning to be researched. Consequently, many of the sorts of genetic tests that might be used to monitor for effects of pollution on non-marine species simply could not be effectively or practically carried out on marine organisms. However, chromosome damage to the spawned, externally fertilized developing eggs of certain chosen test species could easily and economically be used as an assay for genetically harmful contaminants.

Assays using chromosome damage as a criterion have been standard tests for years in determining safety levels of radiation, drugs and food additives. The development of a simple, rapid method for removing interfering yolk material from eggs removes what

was once the major obstacle to using the egg cell in any chromosome assay. The easy availability of marine eggs for assays on contaminants, and their use by NMFS, should attract special scientific and other interest to the results of these assays becuase of the involvement of reproductive cells and developing embryos. Most mammalian and plat tests have to be conducted with less critical somatic cells.

Such monitoring of marine organisms would specifically be done on (1) samples of fertilized, meiotic and cleaving eggs of representative animals from polluted areas which have been obtained by artifically induced spawning of the collected specimens in the laboratory. Comparisons would be made with eggs from animals living in non-polluted waters; (2) the eggs of animals treated with known concentrations of various pollutants under laboratory conditions at critical stages of their gametogenesis; (3) eggs spawned from healthy, unexposed animals which are treated after being spawned with known levels of specific pollutants or with natural seawater from areas suspect of pollution under controlled laboratory conditions.

To begin this work in FY 1973 the gametes of American oysters can be exposed to various levels of concentrations of 11 heavy metals. Cytological fixations are made of the eggs when they are in genetically critical stages of fertilization, meiosis and early cleavage. Cyto-genetic examinations of the chromosomes and cell divisions are then

made with a phase contrast microscope. Any pollution-induced abnormalities in the orderly distribution of the gene-bearing chromosomes should be readily detectable, since breakage and rearrangement of the chromosomes , as well as metabolic disturbances to the chromosomes, affect their gross morphology and lead to errors in cell division.

Phase contrast microscopic equipment and facilities for holding, spawning and fertilizing the animals collected for tests are necessary. Supervisory and assistant personnel with some cytogenetic expertise would be required.

Oysters and clams would be particularly suitable for such studies because they are readily spawned in the laboratory, a knowledge of their chromosomes already exists, and their chromosomes are large enough in size and small enough in number to score readily.

When methods become available in FY 1974-1975 for routinely obtaining embryos of other shellfish species (see Phase No. 1), in particular the surf clam, Arctic clam and deep sea scallop, studies will be made to determine the effect of pollutants on their chromosomes, Also, as routine methods are developed for rearing the rock crab and windowpane flounder in the laboratory in FY 1975-1976 (see Phase No. 1), it will be possible to make the same determinations for these organisms.

Baseline studies of this type can be easily handled at the NMFS laboratory in Milford, Connecticut. For more intensive studies of this type, such as the effect of sub-lethal concentrations of pollutants on the entire life cycle of an organism through one or two generations, it may be necessary to seek outside assistance. Possible contractors for such studies would be universities and, perhaps, Clapp Memorial Laboratory at Duxbury, Massachusetts.

In-house acute studies on shellfish, crustaceans and fish can be initiated in FY 1973 and completed in FY 1976 at minimum cost, since the expertise and capabilities for doing such studies are already available.

For sub-lethal chronic exposure studies to determine long-term genetic damage, it will be necessary to commence work in FY 1974 and continue through FY 1976. The cost of this study is estimated at $33,000 in FY 1974 and $30,000/year thereafter. The estimated cost over 5 years would be $123,000.

The work output derived from these studies would be used by management and natural resources agencies to assist them in conserving marine resources and aquatic ecosystems.

Phase No. 8 - Analytical Chemistry

It is essential that the animals exposed to the various pollutants in the bioassay studies (see Phase 2) be analyzed in order to determine the uptake of the pollutant. In particular, vital organs, such as liver, kidney, muscle and gonad, should be analyzed so that correlations can be made between specific amounts of pollutant buildup in these vital organs and the observations of physiological stress conditions.

During the course of these bioassay studies, the water in which the organisms are exposed must be analyzed to determine the concentration of pollutant in the water at the beginning of the test and at the end of the test in order to establish the quantity of pollutant exposure during the test period.

During the first phases of the physiological stress studies, conventional analytical chemical techniques will be employed to determine the pollutant concentration in vital organs and water. Heavy metal concentrations will be determined by atomic absorption spectrophotometry and neutron activation analysis. Pesticides will be determined by gas chromatographic techniques. Some organs, because they are so small, will require development of micro-techniques for analyzing pollutants. These techniques are available in many instances, but will require refinement to be applied to this analytical problem.

The ideal way to determine the specific concentration of contaminant uptake in the vital organs of marine organisms would be to expose the organisms to radioisotopic labeled compounds. This radioisotopic "tracing" of pollutants in the vital organs can be coordinated with other studies (see Phases 3 and 5) and instrumentation can be shared with these programs.

The chemical analyses needed for such studies can be provided by the in-house unit that will be making the survey of chemical contaminants in marine organisms in the New York Bight baseline study. The cost of such work is estimated at $10,000 for FY 1973 with a total projected cost of $60,000 for the 5-year study.

EQUIPMENT NEEDED FOR STUDIES OF PHYSIOLOGICAL STRESS

1. Temperature Control Bath (FY 74)
2. Software
3. Supplies (expendable)
4. Fish holding tanks (FY 74) 8
5. Proportional diluters (FY 74) 4
6. Waste treatment disposal system (FY 74)
7. Chemicals
8. Micro-osmometer (FY 73)
9. Chloride meter (FY 73)
10. Polarograph (FY 73)
11. Polygraph (FY 74)
12. Liquid scintillation counter (FY 73)
13. Biochemicals
14. pH meter (FY 73)
15. Analytical balance (FY 73)
16. Double-beam, ratio recording spectrophotometer (FY 73)
17. Electrophoretic apparatus (FY 73)
18. Preparative ultracentrifuge (FY 74/2)
19. Autoclavable tanks (FY 74)
20. Virological equipment (FY 74)

ALGOLOGY STUDIES

In addition to basic on-going research programs in algology, the effects of contaminants on marine algae and the specific concentration factors for known taxa should be determined.

Studies of algology require the following phases:

1. Continued refinement of techniques for culturing marine algae, both unicellular and multi-cellular forms, in the laboratory.
2. Exposure of these marine algae to precise levels of pollutants.
3. Studies with radioisotopic tracers to determine uptake by marine algal species belonging to different taxonomic groups.

A study of this type is a self-contained unit in which all phases (requirements 1-3) should be conducted by one organization (or cooperating agencies). A good deal of expertise in this area of research is already available at the NMFS's Milford Laboratory and numerous University and Institute laboratories of the Northeast Region. Cooperative work between these agencies is possible.

Since phytoplankton is at the base of the food chain of all marine animals, it is essential that studies be made on the effects of contaminants on these organisms. If pollution were to eliminate phytoplankton or the normal species composition in a particular area, it could have a drastic effect on the remaining marine animals in the area, since the basic link in their food chain would be destroyed.

Certain types of pollution may be toxic to some or all species of phytoplankton, or may enhance growth to the degree of being harmful to physiological processes of other species and so reduce productivity.

These studies will be essential to those studies undertaken in the field to examine the correlation between nutrient levels, contaminants and phytoplankton composition and abundance in the New York Bight region to assess negative aspects of environmental degradation. Additionally, phytoplankton species may be excellent " indicator organisms " as means of assessing changes in environmental characteristics.

The techniques for culturing marine micro-algae in the laboratory are fairly well defined, but studies of specific requirements are necessary when work on species of algae that are not already in culture is attempted. Once the various indicator species are identified in baseline studies, it would be possible to expose laboratory reared specimens to particular pollutants. Some on-going studies of this nature are already available from current and past NMFS work. It would be most appropriate to initiate bioassay studies with marine algae in FY 1973 and continue them through FY 1975. In FY 1974 it would be possible to examine the effects of pollutants more closely by using radioisotopic tracers to determine actual uptake of pollutants by marine algae, hence, a determination of which pollutants are passed

on through the food chain. These studies through necessity will continue through FY 1976. In FY 1976-1977 through data analysis in FY 1976-1977 it will be possible to correlate the findings of field and laboratory information to determine the impact of pollution on phytoplankton in the New York Bight region, hence, the impact on the living marine resources of this region. It may also be possible at this time to predict what effect a particular pollutant will have on environmental degradation.

The cost of such studies over a 5-year period will be $235,000, and it is anticipated that funding for FY 1973 will be $35,000 and for FY 1974 will be $50,000.

The output of such studies will be most important to those agencies responsible for protecting our marine resources.

OBJECTIVE NO. 3

In order to be able to determine which species or organisms would be " indicator organisms " as a means of assessing changes in environmental characteristics, Objectives 1 and 2 and their related studies must be well underway or near completion. Once it is established through field inventory surveys what resources are present in the New York Bight and how these resources are affected by pollutants in the laboratory, it would be possible to determine which species of marine organisms in the New York Bight region would be " indicator species " (those species of organisms which demonstrate low tolerance to pollution). From the previous studies it will be possible to determine " indicator species " for the various trophic levels. The absence or reduction of these indicator organisms in a particular environmental situation will assist the environmental manager in predicting " alert conditions " and who, in turn, can take the proper steps to avoid environmental degradation. The possibilities of observing " alert conditions " will result from the predictive model developed in the baseline studies and the observed results in the laboratory.

FIVE YEAR STUDY PLAN OF THE BIOLOGICAL AND CHEMICAL BASELINES AND PHYSIOLOGICAL STRESS OF MARINE ORGANISMS IN THE NEW YORK BIGHT

	FISCAL YEARS				
	73	74	75	76	77
ECOLOGICAL BASELINES					
Estuaries and Nearshore					
Initial survey	—				
Seasonal sampling program	———	———			
Hudson Shelf Valley and North and South Coastal Zones					
Initial survey	—				
Seasonal sampling program		———	———		
Laboratory processing of Samples		———	———	———	
Data analysis		———	———	———	———
Model studies and testing			———	———	———
Monitor program design and tests			———	———	———

	73	74	75	76	77
MICROBIOLOGICAL BASELINES					
Development and evaluation of microbial techniques	———				
Qualitative and quantitative identification of microbial types	———				
Systematic microbial sampling		———			
Intensive sampling			———		
Commence monitoring					—
CHEMICAL BASELINES					
Survey of contaminants in invertebrates and fish	—				
Establish monitoring program for contaminant levels in invertebrates and fish			———		
Radionuclides - distribution, abundance and biologic availability	———				
Organic pesticides - distribution, abundance and biologic availability	———				
PHYSIOLOGICAL STRESS					
1) Development of Rearing Techniques					
Embryonic and larval shellfish	———				
Embryonic and larval crustaceans		———			
Embryonic and larval fish		———			

	73	74	75	76	77
2) Bioassay (Exposure)					
Acute Tests (Short Term)					
Embryonic and larval shellfish	———	———	———		
Adult crustaceans	———	———			
Adult fish	———	———			
Embryonic and larval crustaceans			———	———	
Embryonic and larval fish			———	———	
Chronic Tests (Long Term)					
Adult shellfish	———	———	———	———	———
Adult crustaceans	———	———	———	———	———
Adult fish	———	———	———	———	———
3) Physiological Processes					
Osmoregulation Studies					
Fish	———	———			
Crustaceans	———	———			
Oxygen Consumption Studies					
Fish	———	———			
Crustaceans	———	———			
Larval shellfish	———	———			
Larval fish			———	———	
Larval crustaceans			———	———	
Neuromuscular Studies		———	———	———	
Radioisotopic Studies			———	———	———

	73	74	75	76	77
4) Biochemistry-Enzymology					
Exploratory Studies	———				
"In Vitro" Studies of Tissues or Tissue Extracts	———	———	———		
Enzymatic Aspects of Acute Exposure of Whole Animals "In Vivo"	———	———	———		
Enzymatic Aspects of Chronic Exposure of Whole Animals "In Vivo"		———	———	———	———
5) Microbiology-Antibody Response					
Immune Response Studies	———	———			
Bacterial Exposure - Dose Effects			———	———	
Dose Response - Immune Assay			———	———	
6) Histopathology					
Acute Tests (Short Term)					
Adult shellfish, fish and crustaceans	———	———			
Chronic Tests (Long Term)					
Adult shellfish, fish and crustaceans	———	———	———	———	———

	73	74	75	76	77
7) Genetic Stress					
Embryonic Shellfish	———				
Embryonic Crustaceans			———		
Embryonic Fish			———		
Entire Life Cycle (Long Term)		———	———	———	
8) Chemical Analyses	———	———	———	———	
ALGOLOGY STUDIES					
Culture of multicellular species not available for laboratory studies	———	———			
Exposure/Bioassay of algae to pollutants	———	———			
Radioisotopic tracer studies of contaminant uptake		———	———		
Data Analysis				———	———
IDENTIFICATION OF INDICATOR ORGANISMS				———	———
ALERT CONDITIONS				———	———

PROPOSED BUDGET DISTRIBUTION IN NEW FUNDING
(in thousands)

	FY 73	FY 74	Total FY 73-77
BIOLOGICAL BASELINES			
Ecological Baselines			
Field Inventory	115.0/2	350.0/2	615.0
Laboratory Workup	90.0/2	300.0/2	825.0
Data Analysis	12.0	40.0/1	280.0
Model Systems		10.0	150.0
Monitoring Design		10.0	170.0
Microbiological Baselines	60.0/1	170.0/4	680.0
Chemical Baselines (including radioecology)	100.0/3 377.0/8	350.0/1 1,230.0/10	1,150.0 3,870.0
PHYSIOLOGICAL STRESS			
Development of Rearing Techniques		42.0/2	168.0
Bioassay	50.0	110.0/3	490.0
Physiological Processes	45.0/1	72.0/1	306.0
Biochemistry	51.0/1	65.0/1	275.0
Microbiology	30.0/1	40.0	182.0
Histopathology	51.0/1	88.0/2	355.0
Genetic Stress		33.0/1	123.0
Chemical Analyses	10.0 237.0/4	15.0 465.0/10	60.0 1,959.0
ALGOLOGY STUDIES	35.0	50.0	235.0
	649.0/12*	1,745.0/20*	6,064.0

* In-house positions required
Budget figures include possible contract work