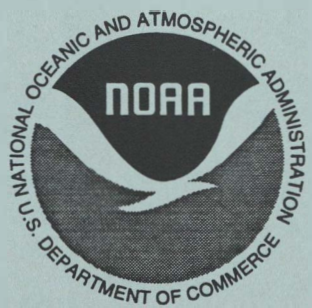
COASTAL OCEAN PROGRAM

IMPLEMENTATION PLAN FOR THE TOXIC CHEMICAL CONTAMINANTS THEME AREA FY95

FINAL
SUBMITTED TO THE
NOAA COASTAL OCEAN PROGRAM OFFICE
December 20, 1994



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SYNOPSIS

This Implementation Plan describes the scientific and technical program proposed for FY95 under the Toxic Chemical Contaminants Theme Area of NOAA's Coastal Ocean Program (COP). NOAA's COP was designed to strengthen and provide improved focus for various programs ongoing in the coastal marine environment. The Toxic Chemical Contaminants Theme was initiated under the COP in 1990, and is directed at developing information needed by decision makers concerning the effects of contaminants on coastal resources. The program of this COP Toxics Theme Area augments, integrates, and expands previously ongoing efforts of the NS&T Program and related research and development efforts within the Environmental Conservation Division of the National Marine Fisheries Service Northwest Fisheries Center.

INTRODUCTION

The following presents the implementation plan proposed for conducting the scientific and technical program of the Toxic Chemical Contaminants Theme Area of NOAA's Coastal Ocean Program (COP) in FY95. This plan primarily proposes studies to continue the technical and scientific program that was initiated in this theme area several years ago to assess and predict the levels and effects of toxic contamination in U.S. coastal and estuarine waters and to develop improved means for making such assessments.

However, it is clearly recognized that toxic chemicals are only one of the categories of serious threats to ecosystem health in coastal and estuarine waters. Nutrient overenrichment, overfishing, habitat destruction, and other factors all can play a major role in contributing to environmental degradation in specific areas. Furthermore, these various threats influence and interact with each other and with other properties of the environment in various and multiple ways leading to a complex network of interactions and relationships. Thus, although we can often detect situations where anthropogenic influences are seriously affecting our near-coastal and estuarine environments, in most situations we are not able to specify the relative importance of the various anthropogenic stresses in causing the observed ecological consequences. For example, we observe the decline in an important fish species, but we find it difficult to determine what role various stressors such as direct and indirect toxic effects, changes in food availability and oxygen conditions due to nutrient overenrichment, destruction of wetland breeding and nursery areas, and fishing pressures have had on the overall population decline.

The Toxic Chemical Contaminants Theme area has been investigating expanding the scope of the studies it proposes to support to include consideration of the levels and effects

of all anthropogenic stresses related to effects on specific fishery and fishery-related resources. The goal of this expansion would be to develop a predictive capability to assess and quantify the effects not just of toxic chemical contamination, but of the combined effects of all the anthropogenic stresses applied to particular coastal and estuarine resources. Discussions have been held with the Nutrient-Enhanced Productivity and the Estuarine Habitat theme areas regarding combining activities for the development of a program directed toward this goal. These discussions have not, as yet, led to a fully integrated plan for combining the work in the three theme areas, however.

BACKGROUND

Toxic Contaminants and the Coastal Environment

The technologically advanced society of the United States releases many different potentially toxic substances to the environment. Domestic use of a wide variety of chemicals results in their disposal in garbage, sewage, and other means that lead to their ultimate escape to the environment. Waste waters and solid wastes from our industrial and municipal treatment facilities often contain appreciable quantities of toxic trace metals and/or organic chemicals such as polychlorinated biphenyls (PCBs). Our intensive agriculture spreads pesticides and herbicides across many millions of acres each year. The exhausts from our millions of automobiles discharge polycyclic aromatic hydrocarbons (PAHs) and other substances resulting from the incomplete combustion of fuel. Power plants that burn coal and other fossil fuels for energy production release large amounts of sulfur and nitrogen gases and other pollutants to the atmosphere. The usage, with concomitant environmental dispersal, of potentially toxic chemicals has provided a major underpinning for the development of our society to date.

Toxic substances may be immobilized for long periods in specific areas on the land, as, for example, in well-designed land fills. While such immobilization also occurs naturally through adsorption of the chemicals onto soil particles, much of the released toxic material rather quickly finds its way into coastal and estuarine waters (including the Great Lakes), either through direct discharges, or indirectly through atmospheric deposition, surface runoff and ground water inputs. NOAA's recent environmental studies, including the National Status and Trends Program, have generated a great deal of evidence demonstrating the presence of appreciable quantities of anthropogenic contaminants in certain U.S. estuarine and coastal areas, especially in the vicinity of major urban areas (e.g. McCain et al., 1989, 1992; National Oceanic and Atmospheric Administration, 1988, 1989; O'Connor et al., 1989; Robertson, 1989; Robertson and O'Connor, 1989; Varanasi et al., 1988, 1989a).

Much less is known about the ultimate fates of these contaminants and especially about their effects on living resources and other organisms in contaminated areas. Very low trace concentrations of toxics in coastal environments seem to cause no demonstrable harm. However, when the exposures of the organisms in the environment exceed certain poorly understood and largely unquantified threshold levels, undesirable biological effects start to occur. These effects range from obvious direct consequences (such as fish kills) to effects that may be subtle and difficult to detect but nevertheless often are serious, such as changes in feeding or predator avoidance behavior or in vital life processes such as respiration, growth, and reproduction. Toxic contaminants may also accumulate in living marine resources at levels that pose a threat to human consumers of these resources.

Numerous instances have been documented where toxic contaminants have caused deleterious effects in coastal environments. For example, there is a rapidly expanding body

of evidence linking high prevalences of hepatic lesions, including neoplasms, in bottom-dwelling fish to exposure to the mixtures of chemical contaminants found in certain coastal urban bays and in rivers connected to the Great Lakes. Hepatic neoplasms have been reported in English sole (*Parophrys vetulus*) residing in urban bays of Puget Sound, Atlantic tomcod (*Microgadus tomcod*) from the Hudson River Estuary, winter flounder (*Pseudopleuronectes americanus*) from Boston Harbor, white croaker (*Genyonemus lineatus*) in coastal waters adjacent to Los Angeles, and brown bullhead (*Ictalurus nebulosus*) in waterways associated with the Great Lakes. Other studies have identified pollution-associated reproductive impairment in marine fish species, including English sole and white croaker. Well-documented cases of the effects of toxics are relatively few, however, and are generally located in areas of relatively very high contamination, or where long-term study by local research institutions have generated the required data base. There are many more situations where toxics are known or suspected to be at appreciably elevated levels, but where adequate information is not available to determine what degree of degradation, if any, has been caused in the exposed biological community. The magnitude and extent of the threats from toxic substances to the Nation's coastal environments at large are not well documented nor are the specific causes of these threats well understood.

The resource managers and others who make vital decisions on regulation and protection of our coastal environments need accurate and reliable information on toxics, their sources, their accumulation and fate in the environment, and their effects on populations and communities of exposed organisms. Such information is crucial to provide the basis for making well-informed decisions on how to proceed with the development needed for our economic growth, while still protecting our coastal environments and the resources they provide so these will be available for the benefit of future generations.

NOAA's Programs

In 1984, the National Oceanic and Atmospheric Administration (NOAA) initiated the National Status and Trends (NS&T) Program, to help provide, on a national scale, the needed information on toxic contaminants in the coastal environment. The purpose of this program is to document the changing status of environmental quality of our nation's estuarine and coastal waters. To date the primary focus of the NS&T program has been on toxic contaminants. The interrelationships and evolution of the NS&T program and related research efforts are introduced briefly here.

The NS&T Program has three primary assessment components: nationwide monitoring, historical trends assessment, and biological effects surveys. Nationwide monitoring is conducted primarily through the Mussel Watch Project, which measures the levels of toxic chemicals in biota and sediments. The data from this project support spatial and temporal comparisons of contaminant levels, and help to determine which regions around our coasts are of greatest concern with respect to environmental degradation. Mussel Watch measures the concentrations of 24 polycyclic aromatic hydrocarbons (PAHs); 20 congeners of polychlorinated biphenyls (PCBs); DDT and its breakdown products DDD and DDE, along with 9 other chlorinated pesticides; butyltins; and 13 trace elements in sediments and in mussels and oysters. Mussel Watch biennially samples bivalve mollusks at about 250 regionally representative sites. The resultant data are stored in NOAA national data bases, and are made available to coastal and marine resource managers and to the public through a variety of interpretive reports and other media.

The second NS&T component is Historical Trends Assessment. For those regions that are indicated to have the greatest potential for environmental quality problems, the

available NS&T data are synthesized with literature information on the distribution and effects of toxic contaminants to assess the magnitude and extent of degradation to living resources and to identify further information needs. In some of the areas, sediment cores are being analyzed to provide improved understanding of the historical trends in contaminant inputs.

Under the third NS&T component, and with support from NOAA's Coastal Ocean Program, more intensive Biological Effects Surveys are carried out in those regions where the first and second components have identified needs for better information to assess the potential for environmental degradation. These studies are designed to document the occurrences of biological effects in living marine resources, especially in bivalve molluscs and bottom-dwelling fish, and to quantify the magnitude and extent of ecosystem degradation. Surveys are generally conducted along concentration gradients of contaminants and focus on such measures of biological effects as histopathology, reproductive impairment, genetic damage, and sediment toxicity. New indicators of contamination and bioeffects are also evaluated in these studies. These surveys lead to updated assessment reports on the environmental quality and biological effects in the region, which in turn are establishing the basis for broader assessments that will evaluate the present understanding of the distribution and possible threat from these contaminants to U.S. coastal waters nationwide.

The NS&T Program is very closely coordinated with the Estuaries component of EPA's Environmental Monitoring and Assessment Program (EMAP-E). A joint NOAA-EPA agreement provides a mechanism for coordination of NS&T and EMAP-E planning activities leading to the establishment of an unified NOAA-EPA program for monitoring the status and trends of estuarine and coastal environmental quality and ecological conditions. Where the two programs are making closely related measurements, i.e. contaminant concentrations in fish and sediments, care has been taken to assure that the same contaminants are measured with documented quality assurance so that the results can be merged. The data from the two programs are being exchanged quickly and joint reports are being developed.

The work carried out under this Toxic Chemical Contaminants Theme Area of the Coastal Ocean Program has very substantially expanded the level of effort placed on the intensive Biological Effects Surveys in the NS&T Program. To support and promote the survey effort, this COP Theme Area also places major emphasis on the development and testing of bioindicators of contaminant exposure and effects and on research to establish definitive links between contaminant exposure and response. This COP Theme Area is directed at accelerating the development of information needed by decision makers concerning contaminant effects. This FY95 Implementation Plan identifies and justifies the continuing activities of the Toxic Chemical Contaminants Theme Area of the Coastal Ocean Program and describes how those activities are coordinated and integrated with activities ongoing in the NS&T Program and other pertinent NOAA programs. Recent progress under each of the various program elements is described briefly in a subsequent section of this document.

GOALS AND OBJECTIVES

The ultimate purpose of this program is to develop and provide up-to-date and reliable information to support resource management decisions involving toxic contamination of our coastal environments. The Coastal Ocean Program has established two long-term goals for its Toxic Chemical Contaminants Theme Area to promote development of this needed information:

- Assess the status and trends of environmental quality in relation to levels and effects of toxic contamination in U.S. marine, estuarine, and Great Lakes environments.

- Develop a predictive capability for effects of toxic contamination on marine resources and human uses of these resources.

The following more specific objectives have been established to ensure the development of a monitoring, research, and assessment program that will make a major contribution toward achievement of these goals:

Objective 1. To assess the magnitude and extent of environmental degradation related to contamination by toxic chemicals, using a series of regional studies as a basis for integration on a national scale.

Objective 2. To establish the physical, chemical, and biological factors that control uptake and accumulation of toxic chemicals in marine organisms and the quantitative relationships between these factors and the levels of contaminants measured in these organisms.

Objective 3. To develop new, or improve existing, methodologies for quantifying biological effects associated with exposures to environmental contaminants, resulting in the enlargement of the suite of bioindicators (e.g., measures of biochemical, immunological, physiological, and histopathological changes) that best assess contaminant-induced adverse biological effects in marine species.

Objective 4. To establish links between contaminant exposure and significant biological effects in individual organisms, with focus on effects with potential implications at the population and community levels.

STRATEGY AND APPROACH

Four interactive program elements are included in this Implementation Plan, each focused primarily on one of the objectives listed above, but each designed to provide information or methodologies needed to facilitate and improve operations in one or more of the other elements, and to support the continued development of the large-scale objectives of the National Status and Trends Program. The elements, listed in the same order as their primary objectives (above), are:

A. Assessment of the Extent and Magnitude of Environmental Degradation Related to Contamination by Toxic Chemicals

B. Evaluation of Factors and Relationships that Control Uptake and Bioaccumulation of Toxic Chemicals

C. Development of New and Improved Methodology for Quantifying Bioeffects of Toxics

D. Bioeffects Research to Establish Links Between Contaminant Exposure and Significant Effects

These elements support each other and the ongoing national objectives of the NS&T Program. The Mussel Watch and coordinated monitoring with EMAP-E projects of NS&T

are designed to support a continuing assessment of the status of contaminant distributions and associated biological effects on a national scale, and evaluation of the temporal trends on that scale in relation to contaminant regulatory practices. These nationwide projects also help to identify those specific regions where contaminants are more likely to elicit biological effects in resident organisms, and in those regions, Intensive Bioeffects Surveys (Element A) are initiated to determine the occurrence of effects and to quantify their areal extent. Contaminant accumulation and bioeffects depend, however, upon both the environmental conditions surrounding an organism and the physiological condition of the organism itself. Complementary laboratory and field research is needed to improve our understanding of the relationships among contaminant loadings and distributions in the environment, tissue levels of contaminants bioaccumulated by resource organisms, and potential deleterious effects on these organisms. Element B of the Toxics Theme Plan provides a mechanism for testing and documenting the rates and levels of contaminant bioaccumulation under different exposure regimes. Although Element B has received no direct support during the program of the Toxic Chemical Contaminant component of the Coastal ocean Program, and none is proposed for FY95, Objective 2 above has been pursued indirectly through ancillary determinations of chemical body burdens in association with Elements A and C.

The specific measures of contaminant effects used at both the national and regional levels require extensive testing to verify and document their sensitivities and specificities to contaminants. Elements C and D of this Toxic Chemical Contaminants Theme Plan provide the needed vehicles for this complementary research. Under Element C, NOAA carries out detailed laboratory and field research necessary to verify the underlying contaminant-causality and dose-response relationships for different biomarkers. In turn, Element D provides for a broad, academically based research effort to identify and quantify new contaminant-related problems, and to support further development and testing of biomarkers and analytical methods. Biomarkers tested under Element C may be field-validated under Element A, in the nationwide monitoring program, or under Element D. An important concern throughout this program is to develop an understanding of the factors and relationships that control uptake and bioaccumulation of toxic chemicals from the environment. In all three active theme elements, therefore, attention is placed where practical on defining the physical, chemical, and biological factors that control uptake and accumulation of toxic chemicals in marine organisms and on establishing the quantitative relationships between these factors and the levels of contaminants measured in these organisms.

The ultimate indicators of success in this program include: (1) the extent to which our understanding of the basic processes underlying contaminant bioaccumulation and effects is improved (measured by numbers and quality of peer-reviewed scientific contributions); (2) the level of support provided to the long-term monitoring efforts in the NS&T Program and other federal and state monitoring programs (measured by the adoption within those programs of candidate biomarkers and analytical methods tested under the COP Toxics Theme); and perhaps most important, (3) the demand for, and use of, the data and information generated under this program by environmental planners and resource managers to support regulatory decision-making.

The Coastal Ocean Program initiated the Toxics Chemical Contaminants Theme area in FY90, with studies directed at the first and third elements (A and C). These ongoing activities were expanded in FY91, and have continued through FY94. A series of multiyear studies related to the fourth element (D) was initiated near the end of FY91, and these studies are reaching the conclusion of their efforts. Preliminary results from some of these ongoing studies are provided in this Implementation Plan under the appropriate programmatic element (or in the Appendices). The work to be implemented in FY95 under

the Toxic Chemical Contaminants theme area is outlined in the following sections, which deal with each program element in succession.

Element A: Assessment of the Extent and Magnitude of Environmental Degradation Related to Contamination by Toxic Chemicals

A series of systematic multi-year field surveys was initiated in FY90 as part of the Coastal Ocean Program to provide estimates of the magnitude and extent of appreciable ecological degradation in our coastal areas as a result of exposure to anthropogenic toxic materials. Selected biological indicator properties are being measured in selected coastal areas where substantially elevated levels of toxics have been found by the NS&T Program. The results will be used to develop estimates concerning magnitude and extent of degradation in each of the areas studied and, when surveys are completed for a sufficient number of individual areas, the cumulative data will be assembled to provide an overall national assessment. The individual regional surveys comprising this national effort will also provide valid baseline measurements against which future trends in the magnitude and extent of regional contaminant bioeffects can be documented.

Previously-funded field work in Tampa Bay, Hudson-Raritan Estuary, Newark Bay, San Pedro Bay, San Diego Bay, and Boston Harbor were completed before or during FY94. Bioeffects surveys were continued in coastal South Carolina/Georgia , in the bays of the Florida panhandle, and in the estuaries and bays of Southern California. Analyses of samples collected in these areas are projected to be completed in FY95. A bioeffects survey was initiated in FY94 in South Florida.

Based on the results obtained to date in these study areas, we plan to complete a preliminary assessment of the general extent and distribution of contaminant-related biological effects in U.S. coastal waters in FY95 (see Appendix A). This assessment will provide a synopsis of our current understanding of the scope and severity of toxicant bioeffects problems in the selected embayments studied thus far.

With funding anticipated from the South Florida Water Management District, a survey of sediment toxicity in portions of Biscayne Bay and adjoining canals will be initiated early in FY 95. Additional studies are proposed for FY95 in the coastal systems of South Florida, encompassing Biscayne Bay to the East and Florida Bay to the west. In addition to inputs of toxic chemicals from municipal, industrial and agricultural practices in the area, this coastal system is also subject to perturbations from inputs of municipal and agricultural nutrients, and also to extensive disruption and destruction of coastal habitat. Studies on the distribution and effects of toxic chemicals in this system will be planned and undertaken with the view toward eventual development of an integrated program on the combined effects of multiple stresses on the ecosystem and its resources. This research in South Florida will represent a major concentrated effort, involving an assessment of the combined effects of different anthropogenic stresses. It is likely that this research will extend for 5 years or more. The South Florida area is likely to be highly stressed and the COP surveys will quantify the degree of stress. Principles learned in the South Florida area should be applicable to new survey areas that are, perhaps, less stressed.

The intensive bioeffects surveys also provide a means to test promising new bioeffects indicators under operational field conditions. Biomarkers, including those currently undergoing laboratory testing under Element C, are also being field tested in the regional surveys. As the bioeffects surveys in each area involve several independent measures of contaminants effects, the surveys provide an excellent and relatively inexpensive opportunity to compare the performance of promising new indicators with the results from

well-established measures to aid in evaluating and interpreting the results from the developmental tests.

The bioeffects surveys include sampling and analyses to determine such properties as indicators of contaminant exposure and contaminant-induced stress both in fish and in sessile indigenous invertebrates, reproductive impairment and genetic damage in important fish species, sediment toxicity to sensitive organisms, and, in some areas, community structure of bottom fauna related to contaminant levels. Teams of experts from both inside and outside of NOAA cooperate and collaborate in these surveys. Much of the field and laboratory work is carried out by scientists from participating state and other federal agencies, academic institutions, and private enterprise, working cooperatively with NOAA scientists. Data interpretation and synthesis is performed primarily by NOAA scientists within both NOS and NMFS, in collaboration with other project participants. In the preliminary stages of each survey extensive discussions are conducted through phone calls, correspondence, and visits with organizations that have marine environmental monitoring and assessment responsibilities and are conducting research and monitoring related to marine and estuarine toxic contamination in the specific region proposed for study. Complementary and cooperative projects are developed and carried out wherever practicable.

While the primary criterion for selection of a survey area is its degree of contamination and the associated likelihood of biological effects, a secondary criterion is the potential for collaboration with other federal, state and local agencies. Many of the participating agencies have identified management needs for the information to be generated and are therefore willing to assist in the overall financial support of the program (See Budget Description). This type of cooperation and collaboration has led to a larger and more effective program than could have been carried out solely under NOAA support and helps to ensure that the program results have direct utility to regional environmental managers. Requests-for-proposals have been used widely for competitive selection of non-federal participants in this program. In several cases, however, highly qualified scientists (usually at nearby state universities) have carried out particular aspects of the program through subcontracts from the participating state agencies.

In FY95, major emphasis will be placed on completing the analyses and interpretation of results from surveys initiated previously in the Hudson-Raritan Estuary, Newark Bay, San Diego Bay, and Boston Harbor; and on preparing and publishing status reports for those systems. Samples and data collected in the third year of the Southern California surveys with FY 94 funds will be analyzed during FY 95. Also, intensive Bioeffects Surveys will be continued in the estuaries of South Carolina/Georgia, in the bays of the northwest Florida panhandle and in South Florida. Chemical analyses of sediments collected and tested for toxicity in FY 94 will be conducted during FY 95. Oyster biomarker assays will be conducted in several Florida bays during FY 95. The current status and planned activities for these bioeffects studies are described below in greater detail. Updated summaries of the recent results and progress for the studies in most study areas are provided in Appendix A.

Hudson-Raritan Estuary

Work funded in FY90 (but carried out largely in FY91) in the Hudson-Raritan Estuary measured a number of bioindicators of reproductive impairment in winter flounder, acute sediment toxicity to several indicator organisms, contaminant trends in sediment cores, and ambient water toxicity of copper and zinc at various sites in this estuary. A major sediment toxicity survey (Phase 1), using 3 test species and 4 toxicity endpoints, was carried out at

127 stations in FY91. Preliminary results from these studies were briefly summarized in Appendix A of the FY 94 Implementation Plan. Chemical analyses of many of these sediment samples were completed in FY 94 by Battelle Ocean Sciences with funding provided by U. S. EPA Region 2.

In Phase 2, a supplemental sediment toxicity survey was funded in FY 92 and implemented in early FY93 to provide greater spatial resolution in the Passaic and Hackensack Rivers and adjoining Newark Bay. This area was expected to cause acute toxicity from elevated levels of multiple toxicants and potential genotoxicity due to dioxin contamination of sediments. The sampling strategy for this area (and all intensive survey areas sampled subsequently) followed a stratified, random sampling design, which allowed us to quantify, in a statistically valid manner, the spatial extent of toxicity within the region. Sampling for this survey was completed in April-May, 1993, with logistics support from EPA Region 2 and the New York District of the Corps of Engineers. Amphipod toxicity tests were conducted by SAIC (Narragansett), and chemical analyses and dioxin equivalency bioassays were conducted at the U.S. National Biological Survey's Midwest Science Center in Columbia, MO. All of the Phase 1 work has been completed and data analyses are nearly completed. All of the Phase 2 work, except the dioxin equivalency bioassays, has been completed and results delivered to NOAA/ORCA. Results from both phases will be combined into a single summary report, a technical memorandum, in FY 95.

Boston Harbor

Bioeffects studies were carried out in Boston Harbor (mainly in FY 89 and FY90) using base funds. In the studies to date, NOAA has surveyed the incidence of histopathological disorders and other measures of bioeffects, including possible reproductive impairment, in resident winter flounder; the biochemistry of reproduction and reproductive success of resident mussels and soft-shell clams; and the toxicity of sediments (measured by inhibition of bacterial bioluminesc-ence). The study on reproductive impairment in bivalves (by Drs. McDowell-Capuzzo and Farrington at Woods Hole Oceanographic Insti-tution) was extended in late FY91 with COP support, and then continued into FY93, supported once again under base funds. This work is exploring the relationships between organic contaminant exposure and reproductive impairment in mussels (*Mytilus edulis)* and soft-shell clams (*Mya arenaria*) and will lead to the development of specific biomarkers of disease and contaminant exposure that may be linked to population-level effects in bivalve molluscs.

The sediment toxicity survey conducted in the Boston Harbor area represented an early pilot study, of very limited scope compared to surveys carried out more recently in other intensive study areas. Only 16 samples were collected throughout Boston Harbor, and these samples were unreplicated. Also, only one test endpoint of limited ecological relevance (Microtox^{R}) was performed.

To complete the assessment of Boston Harbor, an intensive survey of sediment toxicity was conducted in FY93, using tests of *Ampelisca* mortality, sea urchin egg fertilization, and microbial bioluminescence. This survey was carried out in June/July 1993, in the four major regions of Boston Harbor: Inner Harbor, Northwest Harbor, Central Harbor, and Southeast Harbor. Many of the sites coincided with the locations in which sediments were sampled in the previous survey. As of this report, analyses are still underway. A draft report is expected in FY95. The data from the initial sediment toxicity survey and comparative tests of bacterial bioluminescence (Demuth et al., 1993) have been published. Following completion of the chemical analyses of samples from this year's

survey, the data will be analyzed and major effort will be placed in FY95 on the synthesis of these results, along with those of previous studies, and the publication of a summary report. This report will synthesize chemical and bioeffects data from several sources, including all work supported by COP and NS&T funds. Previous data from several dredging and construction projects in the Harbor will also be evaluated, and reports of surveys of sediment chemistry, benthic community structure, sediment physical-chemical properties, and discharge points will be reviewed. The distribution of non-toxic and significantly toxic samples in Boston Harbor are illustrated in Appendix A.

Southern California

A bioeffects survey of Southern California coastal bays and estuaries (between Los Angeles and the Mexican border), was initiated in 1992 under a Cooperative Agreement with the California Water Resources Control Board. The third year of field effort is currently underway, and the time frame for completion of field work in this study area will extend through1994, with final reporting in FY95. The statements of work for all three years (from the Cooperative Agreement) were included in the FY 94 Implementation Plan. This work is coordinated with a new State of California program to assess and quantify toxic hotspots in marine and estuarine waters and sediments of the state. The California program encompasses the entire coastline of the state, focusing on surveys of sediment toxicity and contaminant distributions in sediments, with eventual measures of biological response to contaminants in molluscs (both indigenous and caged). Some of our sampling locations in the Southern California Bight coincide with some of those in the state survey, in order to take advantage of that extensive source of data. Data from sampling stations not supported by the NOAA funds have been provided by the state.

The first year's focus in this survey was on inshore areas in San Pedro Bay, Long Beach Harbor, Los Angeles Harbor, Alamitos Bay, and Huntington Harbor; while the year 2 effort was focused on San Diego Bay and Mission Bay; and the third year's effort is completing the study by surveying the small coastal lagoons and estuaries along the intervening coastline. Through participation in the planning for, and subsequent cooperation with, the California program, NOAA has greatly increased the expected value from our effort in this region.

The Los Angeles-San Diego coastal area was chosen as an intensive study area because past work in the Southern California Bight indicated a variety of biological responses to contaminants, especially in the vicinity of Palos Verdes, San Pedro Bay, and San Diego Bay. Hepatic mixed-function oxidase (MFO) activities have been found to be elevated in white croaker from these regions compared with fish from Dana Point (Cross and Hose 1988; Collier et al., 1989). Four types of pollution-associated liver lesions (neoplasms, specific degeneration/necrosis, proliferative lesions, and nonspecific necrosis) were found in white croaker from this region, with the highest prevalences in fish from the San Pedro Bay Outer Harbor (Varanasi et al. 1989a, Malins et al. 1987). White croaker with hepatic neoplasms were also found at sites near Long Beach. Black croaker from San Diego Bay exhibited elevated prevalences of liver neoplasms and (along with several other species) elevated prevalences of fin rot (McCain et al., 1992). Sculpins and other species from these same regions exhibited elevated hepatic glutathione levels relative to those found in Santa Monica Bay (Brown et al.,1987). Female white croaker and kelp bass from San Pedro Bay both exhibited reduced rates of spawning (in response to gonadotropin injection), decreased fecundity, and reduced fertilization success, compared to reference fish from Dana Point (Cross and Hose, 1988, 1989; Hose et al., 1989).

The spatial extent of sediment toxicity and the contaminant bioeffects in fish are being explored in greater detail in the present survey. Sediment chemistry and toxicity bioassays were performed during the summer of 1992 at 105 stations distributed throughout San Pedro Bay and adjoining areas. Toxicity was widespread in this area (Appendix A). All work in San Pedro Bay has been completed, and several iteration of a final report have been produced by the state for review and revision by NOAA. In FY93 a total of 121 stations were sampled in San Diego Bay and vicinity, and samples were gathered for determination of toxicity and chemical concentrations. The toxicity tests have been completed and the chemical analyses are underway. Also, portions of some of these samples are being tested by Columbia Aquatic Sciences for cytochrome P-450 induction with a rat hepatoma RGS assay. The data analyses are expected to be completed in FY 95. About 43 stations in other small coastal lagoons and embayments within the study area (focusing on Newport Bay) will be sampled during the fall of 1994. Each sediment sample gathered during this survey has been or will be tested by scientists at the University of California-Santa Cruz for toxicity with two independent tests: a 10-day solid phase test with amphipods (*Rhepoxynius abronius*) and a 48-hour pore water test with abalone (*Haliotis rufescens*) embryos (first year) or a 1-hour sea urchin egg fertilization (*Strongylocentrotus purpuratus*) test of pore water (second and third years). Also, in the third year of research a toxicity test with the amphipod *Ampelisca abdita* and analyses of benthic community composition at each of the 43 stations will be funded by EPA's EMAP.

In spring of 1993, pilot studies were conducted to evaluate biomarkers of contaminant exposure and stress in bivalve mollusks. Caged mussels (*Mytilus*) were deployed in February 1993 at contaminated and uncontaminated sites in San Diego Bay using methods developed by Salazar and Salazar (1991). The caged mussels were retrieved in May, and resident mussels were collected simultaneously at several of the sites. Genotoxicity (DNA unwinding), proteotoxicity (induction of stress proteins), and whole organism health (growth and reproduction) were measured using a variety of methods. Bioaccumulation was also measured in mussel tissues. This study is directed by Dr. B. Sanders at California State University-Long Beach, and involves cooperative logistics support from the U.S. Navy, and collaboration from several other investigators (See Appendix B for more details).

Studies of contaminant exposure and effects biomarkers in resident fish were not undertaken during the first year of this survey. The sampling strategy and design were finalized and fish were collected in August/September, 1993. The target species were three species of goby (*Lepidogobius lipidus*, *Ilypnus gilberti*, and *Clevelandia ios*). Unfortunately, these species were not caught at all stations. However, a sufficient number of specimens of these and other species were caught to warrant initiation of the assays. The bioindicators quantified in each fish include fluorescent aromatic compounds in bile, hepatic MFO activities, condition indices, and histopathology, including liver lesions. The biomarker assays are being performed by scientists from the University of California at Davis and are nearly completed. Levels of organic and inorganic toxic contaminants will also be measured in the livers of these fish.

Western Florida Panhandle

Bioeffects surveys were initiated in the western Florida panhandle in FY93. The study area extends from Appalachicola Bay to Pensacola Bay and includes St. Andrew Bay (Panama City), and Choctawatchee Bay (Fort Walton Beach).

The plan for this area is modeled after the multi-disciplinary approach applied in the Tampa Bay survey. Over a three-year period, surveys of bioeffects are being conducted in

three media: demersal fish, oysters, and sediments. The data from the demersal fish are for use in evaluating the occurrence of bioeffects in mobile resource species that integrate the effects of contaminants over large areas. The data from oysters provide information from a sessile, resident suspension feeder; and the data from the sediment toxicity tests provide the best possible resolution of the spatial extent of potential effects. This area is being surveyed over a three-year period which began in 1993.

Sediment Toxicity. Sediment toxicity surveys in the Northwest Florida region were conducted in two phases, Phase 1 in Pensacola and St. Andrew Bays and Phase 2 in Choctawhatchee and Appalachicola Bays. The Phase 1 sediment toxicity survey was carried out in FY93, with sediment samples collected from 46 stations in the Pensacola Bay area and 31 stations in the St. Andrew Bay area. Sample collection was performed in a cooperative venture with the State of Florida Department of Environmental Protection. A stratified, random sampling design was prepared following evaluations of existing sediment chemistry data from the NS&T Program, the Department of Environmental Protection (FDEP), and other programs. Sediments were collected from areas expected to be highly, moderately, and not contaminated. In the Pensacola region, samples were collected from Pensacola and Escambia Bays, including East Bay, Bayou Grande, Bayou Texar, the Pensacola harbor, and Bayou Chico. In St. Andrew Bay, sampling areas included West Bay, North Bay, Watson Bayou, and East Bay. The overall area was subdivided into a number of blocks designed to represent the expected distribution of contaminants, and the specific sampling stations were selected randomly within each of these blocks.

Three tests were performed on each of the samples: a 10-day, amphipod (*Ampelisca*) survival test with solid-phase sediments; a microbial bioluminescence test (Microtox) with organic solvent extracts; and a 1-hour echinoderm egg fertilization test with pore water (see Appendix A). Non-toxic control sediments were tested concurrently with the environmental samples. Portions of each sample were retained for possible future chemical analyses. Chemical analyses of the samples from Pensacola Bay have been completed by the Skidaway Institute of Oceanography with funding provide by the State of Florida. The chemical analyses of the St. Andrew Bay samples are underway at Battelle Ocean Sciences with NOAA funding. The data will be evaluated to identify spatial patterns in toxicity, the severity of toxicity, and the relationships between toxicity and chemical concentrations.

A similar strategy for sampling and analysis was employed for Phase 2 surveys in Choctawhatchee Bay and Appalachicola Bay during FY94. Samples were collected at 39 stations in Choctawhatchee Bay and at 9 stations in Appalachicola Bay. Toxicity tests and chemical analyses are underway on these samples.

Preliminary analyses of the western Florida sediment data will be initiated in FY 95. The data from all four bays will be merged for presentation in one technical memorandum.

Oyster Bioeffects. In FY 94 an oyster bioeffects survey was planned for implementation in FY95. This planning drew on the exploratory bivalve biomarker work currently progressing in three areas under the COP Toxics Theme. Specifically field studies are being carried out under Element A within the Tampa Bay as well as in the Southern California region, and experimental work is being conducted under Element C by the Environmental Conservation Division of the Northwest Fisheries Science Center. The principal investigators participating in these studies attended a workshop in FY94 organized by CMBAD to evaluate the results of these ongoing studies and to make recommendations regarding the specific biomarkers to be used in future studies. The performance of various biomarkers was compared using the data available from these and other studies. In adddition to making recommendations on which biomarkers to use in future studies, the

workshop considered what strategy should be used for designing molluscan bioindicator measurement surveys and how to optimize the tradeoff between the number of sites sampled and the number of measurements made at each site.

Four bays (Pensacola, Choctawatchee, St. Andrew, and Biscayne) with six sites per bay will be assessed for molluscan bioindicators in FY95 . To complement work conducted by EPA in Pensacola Bay, a more intensive investigation will be conducted there.

Biomarkers evaluated in Tampa Bay oysters included physical measures of their size and condition; measures and histological evaluations of their fecundity and reproductive condition; determination of incidence of histopathological disorders, cytogenetic/cytologic disorders, and DNA anomalies; and assessment of impairment of immunological competence (See Appendix B for more information). As a result of the biomarker workshop and of the test results from FY94, many of these assays will be used in the western Florida study area in FY95. At some sites the oysters will be monitored periodically to assess their reproductive condition over an annual cycle. During the winter when the stage of gametogenesis is most stable and comparable among sites in oysters, all sites will be sampled for the full suite of measures of bioeffects. The results will be compared statistically among sites and with similar measurements made in Tampa Bay and elsewhere. Samples will be frozen and saved for chemical analyses to help evaluate the effectiveness of the biomarkers being tested.

Fish Bioeffects. In FY 1993, demersal fish were collected from 4 sites in Pensacola Bay: Bayou Chico, Bayou Texar, Bayou Grande, and White Point in East Bay. Fish were also collected from 2 sites in Choctawatchee Bay: Boggy Bayou and Garnier Bayou. Atlantic croaker (*Micropogonias unduatus*) was the only species that was captured at all sites, whereas, hardhead catfish (*Arius felis*) and spot (*Leiostomus xanthurus*) were captured in varying numbers from 5 and 3 sites, respectively. Sediment samples for chemical analyses were collected at all 6 sites. The principle findings were that very high concentrations of polynuclear aromatic hydrocarbons (PAHs) were found in the sediment samples from Bayou Chico, and these concentrations were reflected in the levels of fluorescent aromatic compounds (FACs) in the bile and of DNA adducts in liver of Atlantic croaker from this site. The levels of all these parameters were low at the White Point reference site in East Bay. No contaminant-associated liver lesions were detected in this species from any of the sampling sites.

In FY1994, demersal fish were also collected from 2 sites in St. Andrews Bay and one site in Appalachicola Bay. Samples of sediment and several fish species were collected at each site, with striped mullet (*Mugil cephalus*) and pinfish (*Lagodon rhomboides*) being the most commonly caught species.

During FY1995, follow-up studies will be conducted in Bayou Chico and White Point in order to more fully define the impacts of the severe pollution in Bayou Chico on multiple fish species. At least two fish species (e.g., Atlantic croaker, hardhead catfish) with different habitat requirements and/or feeding stategies will be collected and analyzed.

South Carolina/Georgia Estuaries

Several estuaries along the South Carolina/northern Georgia coast are known to be relatively highly contaminated and under intense pressure from urbanization. Oysters from this area sampled as a part of the Mussel Watch Project show relatively high concentrations of aromatic hydrocarbons, detectable levels of several pesticides, and very high

concentrations of arsenic, silver, and other trace metals. The Coastal Resources Coordination Program of NOAA's Hazardous Materials Response Division ranks the Brunswick (GA) estuary as a very high priority area because of the intense industrialization by potential polluters. The University of South Carolina and the Charleston laboratory of the NMFS have begun a cooperative research program (Urbanization and Southeastern Estuarine Systems-USES) funded through the NOAA Sea Grant Program and focusing upon the effects of urbanization upon coastal estuaries. This cooperative program currently involves research in North Inlet and Murrells Inlet in South Carolina. The Jacksonville and Charleston Districts of the Army Corps of Engineers have begun a series of systematic surveys of sediment toxicity in the Federal channels of the estuaries along the South Carolina and northern Georgia coast. The potential for bioeffects and the potential to cooperate with other programs affords the Coastal Ocean Program an excellent opportunity to provide very useful information for this study area.

A multi-disciplinary survey comparable to that underway in the western Florida panhandle has been designed. The survey consists of measuring bioeffects in three media: sediments, oysters, and fish. Over the three-year period of the survey the following estuaries are being sampled: Winyah Bay, Charleston Harbor/Cooper River estuary/Ashley River estuary, Leadenway Creek in North Edisto Bay, Savannah River estuary, and St. Simons Sound/Brunswick Harbor (GA.).

Sediment Toxicity. In FY 93, Phase 1 of the sediment toxicity survey was carried out by evaluating toxicity in samples from 63 stations in the Charleston Harbor area. The sampling design was prepared based on evaluations of existing sediment chemistry data from the NS&T Program, the Army Corps of Engineers, NMFS, the University of South Carolina, and other programs. As was done in the northwestern Florida bays, a stratified, random sampling design was prepared. The overall area was subdivided into a number of blocks designed to represent the expected distribution of contaminants, and specific sampling sites were selected randomly within each of those blocks. Sediments were collected from areas expected to be highly, moderately, and not contaminated. The sampling design includes the collection of samples in the lower Cooper River, lower Ashley River, lower Wando River, and Charleston Harbor and the area of the harbor entrance.

Following the same design approach, the FY94 (Phase 2) sampling effort for sediment toxicity was extended southwestward from Charleston to include the Savannah River Estuary near the city of Savannah and the St. Simons Sound/Brunswick Harbor (GA) area. The same probabalistic, stratified, random sampling design was used. Toxicity tests on the 60 samples collected from the Savannah River and the 20 samples from St. Simons Sound are underway.

In both phases of the sediment quality survey, three tests are being performed on each of the samples: a 10-day, amphipod survival test with solid-phase sediments; a microbial bioluminescence test (Microtox); and a 1-hour echinoderm egg fertilization test with pore water. In addition, some of the samples are being tested by the University of South Carolina with a meiobenthic, harpacticoid copepod survival and fecundity bioassay comparable with that being used in the USES program. Non-toxic control sediments are being tested concurrently with the environmental samples. Portions of each sample are being retained for possible future chemical analysis. Also, portions of some samples are being tested by Columbia Aquatic Sciences for cytochorme P-450 induction with a rat hepatoma RGS assay. Chemical analyses of the Phase 1 samples are underway at NMFS-Charleston. Chemical analyses of the Phase 2 samples from the Savannah River and St. Simons Sound by NMFS-SEFSC are proposed for FY 95 to complete the survey of this

region. The data will be evaluated to identify spatial patterns in toxicity, the severity of toxicity, and the relationships between toxicity and chemical concentrations.

Oyster Bioeffects. An oyster bioeffects survey will be conducted in Charleston Harbor and several other South Carolina bays in FY95. A number of measures of oyster bioeffects including physical measures of their size and condition, measures and histological evaluations of their fecundity and reproductive condition, incidence of histopathological disorders, incidence of cytogenetic/cytologic disorders, incidence of DNA anomalies, and impairment of immunological competence are being comparatively evaluated as part of the Tampa Bay Bioeffects Survey. Based on the results of this evaluation and taking into account the information and recommendations arising from the Bivalve Biomarker workshop which was held in FY94, an optimal battery of biomarker measurements will be selected for use in South Carolina. The sampling sites are being selected on the basis of the known distributions of contaminants, the availability of native oysters, and the extent to which the sites are representative of areas with integrated inputs of contaminants from multiple sources. Tissue samples will be collected as part of the South Carolina biomarker survey, and composite tissue samples will be frozen and saved for chemical analyses. The chemical analyses will be performed to help establish the linkages between the biomarker measurements and contaminants levels.

Fish Bioeffects. In FY1993, demersal fish were collected from 4 sites in Charleston Harbor: the lower Cooper River, the lower Ashley River, the lower Wando River, and the entrance to Charleston Harbor. The primary target species was red drum (*Sciaenops ocellatus*). Chemical analyses of sediment samples collected at these sites showed the highest concentrations of PAHs in the Ashley River (10 times as high as any other site). Even though the concentrations of PCBs were elevated in the sediments from the Cooper River site relative to the other sites, this value was moderate compared to many other East Coast urban waterways. Concentrations of hepatic PCBs and biliary FACs, as well as levels of hepatic DNA adducts, were highest in red drum from the Ashley River. Values of these parameters were generally lower in the Wando River. No contaminant-associated liver lesions were detected in this species from any of the sampling sites.

In FY1994, demersal fish were collected from 2 sites each in the Savannah River, Winyah Bay and Brunswick River. Samples were taken from striped mullet collected from each of these sites, although too few fish were collected from the Sampit River in Winyah Bay to provide an adequate sample size.

During FY1995, studies will be conducted in the Ashley and Wando Rivers in Charleston Harbor. Three sites will be located along the Ashley River and one site will be in the Wando River (a reference site). The objectives of these studies will be to better define the geographical extent of the pollution problems in the Ashley River and to examine multiple demersal fish species (e.g., juvenile red drum, striped mullet) with different feeding strategies to evaluate the severity of the contaminant-related bioeffects.

South Florida Coastal System: Biscayne Bay

Biscayne Bay, Florida has been identified by the Florida Department of Environmental Protection (FDEP) as a high priority area with regard to potential toxicity. Numerous canals adjoining Biscayne Bay are known to be anoxic and/or hypoxic. Considerable amounts of marine and estuarine habitat in and around the bay have been destroyed through filling, dredging, channelization, and construction. There are 11 National Priority List hazardous waste sites recognized by EPA's Superfund Program that contribute to the degradation of the Biscayne Bay system, and several additional sites are under

consideration for Superfund designation. The bay is known to have extremely elevated concentrations of toxicants, including pesticides and trace metals in water, sediments, and biota. Fish with high incidences of gross pathological disorders have been observed (Overstreet 1988, Skinner and Kandrashoff 1988, Browder et al. 1993).

Several major programs are underway with a number of participating agencies to scope and address environmental problems in the Biscayne Bay area. These programs include the South Dade County Watershed Project being conducted by EPA, Dade County, FDEP, and the South Florida Water Management District (SFWMD). Also, the Jacksonville District of the Army Corps of Engineers is beginning an effort to draw together data from sediment quality surveys and hydrological modeling conducted in Biscayne Bay. Dade County and the University of Miami have assembled a technical library and an annotated bibliography of references on toxicant concentrations in the Biscayne Bay area. FDEP has assembled a GIS-based environmental atlas of toxicant concentrations in sediments. NMFS/SEFSC has compiled information on pesticides use and ambient concentrations in the canals of South Florida.

Sediment Toxicity. The first segments of an area-wide sediment quality survey for Biscayne Bay are scheduled to be sampled early in FY95. This survey is being conducted in cooperation with the state of Florida, the South Florida Water Management District (SFWMD), and, possibly, Dade County's Environmental Resource Management (DERM) agency. It will be expanded to sample the remaining segments of the bay system in the summer of 1995. Two or more toxicity tests will be conducted on each sediment sample including use of the 10-day amphipod survival test with solid-phase sediments, the 1-hour echinoderm egg fertilization test with porewater, and the bacterial bioluminescence (Micortox$^{R)}$ test. The state will conduct chemical analyses of the phase 1 sediment samples through Skidaway Institute of Oceanography to provide data for comparison with the toxicity results, and the phase 2 samples will be analyzed for chemical contaminants in FY96.

Oyster Bioeffects. An oyster bioeffects survey similar to the one conducted in the South Carolina bays will be initiated in Biscayne Bay in FY95. This survey will employ the same biomarkers as selected for use in South Carolina. The results from the two surveys will be compared and contrasted amongst themselves and with the results from the two oyster biomarker surveys conducted previously in Florida bays to evaluate the effectiveness and consistency of these measures of contaminant effects. The sampling sites are being selected on the basis of the known distributions of contaminants, the availability of native oysters, and the extent to which the sites are representative of areas with integrated inputs of contaminants from multiple sources. Tissue samples will be collected as part of this survey, and composite tissue samples will be frozen and saved for chemical analyses to provide data for evaluating the linkages between the biomarker measurements and contaminants levels.

Fish Bioeffects. In FY1994 NMFS/NWFSC began assessments of fish biomarkers and health at selected sampling stations in Biscayne Bay. Sampling operations were conducted at four sites in Biscayne Bay: the mouth of the Little River near downtown Miami, the mouth of the Miami River, the Oleta River, and Elliott Key Harbor in South Biscayne Bay. The primary target species captured at all sites was the sea bream (*Archosargus rhomboidalis*), although samples were collected from a few other species, including striped mullet and hardhead catfish, caught at some sites.

For FY 1995, sampling will again be conducted near the mouth of the highly urbanized Miami River, as well as near the mouths of major canal systems in Biscayne Bay and Florida Bay, for example, Coral Gables Canal and Biscayne Canal in Biscayne Bay and Glades Canal in Manatee Bay near Florida Bay. The sea bream or striped mullet will likely be the target species for these sites. This study will expand our understanding of contaminant-related bioeffects in fish from these bays, with an emphasis on effects of pesticides used in local agricultural operations. So far, biomarkers used in this study were developed to detect the bioeffects of urban-associated contaminants (e.g., PAHs and PCBs) on fish; however, little has been done to assess the impacts of pesticides on these biomarkers. This study will be designed to better understand the relationships between the currently used biomarkers and pesticide exposure.

Element B: Evaluate the Relationships that Control Uptake and Bioaccumulation of Toxic Chemicals

Programs to monitor and regulate toxic contamination in the marine environment commonly use measurements of the tissue levels of toxic chemicals in organisms to assess the contamination conditions at a sampling site. The NS&T Program monitors contaminant levels in fish and bivalve molluscs to obtain data that are compared geographically and temporally to determine national and regional patterns in the status and trends in contamination. The results are taken as representative of ambient contaminant conditions at the specific sites and times that are sampled. A number of exogenous environmental factors and intrinsic biological conditions, however, influence uptake and accumulation of contaminants by biota and thus affect the concentrations measured. These include major physical environmental factors, such as temperature, salinity, and turbidity, as well as intrinsic biological factors including age and reproductive and nutritional state.

In order to minimize the influence of environmental and biological factors other than ambient contaminant levels that might affect the concentrations being measured, the NS&T Program samples the same species of organisms annually at the same sites and at approximately the same times of year. However, it has become clear that detailed, objective comparisons of contaminant levels among locations and times must consider the possible influences of the physical environmental conditions at the site where the organisms were collected, and the physiological condition of the organisms themselves. Since these factors influence uptake and bioaccumulation (that is contaminant exposure), they are likely also to affect indirectly the response of organisms to contaminants. A better understanding of these interactions would improve our ability to forecast the potential biological effects that may be associated with the concentrations of contaminants observed in environmental samples, and especially in tissues of organisms.

Under Elements A, C, and D, measurements are made wherever possible to improve our quantitative understanding of the physical, chemical, and biological factors that control uptake and bioaccumulation of toxic materials. For example, in two areas, Tampa Bay and Southern California, field studies are planned or underway to identify contaminant-related responses in bivalve mollusks. As the results from the Mussel Watch Project and other studies have suggested that salinity can strongly influence uptake and accumulation of toxic substances, especially metals, by molluscs, we are taking care to incorporate stations that not only represent significantly different contamination regimes, but are also in different salinity regimes (including especially some sites in both the 10-15 ppt. and 20-25 ppt zones). Salinity patterns in these areas will be determined from historical information, and in-situ measurements will also be made during sampling periods. In FY93 caged bivalves were placed on an experimental basis along these contaminant and salinity gradients, and tissue analyses have been conducted on these samples in order to ascertain the levels of

contaminant bioaccumulation accompanying the appearance of biological and biochemical responses in the test animals. The focus of our bioeffects attention has been expanded to include growth (of caged juvenile bivalves placed at the test sites) and the induction of stress proteins in the tissues. These pilot studies were conducted in FY 1993-4 as an integral part of the ongoing intensive bioeffects surveys in existing study areas.

Similar to the studies with mollusks in FY93-4, our studies with fish attempt where possible to control for, or to test, the interactive effects of other potential controlling factors on the uptake of contaminants and biological and biochemical responses. In these studies, the concentrations of contaminants in fish from Intensive Bioeffects Survey areas are being determined and related to biological and biochemical responses. In addition, physical factors (e.g., salinity, temperature) and biological factors (age, size, lipid content) that may influence the bioavailability and/or the bioaccumulation of contaminants are documented and incorporated into sampling designs where possible. The influence of physical factors on the accumulation of toxic chemicals and associated biological effects is being assessed in selected territorial species, such as the killifish (*Fundulus sp.*), which are known to inhabit areas with different salinity regimes. This strategy will be continued in FY95 under the mollusk and fish bioeffects projects in the South Carolina and Florida panhandle areas.

No FY95 funds are identified in the budget section of this plan for specific studies related to Element B. If additional funding becomes available in FY95 or in subsequent years, these initial efforts will be supplemented by a program of laboratory and field experiments including well-controlled experiments on the influence of salinity and other major factors, such as temperature and reproductive and nutritional state in mussels and oysters, on the rates and levels of contaminant bioaccumulation in these organisms.

Element C: Develop New and Improve Existing Methods for Quantifying Bioeffects of Toxics: Bioindicator Development

Epizootiological studies can establish the correlation between chemical contaminants and biological effects in the environment, but they do not delineate cause-and-effect relationships. Furthermore, the more chronic the effect, the greater the difficulty in detecting such relationships with statistical certainty. This difficulty has led to increased interest in the use of measures of biochemical and physiological responses (bioindicators) that occur within a short period after exposure to chemical contaminants. These bioindicators can be measured in wild organisms to provide the needed information to strengthen the link between exposure and effects. This approach has gained widespread scientific acceptance, and appears to offer the opportunity to enhance environmental monitoring and assessment, whether directed towards ecosystem health or human health. At present, however, there is a critical lack of bioindicators in fish or invertebrates that are markers of important pathways by which chemical contaminants elicit alterations in biological function and structure. The focus of the present bioindicator development project is on physiological and biochemical effects that have a clear potential to enhance our ability to demonstrate links between contaminant exposure and serious biological effects. Additionally, we have focused on biological effects for which the bioindicators can provide meaningful results in the context of environmental monitoring. Furthermore, the use of a broad suite of bioindicators in biomonitoring studies is mandated by the complexity of the mixture of potentially toxic chemicals present in most polluted areas, and also strongly argues against any single bioindicator being able to adequately discriminate between populations of organisms exposed to different levels of chemical contaminants or to detect effects that arise through various distinct mechanisms. The concerted use of a suite of bioindicators that assess both exposure and effects is needed to provide a comprehensive

assessment of the impact of the complex mixture of chemical contaminants present in near coastal US waters.

The use of a broad suite of bioindicators in fish should substantially enhance the assessment of toxic chemical contamination on aspects of ecosystem health; however, the application of bioindicators to other species would significantly expand the scope for assessing alterations to ecosystem function from anthropogenic chemicals. Another important group of marine species sampled in environmental monitoring studies (e.g., the Mussel Watch component of NOAA's National Status & Trends Program) are the invertebrates. The ability in biomonitoring projects to link contaminant exposure to indicators of effects in invertebrates would significantly expand the scope of the assessment of near coastal ecosystems' quality. However, the development of bioindicators for invertebrates is considerably less advanced than for fish and few, if any, bioindicators have been validated to the extent that they can be used with confidence in large-scale environmental monitoring studies.

The long term objectives of the present project specifically address the need to 1) develop and validate in fish new bioindicators that are markers of altered biological structure and function which are induced by chemical contaminants and are potentially predictive of serious effects, 2) determine in fish the relative sensitivity and species differences, if any, in the response of promising new bioindicators and of bioindicators currently used in biomonitoring projects such as NOAA's National Benthic Surveillance Project (NBSP) and other national and regional projects, and 3) to identify, develop and validate bioindicators of exposure and sub-cellular effects in invertebrates and evaluate their potential to diagnose population level effects.

Objective #1: To develop and evaluate new bioindicators of contaminant exposure and effects in fish species for incorporation in NOAA's NS&T Program.

A major objective of this project is the evaluation, development, validation and field testing of bioindicators in fish that reflect substantial sublethal alterations to cellular or organ structure, function or both, and are linked to exposure to chemical contaminants, including classes of compounds (e.g., metals and organometals) for which we do not have well validated indicators. The initial areas selected for development of new bioindicators were: immunotoxicologic alterations, oxidative damage, and altered porphyrin metabolism. We have expanded the research to include assessment of DNA damage in individual cells and the use of immunohistochemical techniques to improve detection of cellular changes associated with contaminant exposure and progression of histopathological effects. These techniques have the potential to provide greater specificity in identifying specific tissues or cell types affected by contaminant exposure and in specific instances allow examination of contaminant exposure in juvenile fish.

The studies conducted to date, which were started at the end of year 1, have focused primarily on 1) the systematic development of assay procedures, testing the responsiveness of the candidate bioindicators to chemical contaminant exposure using model compounds; 2) the evaluation of dose response using contaminated sediment extracts for those assays showing particular promise; and 3) the evaluation of the applicability of the candidate bioindicators to biomonitoring in field studies in Puget Sound. In FY94, those bioindicators that showed potential were further tested in the Bioeffects Surveys (Element A - Toxic Chemicals Theme).

Progress To Date

Bioindicators of Immunotoxicologic Effects

A properly functioning immune system is important for defense against pathogens and altered cells with neoplastic potential. Dysfunctional immunological responses resulting from exposure to chemical contaminants can lead to opportunistic infections and a weakened host resistance to a variety of diseases. Immuno-modulatory effects of chemical contaminants have been demonstrated to occur in virtually all major components of the immune system of fish (Hetrick et al. 1979, O'Neill 1981, MacFarlane et al. 1986, Anderson et al. 1989, Arkoosh 1989, Wishkovsky et al. 1989, Arkoosh et al. 1991, Arkoosh et al., 1994a).

The major components of the immune system can be broken down into cell-mediated immunity, humoral immunity and accessory cell (macrophage) responses. Cell-mediated immunity can be defined as reactions in which antibody is not involved. Humoral immunity results in the production of antibody so that the response is due to the antibody product of the lymphocyte. Macrophages are involved in nonspecific mechanisms of host defense. Many of the previous chemical contaminant-related immunological studies in fish have focused on and evaluated only one component of the immune system. The objective in the current project is the development and validation of bioindicators of immunotoxicologic effects in marine fish that cover contemporaneously all the major components of the immune system. Bioindicators for the three major components of the immune system should enhance our ability to interpret effects from chemical contaminant exposure on a mechanistic basis, thereby improving the linkage between exposure, altered immune function, and potential effects on survival.

To date we have developed assays for each of the three major components of the immune system in English sole and have examined the effect of contaminant exposure on two of these components in FY94. The mitogen assay, which functionally examines cell-mediated immunity, has been applied in English sole exposed in the laboratory as well as with English sole exposed in the field to contaminants. To evaluate macrophage function, we completed studies in FY 94 determining effective assay conditions for measuring superoxide anion production in activated peritoneal macrophages from English sole. We have used these assay conditions for analyzing macrophages from English sole exposed to contaminants. Finally, to examine humoral immunity, conditions for performing an enzyme-linked immunospot (ELISPOT) assay have been initiated.

The mitogen assay measures the proliferative response of leukocytes stimulated with mitogens. Mitogens are stimulants such as plant lectins and bacterial products. The ability of leukocytes to proliferate is determined by the incorporation of tritiated thymidine into their DNA. In order to conduct the assay, effective *in vitro* culturing conditions for English sole splenic leukocytes were determined. The results of the study, which determined effective assay conditions for English sole, have been accepted for publication in a peer-reviewed journal (Arkoosh et al. 1994). Experiments were subsequently conducted to determine the sensitivity of the mitogen assay in detecting immunotoxicologic effects from chemical contaminant exposure in English sole using both laboratory and field-oriented studies. A manuscript of the results is now in preparation.

In the laboratory study, we demonstrated that English sole injected with a sediment extract containing high levels of aromatic compounds have a significantly augmented leukocyte proliferative (LP) response to the mitogen concanavalin A (Con A) suggesting that the English sole splenic LP response to Con A may be a good bioindicator for detecting PAH exposure in this benthic marine fish. Similarly, English sole placed directly on

contaminated sediment showed an augmented LP response to Con A after 5 weeks of exposure. Normally, English sole leukocytes are unable to respond to Con A, however, upon exposure to aromatic compounds a LP dose-response to Con A is generated.

In an initial field study, an altered LP response was observed in splenic leukocytes of English sole from a chemically contaminated site in Puget Sound, WA relative to fish from a control site. Leukocytes of English sole sampled from the Duwamish Waterway, a highly urbanized waterway, had a statistically significant LP response to Con A. These fish also exhibited an augmented LP response to pokeweed mitogen (PWM) compared to the mitogenic response produced by the leukocytes from English sole sampled from minimally contaminated areas of Puget Sound. Further, elevated biliary FACs, an estimate of recent exposure to PAHs, were found in fish that exhibited altered immune responses suggesting an association between alteration of the mitogenic response to Con A in English sole from the Duwamish Waterway and contaminant exposure. Leukocytes of English sole from another contaminated urban area, Commencement Bay, however, did not show an altered LP response. Although fish from Commencement Bay had elevated concentrations of bile FACs compared to fish from the control site, levels were comparable to the background levels found in our reference fish. Therefore, the lack of an altered mitogen response in Commencement Bay fish may be due to lower contaminant exposure compared to fish from the Duwamish Waterway site.

In summary, the LP response of splenic leukocytes of English sole from urban-associated areas in Puget Sound and in sole exposed in the laboratory to PAH contaminated sediment and sediment extract was examined with the mitogen assay. The findings suggest that the augmentation of the mitogen response to Con A is a promising bioindicator of contaminant exposure and altered immune function. Because environmental factors other than chemical contaminants may also alter the LP response in these fish, their LP response should be monitored in conjunction with a suite of biochemical and chemical markers.

The development of an ELISPOT (enzyme-linked immunospot) assay was initiated in FY94 to measure humoral immunity in English sole. The ELISPOT will be used to measure the number of antigen specific B-cells and thus is a tool for assessing contaminant effects on specific B-cell responses. The cell culture conditions to be used in conjunction with the ELISPOT have been established and the optimization for the ELISPOT itself is in progress.

We have developed effective assay conditions for measuring superoxide anion production in activated peritoneal macrophages from English sole. Superoxide anion production is the earliest product of the oxidative burst and has potent microbiocidal activity. The ability to quantitate this oxygen radical provides a method for determining the ability of the macrophage to destroy bacteria, fungi and protozoa, and thus is a measure of the ability of fish to respond to infectious agents. We have utilized this assay system in determining the amount of superoxide produced by peritoneal macrophages from English sole injected with contaminated sediment extracts. Preliminary results indicate that activated macrophages from English sole injected with the extract have an altered ability to produce superoxide anion after *in vitro* stimulation of the macrophages with phorbol myristate acetate (PMA) or zymosan.

The strength of our approach, however, will not be evident until the full suite of bioindicators covering the major immune functions are evaluated in concert for their sensitivity and specificity to a variety of chemical contaminants in both laboratory and field-exposed English sole. In the near future we will be able to assess contaminant effects on all aspects of immune function in English sole. The components of the immune system are highly interconnected and thus without a comprehensive evaluation of immune function the

ability to link chemical contaminant exposure to altered immune function and ultimately predict potential effects on survival is limited.

Bioindicators of Oxidative Damage

Oxidative stress occurs when the level of reactive oxygen species (ROS, i.e., hydrogen peroxide, hydroxyl free radical, superoxide anion radical) increases beyond basal levels, and has been implicated in the progression of a number of adverse health effects. The biological consequences of increased concentrations of reactive oxygen species include oxidative DNA damage (e.g.. 8-oxo-2'-deoxyguanosine) and alterations in levels of antioxidants [e.g., glutathione (GSH)]. Because tissue GSH may modulate oxidative DNA damage by suppressing levels of ROS, the potential of both biochemical alterations as bioindicators of exposure to prooxidant compounds in fish was investigated. None of the bioindicators (bile FACs, hepatic xenobiotic-DNA adducts and cytochrome P450) currently being used in the NBSP and other monitoring projects specifically respond to compounds capable of inducing oxidative stress.

Oxidative DNA damage: One of the major products of oxidative DNA damage is 8-oxo-2'-deoxyguanosine (8-oxo-dG), which has been shown to induce DNA base mispairing in bacterial systems (Sies 1991). Several studies have shown increased formation of 8-oxo-dG in mammals after exposure to prooxidant compounds, however, information regarding oxidative DNA damage in fish is limited.

Our previous findings showed the potential for using 8-oxo-dG as a bioindicator in biomonitoring studies. A dose response to and a rapid removal of 8-oxo-dG in liver of English sole exposed to a model prooxidant compound, nitrofurantoin, were found (Nishimoto et al. 1991). In addition, laboratory studies with contaminated sediment extracts showed dose responsiveness of 8-oxo-dG in English sole liver, and sole sampled from several sites within Puget Sound, WA, showed significantly higher hepatic concentrations of 8-oxo-dG in fish from the contaminated sites compared to the reference site. However, the between-set variability in the performance of the electrochemical detector used to measure 8-oxo-dG was outside acceptable quality control specifications to be reliably used in biomonitoring studies. To develop 8-oxo-dG as an effective bioindicator for use in large scale field studies, a high degree of reproducibility in the assay is required.

Steps were taken to improve the accuracy and precision of the 8-oxo-dG measurements. However, this task proved to be technically challenging. Our studies demonstrated the extreme importance of using ultra high-purity water, requiring specific methods for it's preparation, and selecting high-grade buffers to achieve acceptable reproducibility and sensitivity. In light of these findings and the need for further development of the 8-oxo-dG method, we redirected efforts in FY94 to focus on the evaluation of an alternate and recently developed, promising method to assess DNA damage. This assay, termed the "comet" assay, or single-cell microgel electrophoretic method, may provide increased specificity with respect to the response of individual cell types to chemical contaminants. In addition, part of our effort was spent on developing methods to enhance the specificity and sensitivity of methods to assess toxicological effects at the cellular level. Specifically, we evaluated the potential of immunohistochemical localization of proliferating cell nuclear antigen and CYP1A activity. The results of studies evaluating these assays are described below in the section entitled: cellular bioindicators--development and evaluation.

Hepatic glutathione: Cellular glutathione (g-glutamylcysteinylglycine, GSH) is widely known for its function in the detoxication of xenobiotics and reactive oxygen species. Alterations in GSH levels arise from conjugation of electrophilic xenobiotics, as well as reduction of reactive oxygen metabolites to form oxidized glutathione (GSSG).

Studies have shown that exposure to a broad spectrum of xenobiotics alters hepatic GSH concentrations in fish. For example, laboratory studies involving exposure of Atlantic croaker (*Micropogonias undulatus*) and striped mullet (*Mugil cephalus*) to heavy metals and organic xenobiotics resulted in increased concentrations of total hepatic GSH (GSH + GSSG) (Thomas, 1992; Thomas, 1984). When catfish (*Ictalurus punctatus*) were continuously exposed to water-borne chlorothalonil, hepatic GSH levels increased significantly after 3 days (Gallagher, 1992). Similar results of elevated hepatic GSH levels were obtained in rainbow trout (*Oncorhynchus mykiss*) exposed for 96 hrs to chlorothalonil dissolved in water (Davies, 1985). Exposure of winter flounder (*Pleuronectes americanus*) to pentachlorophenol for 15 days also resulted in increased hepatic GSH concentrations (Thomas, 1984).

Results of our laboratory study examining GSH levels in English sole exposed to an organic-solvent extract of a contaminated sediment containing PAHs, PCBs, and chlorinated pesticides (CPs) showed an increase in the mean concentrations of hepatic GSH (3-fold) with increasing dosage of the extract at 3 days post-exposure (Nishimoto et al. In press). Analyses of samples from a similar study conducted with winter flounder was completed in FY94. These results also showed a dose-dependent (2- fold) increase in hepatic GSH levels when winter flounder were exposed to the same contaminated sediment extract used in the study with English sole. Moreover, in a time response study, hepatic GSH concentration in winter flounder exposed to the sediment extract was significantly elevated at 3 days post-exposure, but the maximum increase was 1 day post-exposure, suggesting some potential species differences in response of English sole and winter flounder to the sediment extract.

Extensive field evaluation of hepatic GSH was also conducted as part of the Bioeffects Survey. Atlantic croaker, red drum, Southern flounder, sea bream, striped mullet, and hardhead catfish were the major species collected during the intensive bioeffects surveys (Element A). The hepatic GSH data are being included in a database with results for biliary FACs, and hepatic P450 activity and DNA adducts. For the samples analyzed to date, the concentration of hepatic GSH for red drum, southern flounder, and Atlantic croaker exhibited apparent contaminant-associated changes among the sampling sites. However, the differences among sites in hepatic GSH concentrations for each species sampled were less than the differences observed for the other bioindicators. Previous studies (Stein et al. 1992) in Puget Sound with English sole showed substantial contaminant-associated differences in hepatic GSH concentrations among sites. The present results suggest that the magnitude of changes in hepatic GSH concentration in response to chemical contaminant exposure may be species-specific. The issue of whether there are marked species differences in the response of hepatic GSH to contaminants will be further addressed in FY95 in dose-response studies with red drum and English sole. Comparison of dose-response data for winter flounder and English sole from our previous studies suggests that there may be species differences in response of hepatic GSH in fish exposed to contaminated sediment extracts.

Results of studies in FY93 with English sole suggested compound specific differences in the biochemical mechanisms regulating increase in hepatic GSH in response to chemical contaminant exposure. In a study on the effects of chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile, TCIN) on channel catfish (*Ictalurus punctatus*), Gallagher et al (1992) observed an association between increased hepatic GSH and increased g-

glutamylcysteinyl synthetase activity (g-GCS) as well as L-cysteine concentrations, suggesting that induction of g-GCS activity and availability of L-cysteine as a substrate contribute to the increase in the synthesis of GSH. However, in our studies with English sole exposed to a contaminated sediment extract, the results indicated that induction of g-GCS may have a relatively minor role in the increase of hepatic GSH. To further assess the mechanism for the increase in GSH, we conducted a time-response experiment in which juvenile English sole were exposed to TCIN in static aquaria. The results from this study showed that an increase in hepatic GSH level was accompanied by significant induction in g-GCS activity at 7 days post-exposure, which is in accordance with the results of Gallagher et al.(1992); but no significant alteration in hepatic L-cysteine was observed. These results suggest that induction of g-GCS activity exhibits some chemical specificity. This indicated that measurement of both GSH concentration and g-GCS activity may increase the specificity in assessing contaminant exposure.

Another approach to measuring g-GCS is to determine the levels of g-GCS mRNA. The measurment of g-GCS mRNA may have technical advantages over measurement of g-GCS activity for samples collected in biomonitoring studies, such as ease of measurement. Preliminary experiments have been initiated to assess the feasibility of developing a cDNA probe for measuring g-GCS mRNA. If a probe can be developed, further investigations of the mechanism for increased hepatic GSH in fish and the potential of g-GCS mRNA levels as a bioindicator will be assessed.

Additionally, in FY94, experiments with English sole and analyses of samples from winter flounder exposed to whole sediments were completed. These experiments were conducted to aid in validating the environmental relevance of the studies with sediment extracts. Both species were exposed to a contaminated and a reference sediment. The results with winter flounder corroborated the findings with sediment extracts in that hepatic GSH rapidly increased in flounder exposed to contaminated sediments but not in flounder exposed to the reference sediment. The results also showed that hepatic GSH was responsive to changes in the level of exposure; as FACs in bile decreased with decreasing exposure to PAHs, the levels of GSH also declined. These findings are consistent with the dose-response data with sediment extracts showing that there is an apparent threshold for response to contaminants (Nishimoto et al. In press). Furthermore, preliminary results of the study with English sole also confirmed that there is an apparent threshold for induction of hepatic GSH shown by the studies with sediment extracts. In this study, the level of exposure to PAHs was less than in the experiment with winter flounder (see Progress FY94, Objective 2) , and we observed minimal increase in GSH in sole exposed to contaminated sediment. Thus, overall, these results further substantiate that exposure of fish to contaminants, such as PAHs, can increase hepatic GSH concentrations.

Porphyrin profiles - Bioindicator of impaired heme biosynthesis

The effects of chemical contaminants on the heme biosynthetic pathway results in a sequence of biochemical disorders leading to alterations in the composition and size of the porphyrin pool, and has been demonstrated to occur in humans, rats and birds (Rank et al. 1990, Elliot et al. 1990, Woods et al. 1991). Only a few studies have examined alterations in heme biosynthesis in aquatic species (Koss et al. 1986). Thus, in the present study, the feasibility of measuring porphyrin profiles in fish tissues and bile as a bioindicator of contaminant exposure was evaluated.

Efforts in FY94 were directed toward completing analyses of liver and kidney of English sole for porphyrins that were exposed to chronic and acute doses of methyl mercury. Because non-porphyrin bile components, such as riboflavin, were initially found

to coelute with the uroporphyrins, correction of the porphyrin concentrations for flavanoid-interfering components in fish tissues and bile was resolved by using two fluorescence detectors in series, one to monitor only riboflavin, during the HPLC analysis. Results of these analyses showed low levels of porphyrins in liver and kidney of fish exposed to relatively high concentrations of methyl mercury. Moreover, in FY93, no significant differences in porphyrin profiles were found between fish exposed to relatively high concentrations of HCB or TBT and control fish. Additionally, English sole exposed to extracts from a contaminated sediment (containing high concentrations of PAHs) and sole collected from a contaminated urban and pristine non urban sites in Puget Sound, showed no differences in porphyrin profiles. Thus, the results of laboratory and field studies conducted in the last two years suggest that alterations in porphyrin profiles in fish are relatively insensitive indicators of exposure to the model chlorinated hydrocarbon, HCB, or the toxic metals, methyl mercury, and TBT, even at extremely high doses. Thus, in FY95, no further evaluation of porphyrin profiles as a bioindicator will be conducted.

Cellular Bioindicators--Development and Evaluation.

Immunohistochemical analysis of cytochrome P4501A (CYP1A): In the past year, efforts have focused on the use of image analysis techniques to quantitate immunochemically detected CYP1A in fixed tissue samples from marine fish. The use of image analysis will allow us to determine enzyme induction in specific cell types, examine induction in archival samples, and may eliminate the need to assay for CYP1A induction within twelve months of sample collection.

In the initial studies, quantitation of CYP1A in a variety of cell types from the liver of two flatfish species, English sole and starry flounder was conducted. While there were definite species differences in the relative induction in different cell types, our results have shown that staining intensity in hepatocytes is linearly related to catalytic activities in the whole tissue, for both species. Currently the relationships between staining intensities and the catalytic assay activity is being determined. The successful development of this assay will increase the utility of CYP1A as a bioindicator in assessing exposure and in evaluating CYP1A expression in various hepatic lesions, and thus effects of contaminant exposure.

Immunohistochemical localization of proliferating cell nuclear antigen: Identifying the status of cells within the cell cycle is critical to determining the proportion of replicating cells in a tissue in assessing toxicity and carcinogenesis. Localization of proliferating cell nuclear antigen (PCNA), a highly conserved endogenous nuclear protein cofactor of DNA polymerase delta required for DNA replication, is useful in identifying the various stages in cell replication in actively cycling cells. As such, it is useful in estimating the growth fraction in tissues to better assess the role of cell proliferation in certain contaminant exposure-related lesions involved in the histogenesis of hepatic neoplasia.

Our first application of this new technology, done in cooperation with Lisa Ortego of the Gulf Coast Research Lab in Ocean Springs, MS, was to estimate the proportion of actively cycling cells in hepatic neoplasms, neoplasia-related lesions such as putatively preneoplastic foci of cellular alteration, and normal liver in English sole. PCNA expression was localized immunohistochemically with anti-PCNA monoclonal antibody (PC10) following standardized antigen-retrieval techniques. PCNA expression was then quantified in liver of normal and affected sole from a chemically contaminated waterway in Seattle, WA. The results showed that this technique improves the ability to assess cell proliferation in these lesions, as labeling indices were increased in putatively preneoplastic focal lesions, hepatocellular adenomas, and both hepatocellular and cholangiocellular carcinomas.

In addition to evaluating the utility of PCNA for assessing the proportion of actively cycling cells, significant progress was made in improving quantification of cell labeling using unique thresholding techniques in computerized image analysis that substantially reduced subjectivity in determining positivity/negativity in labeling. Preliminary results indicate a higher growth fraction and labeling index in hepatocytes and cholangiocytes composing hepatocellular and cholangiocellular carcinomas, hepatocellular adenomas, and foci of cellular alteration, as compared to normal surrounding parenchyma. The results to date demonstrate that the use of state-of-the-art imaging techniques can significantly improve our ability to objectively and consistently quantify labeling for PCNA in tissue sections in a way that significantly reduces human subjectivity in scoring tissue sections for PCNA expression. This improves the potential for using PCNA in enhancing the use of histologic lesions as bioindicators of contaminant exposure and sub-lethal effects.

DNA damage in individual cells: We continued evaluation of the single-cell microgel electrophoretic method, or comet assay, for measuring strand breaks and alkali-labile sites in nuclear DNA of individual cells. This assay has the potential to detect damage to cellular DNA from exposure to compounds other than PAHs. DNA damage by polycyclic aromatic compounds, including PAHs is assessed using the ^{32}P-postlabeling assay (see Objective #2).

Similar to the immunohistochemical studies, the experiments with the comet assay were directed towards adapting the assay to our new imaging system, and increasing the sensitivity of the assay. In addition, initial studies with a model compound were conducted. The use of the imaging system has increased our ability to rapidly and more objectively assess the magnitude of DNA damage in individual cells. Furthermore, we have increased the sensitivity of the assay by modifying the DNA detection steps, and have shown that both contaminated sediment extracts and pro-oxidant compounds, which induce oxidative damage, increased DNA damage detected by the comet assay. The experiment with the model compound also showed that DNA damage increased rapidly and then showed relatively rapid return to background levels. These experiments demonstrate responsiveness to contaminant exposure, but the studies also showed that non-contaminant factors (e.g.. physiological stress) also appear to induce some DNA damage. This finding shows that the comet assay must be used in conjunction with other bioindicators.

Studies were also initiated to validate the comet assay for use in mussels in support of its use in Element A and anticipation of its use in Element C, Objective #3 in FY95. The comet assay has been used in the bioeffects survey conducted in San Diego, CA. A recent study (Vukmirovic et al. 1994) using another technique (alkaline elution) that also detects DNA strand breaks has ascribed DNA single strand breaks in natural populations of mussels (*M. galloprovincialis*) to contaminant exposure.

Overall, these studies are building on our findings which clearly show that the use of a suite of validated bioindicators is necessary to adequately assess contaminant exposure and effects in indigenous fish. Moreover, as the bioindicators are developed, it will be possible to tailor the suite to effectively address the objectives of individual biomonitoring efforts.

Current Approach (FY95)

Bioindicators of Immunotoxicologic Effects

Flatfish

Humoral Immunity: We will continue development of the assay conditions for the ELISPOT in English sole. Once the ELISPOT is optimized in English sole we will adapt it for the development of the more versatile immunofluorescence-linked immunospot (ILSPOT) assay. In conjunction with immunofluorescence digital processing (IDIP) this assay will not only allow us to enumerate the number of B-cells producing antibody to a specific antigen it will also allow us to quantify the amount of antibody that particular B-cell is producing. When the effective assay conditions for the ILSPOT-IDIP assays are determined, we will then conduct time- and dose-response studies for this immunological bioindicator in English sole exposed to the standard urban sediment extract and selected model compounds. Studies with model compounds are warranted because there is little information on the specific ability of different classes of chemical contaminants to alter immune function in fish.

In addition, we will initiate studies to determine basic biochemical characteristics of the antibody from English sole and compare to the basic biochemical characteristics of antibody from another flatfish species, winter flounder. The differences in antibody structure may relate to species differences in immune function. Features to be determined include molecular weight determination of heavy and light chains of the antibody, isoelectric point, subunit structure of whole antibody molecule, assess amino acid composition, and determine heavy and light chain glycosylation between species.

Cell-Mediated Immunity: We will complete a manuscript describing laboratory and field findings on the splenic leukocyte proliferative response in exposed and non-exposed English sole in the presence of stimulating mitogens.

Macrophage Function: Based on our laboratory studies examining superoxide production in macrophages from English sole exposed to a contaminated sediment extract, we will apply our recently developed superoxide anion assays to field-exposed fish from a variety of sites in Puget Sound to evaluate further the potential of macrophage generated superoxide as an immunological bioindicator of contaminant exposure in fish. The assay for superoxide production by activated macrophages will provide a measure of the functional capacity of the macrophage to react to foreign organisms.

Disease Challenge: Chemical contaminant exposure has the potential of compromising immune function. This in turn can lead to altered host resistance to pathogens. We have demonstrated this relationship in juvenile salmon in which altered immunological function in field-exposed juvenile salmon are more susceptible to mortality from a marine pathogen than unexposed salmon (Arkoosh et al. unpublished obs.).

We have determined that English sole exposed directly to a contaminated sediment exhibit characteristics of a dysfunctional immune system and hypothesize that they may have an altered disease resistance to a pathogen, similar to our findings in contaminant-exposed juvenile salmon. To evaluate disease resistance of English sole exposed to contaminants, we will conduct disease challenge experiments to determine if exposure to contaminants can alter (decrease) their resistance to a natural pathogen of the marine environment. This study will aid in establishing the link between bioindicators of immune function and potential effects on survival.

Red Drum

Assays of immune function have been developed for red drum (Burnett et al., 1994). As part of our studies on development and validation of bioindicators in this important

resource species from the southeastern US, we will evaluate immune function in relation to chemical contaminant exposure.

Because the leukocyte proliferative response of splenic leukocytes is a promising bioindicator when used in conjunction with other chemical or biochemical markers in English sole, we will initiate our studies with the red drum by examining their cell-mediated immune response to a variety of mitogens after exposure to a contaminant. In support of these efforts we will begin preliminary trials to evaluate disease susceptibility in relation to chemical contaminant exposure by conducting disease challenge studies. Disease challenge in the laboratory represents a means by which extrapolation to effects of contaminants in the natural environment can begin to be made.

Bioindicators of Oxidative Damage

Oxidative DNA damage: We will complete the quality assurance improvements in measuring 8-oxo-dG with electrochemical detection. If results are positive, selected samples from red drum collected during the intensive field surveys under Element A will be analyzed in conjunction with laboratory exposure studies with red drum and English sole. Results of time- and dose-response experiments with 8-oxo-dG will be compared to those for DNA adducts and the "comet" assay, as applicable, to assess the relative sensitivity and potentially the specificity of each of these measures of DNA damage in relation to contaminant exposure.

Hepatic Glutathione: Laboratory results to date show that hepatic glutathione in English sole and winter flounder increased upon exposure to contaminated sediments, supporting the use of hepatic glutathione as a bioindicator. A manuscript describing the laboratory and field study on the alteration of hepatic GSH in English sole upon exposure to a complex mixture has been accepted for publication (Nishimoto et al. in press). Furthermore, a previous field study in Puget Sound showed that increased levels of glutathione were observed in liver of three flatfish species from contaminated sites. These findings indicate that expanded field testing of hepatic glutathione as a bioindicator of exposure and sublethal effects is warranted. For this year, we will continue to analyze samples collected in the Bioeffects Surveys (Element A) for GSH and g-GCS (in selected samples). In laboratory studies, red drum will be exposed to sediment extracts to determine dose-response of hepatic GSH concentration, for comparison to results from the surveys.

In the studies conducted to date with English sole, the results suggest that induction of hepatic g-GCS may have a minor role in the increase of hepatic glutathione in sole exposed to contaminated sediment extracts but appears to be a factor in sole exposed to the fungicide chlorothalonil. To further assess the mechanism for the increase in glutathione, red drum will be exposed to sediment extracts and to chlorothalonil, which has also been shown to induce g-GCS in channel catfish (Gallagher et al. 1992). The results from these experiments will further establish the role of g-GCS in regulation of GSH in fish exposed to contaminants and identify whether compounds in the sediment extracts typically induce this enzyme or that induction is species-specific. Moreover, we will continue molecular biological studies initiated in FY94 to explore the potential for developing a cDNA probe to g-GCS mRNA. If the cDNA probe is successfully developed, studies with the probe will further clarify the mechanism of regulation of GSH in fish exposed to contaminants and provide information to assess the potential of using measurements of both GSH and g-GCS mRNA as a bioindicator that may have greater chemical specificity.

Cellular Bioindicators--Development and Evaluation

Immunohistochemical analysis of cytochrome P4501A (CYP1A): After finishing the work relating CYP1A measured by imaging analysis to catalytic assays, for two benthic fish species, we will prepare a publication for submission to a peer-reviewed scientific journal. Subsequent research will be directed towards application of imaging technology to whole body sections of fish, such as juvenile salmonids as a surrogate for examining juveniles of other species. This will allow us to semi-quantitatively determine routes of uptake and points of exposure for aromatic contaminants in field sampled animals. Such information will be used to identify tissues other than liver (e.g. olfactory epithelia, lateral line organs, and visual structures) that may be susceptible to deleterious effects of toxic contaminants. The ability to examine juveniles will be important in relation to our long-term objectives assessing linkages between exposure and population effects, such as decreased recruitment.

Immunohistochemical localization of proliferating cell nuclear antigen: Research in FY95 will explore the utility of this relatively new method for assessing and objectively quantifying cell proliferation in liver lesions of archived tissues from other fish species and in laboratory contaminant-exposure experiments focusing on hepatotoxic effects. These results will be useful in a number of field and laboratory studies examining cellular proliferative response to contaminant exposure in multiple target tissues. Thus, the use of PCNA labeling in conjunction with histologic examination of lesions will further enhance the utility of a range of histologic lesions as bioindicators of contaminant exposure and effects.

DNA Damage in Individual Cells: The single-cell microgel electrophoretic method (comet assay) will continue to be evaluated for its use as a bioindicator in fish and bivalves. The studies will focus on laboratory studies to assess dose-responsiveness to model compounds and sediment extracts using fish and mussels. As appropriate, these laboratory studies will be complemented by field studies in Puget Sound. These studies are needed to evaluate contaminant specificity to complement further testing in the Bioeffects Survey.

Objective #2: To further validate bioindicators currently being measured in target fish species of biomonitoring studies.

The effective use of bioindicators in marine environmental monitoring programs requires that the bioindicator has the necessary sensitivity and specificity to chemical contaminant exposure. Additionally, in environmental monitoring programs with wide spatial coverage, several different target species must be sampled. This requires knowledge of species differences in the response of the bioindicator. Thus, an important component in the process of validating a bioindicator for effective use in biomonitoring projects is the determination of dose-response and evaluation of species differences in the response. Additionally, the use of complex mixtures of chemical contaminants is needed in validating bioindicators, because coastal waters near urban/industrialized areas are contaminated with a wide range of anthropogenic chemicals (e.g., Varanasi et al. 1989a), and the potential exists for interactions among contaminants to influence the response of the bioindicators. Results from such validation studies will provide the foundation to better interpret results from field studies using bioindicators.

The experimental studies under this objective were completed in FY94. In FY95 the major focus will be to complete publication of the findings on time- and dose-response relationships in the benthic flatfish English sole, starry flounder and winter flounder. The

three bioindicators that are currently used in biomonitoring studies and examined under this objective are:

- hepatic cytochrome P450 (CYP1A) activity (specifically, aryl hydrocarbon hydroxylase),
- levels of DNA adducts in liver, and
- levels of fluorescent aromatic compounds (FACs) in bile.

Cytochrome P450 (CYP1A)

The biotransformation of many xenobiotic chemicals is mediated by the enzyme cytochrome P450. The level of this enzyme increases on exposure of fish to low levels of a broad spectrum of chemical contaminants, including carcinogenic aromatic hydrocarbons, certain chlorinated hydrocarbons including planar PCBs and several dioxin and furan congeners, as well as several other petroleum-derived compounds and pesticides. Hence, the induction of cytochrome P450 is a very early and sensitive biochemical response to exposure to toxic chemicals (Payne et al 1987) and has been linked to serious biological effects (e.g., reproductive dysfunction) in fish (Spies et al. 1988; Johnson et al. 1988) and mammals (Guengerich 1990).

Hepatic DNA Adducts

During the biotransformation of xenobiotic chemicals, reactive metabolites are formed that can covalently bind to the genetic material, DNA, to form xenobiotic-DNA adducts. The binding of a chemical carcinogen to DNA is believed to be an essential early step in the process of chemical carcinogenesis. Recently, a very sensitive technique (^{32}P-postlabeling) has been developed (Gupta and Randerath 1988) and shown to be able to detect DNA adducts in feral fish (Dunn et al. 1987; Varanasi et al. 1989c; Stein et al. 1989). The measurement of these adducts provides an assessment of exposure to genotoxic compounds, and because this type of DNA damage can be linked to more severe biological effects, such measurements may be very important in establishing causal relationships between contaminant exposure and damage to living marine resources (Stein et al. 1989; Schiewe et al. 1991).

Biliary FACs

The measurement of FACs is useful because it provides an estimation of exposure to aromatic compounds of toxicological concern, such as polycyclic aromatic hydrocarbons (PAHs), which have been strongly linked to liver cancer and impaired reproductive processes in fish (Johnson et al. 1988, Myers et al. 1993, Myers et al. 1994). These compounds usually cannot be measured directly in tissues because they are extensively and rapidly metabolized by fish and predominantly excreted into the gall bladder (Krahn et al., 1986; Varanasi et al., 1989b).

Progress To Date

We completed the determination of dose-response relationships for the three bioindicators (biliary FACs, and hepatic DNA adducts and cytochrome P450 activity) in three marine fish species exposed to organic-solvent extracts of contaminated urban

sediments. The results of these studies have provided a multi-species and comprehensive assessment of the relative responsiveness of the bioindicators. Our findings demonstrated that the responses of various species to exposure to chemical contaminants were relatively consistent over the range of extract concentrations tested. Knowledge of the differences in time- and dose-response among the three bioindicators improve the ability to assess the extent and duration of contaminant exposure in fish sampled in field studies. Without an understanding of concentration-response relationships in multiple species, comparisons among studies using different species is very limited.

The success of this comprehensive strategy of evaluating time and dose responsiveness of bioindicators in multiple species using an environmentally relevant complex mixture of contaminants is applicable, and needed, for any new bioindicator that shows sufficient potential as a useful tool in assessing contaminant exposure and response in wild species. The availability of a validated approach for determining concentration-response relationships will allow evaluation of all promising bioindicators of toxic chemical exposure and effects that are identified in the COP or in other NOAA programs. Thus, since we have developed and tested methods for preparing sediment extracts for laboratory exposure studies as well as an experimental protocol for assessing time and dose responsiveness, we will continue to use this approach for all bioindicators to be used in the Intensive Surveys (Element A) first, or at least concomitantly, be evaluated under Element C. This applies to bioindicators to be used with fish or invertebrates (bivalves).

Studies with whole sediments

In the completed studies on time- and dose-response with sediment extracts it was critical that the dosage of contaminants was well-defined and controlled so that the results were most useful for assessing both the relative response of the bioindicators as well as species differences. For these reasons, the fish were exposed via intermuscular injection to sediment extracts that are chemically characterized in detail. However, to better use the findings of the laboratory dose-response studies for interpreting results for indigenous fish, the bioavailability of chemicals from sediment and effects of chronic exposure to contaminants are issues that needed to be addressed. To address these issues, experiments with whole sediments were conducted to estimate the range of environmentally relevant response in the dose-response studies with the sediment extracts.

Analyses of samples from an experiment in which winter flounder were exposed to a contaminated and reference sediments for two months were completed. The results confirmed that biliary FACs and hepatic DNA adducts are specifically responsive to PAHs present in the contaminated sediment. Neither the levels of FACs nor DNA adducts increased in fish exposed to the reference sediment, which was found to have low concentrations of PAHs. These findings confirm results of studies with reference sediment extracts, and that alterations in these two bioindicators are due predominantly to exposure to anthropogenic compounds and not to naturally occurring compounds in sediment. In addition, chemical analyses of sediments showed some loss of PAHs from the sediment during the 2 months of the experiment. The loss of contaminants was reflected in the response of biliary FACs but not in hepatic DNA adducts, which also confirms the findings of studies with sediment extracts that showed that adducts are persistent in fish, whereas, FACs depurated relatively rapidly. Moreover, the results showed that hepatic P450 (CYP1A) was more sensitive to contaminant exposure than either biliary FACs or hepatic DNA adducts, as evidenced by the lack of a consistent decline in CYP1A activity in fish exposed to the contaminated sediment. These findings are also consistent with the studies with sediment extracts which showed maximal induction of CYP1A activity at relatively low dosages of the contaminated sediment extract.

A whole sediment exposure study was conducted with English sole at four levels of PAH contamination. The study focused on the response of the above three bioindicators as well as response of an immunological bioindicator, the mitogen assay. The majority of the analyses have been completed. The results for the immunological bioindicator are discussed under Objective 1 above. The results of the study provided additional evidence of differences in specificity of biliary FACs and hepatic CYP1A and DNA adducts with respect to exposure to PAHs and suggested that the differences in the uptake of high molecular weight PAHs would appear to account for the differences observed between the studies with English sole and winter flounder. The results showed that biliary FACs increased significantly with increasing levels of PAHs in sediment and remained elevated throughout the experiment, whereas the levels of hepatic DNA adducts increased at the higher levels of sediment contamination and accumulated throughout the course of the experiment. No appreciable increase in CYP1A activity was observed during this period. In contrast, as noted above, all three bioindicators significantly increased in the study with winter flounder exposed to contaminated sediments.

The findings with English sole would appear to contradict, in part, the dose-response relationships determined using sediment extracts. In those studies, P450 activity increase at much lower doses of extracts than either biliary FACs or DNA adducts, while the dose-response relationships for biliary FACs and hepatic DNA adducts are comparable. However, there are also differences among these bioindicators in their responsiveness to low and high-molecular weight PAHs as well as the relative persistence of the response following exposure that must be considered. Previous studies (Krahn et al. 1992) have shown that biliary FACs measures a broad suite of PAH metabolites. For example, when levels of biliary FACs are measured at benzo(a)pyrene wavelengths, metabolites of 3-4 ring PAHs as well as ≥5 ring PAHs are detected. In contrast, PAHs that induce P450 and readily form DNA adducts are primarily the 5 ring PAHs and a more select set of 4 ring PAHs. Moreover, the interaction of PAHs with sediment particles increases with increasing molecular weight of PAHs and with organic carbon content of the sediments, which consequently decreases PAH bioavailability. The physical characteristics of the sediments used in the two experiments and the results of analyses of the sediments for PAHs suggest that the PAHs were more avidly bound to the sediment used in the study with English sole. Thus, in combination, these factors are consistent with exposure of English sole to lower levels of high molecular-weight PAHs than were winter flounder, and consistent with the ability of hepatic DNA adducts to accumulate with continued exposure, because they are relative persistent. Furthermore, because PAHs are rapidly metabolized and the parent compound does not accumulate, their ability to induce CYP1A activity does not increase with continued exposure.

Studies with New Target Species

Studies with black croaker (*Cheilotrema saturnum*), a target species for Pacific Coast biomonitoring studies, were to be conducted. Concerns on availability of fish, the lack of specific knowledge on the culture of this species, and the need to conduct experiments at an offsite location led to the selection of an alternative test species.

Red drum (*Sciaenops ocellatus*), which is a target species in biomonitoring studies (Element A) conducted in the Southeast, was chosen for the following reasons. Biomonitoring surveys have shown evidence of significant contaminant exposure in red drum from contaminated waterways (McCain et al. in preparation). Additionally, red drum is a commercially and recreationally important species, has a wide geographical distribution, and is tolerant to a wide range of environmental conditions. Because red drum

are available from commercial hatcheries at any time during the year, it is feasible to hold red drum in our sea water facility allowing studies on this species regardless of season. This will allow a more extensive evaluation of all bioindicators than could be done if we conducted studies at an offsite location. Moreover, culturing red drum at our sea water facility will allow additional experiments to be conducted to assess linkages between bioindicator responses and other effects, such as disease resistance and impaired growth.

In FY 94, we adapted a laboratory to specifically meet the aquacultural needs of red drum. Incoming sea water is heated, passed through a UV sterilizer and then distributed to the holding tanks. Effluent water from the holding and exposure tanks is treated with chlorine and discharged. The water is heated for holding of the fish and for the determination of bioindicator responsiveness at temperatures that are environmentally relevant for the red drum. Additionally, we developed a method for shipping red drum from the hatchery in Texas to our facility and currently we are culturing approximately 1800 red drum for use in experiments in FY 95. Our current rearing facility will accommodate up to 4000 red drum at a size of 50 grams per fish. Additional tanks are also in place for holding numerous treatment groups at a temperature in the range measured in the Bioeffects field surveys.

Current Approach (FY95)

The majority of studies under this objective have been completed. The major emphasis in FY95 will be completion of publications on the research findings. The studies with red drum will focus on the newer bioindicators being developed (e.g., immunological bioindicators, oxidative damage) under Objective #1. However, in support of these studies and to validate the findings of the studies conducted under the Bioeffects Surveys, dose responsiveness of hepatic DNA adducts and P450, and biliary FACs in red drum will be examined as needed.

Objective #3: Bioindicator identification, development and validation in invertebrates

The Mussel Watch Program of NOAA's NS&T program has yielded a comprehensive data base on the magnitude and extent of exposure of mussels to chemical contaminants in our coastal environment. There is, however, virtually no information regarding the effects of exposure to chemical contaminants on the natural populations of bivalves or other invertebrates. Development of bioindicators in invertebrates has been limited because much less is known about the mechanisms of interaction of chemical contaminants with biochemical systems in these species compared to the knowledge base for fish. The overall current project goal is to identify potential bioindicators that may be predictive of serious biological effects. The studies on bioindicator development in bivalves were initially conducted as a NMFS base-funded program in the Environmental Conservation Division. The success of the initial studies showing the potential of cytochemical parameters to serve as bioindicators of contaminant exposure and effects and the completion of validation studies for bioindicators in fish has allowed greater emphasis under Element C in the last two years on the further development and validation of bioindicators in *Mytilus edulis*. We therefore initiated studies to assess the relationship between bioindicator response and population-level parameters, such as reproductive endpoints, population age structure, and growth.

Our initial studies were field evaluations, at multiple sites in Puget Sound, on the potential of the cytological alterations as bioindicators. The results showed that changes in

these parameters were associated with contaminant levels at the sites and also showed that features such as lysosomal membrane stability and lipofuscin and neutral lipid content in the digestive cells, irrespective of site, were associated with tissue burdens of PAHs and PCBs, indicating that contaminant exposure was a key factor in the response of these potential bioindicators. The results of the study were published (Krishnakumar et al. 1994). These studies have been continued to evaluate additional bioindicators (NADPH-dependent ferrihemoprotein reductase, catalase, DT-diaphorase, and g-glutamyl transpeptidase activity) and to assess relationships between bioindicator responses and effects on populations, as described below. The results from these studies will allow the selection of the most promising cytological bioindicators for assessing contaminant exposure.

Progress To Date

The systematic evaluation of cytochemical and enzymatic indices as bioindicators of contaminant exposure and altered cellular function was further assessed using *M. edulis* from urban and non urban sites, and a second manuscript arising from these studies has been drafted and is undergoing internal review. The sites included minimally contaminated areas in northern Puget Sound (Double Bluff, Coupeville, and Oak Bay) and southern Puget Sound (Saltwater Park) and urban areas in Elliott Bay (Seacrest and Four Mile Rock-an NBSP Mussel Watch site, Sinclair Inlet, Commencement Bay, and Eagle Harbor). The latter two sites are in EPA Superfund areas. Activity of enzymes known to be involved in metabolism of toxic chemicals or altered after contaminant exposure were measured in *M. edulis* collected from nine sites in Puget Sound, Washington (USA). Mussels from urban-associated sites (areas with elevated concentration of anthropogenic contaminants such as PAHs, PCBs, DDTs, and toxic elements in the sediment) showed increased activities of NADPH dependent ferrihemoprotein reductase (NFR), NADH dependent DT-diaphorase (DTD), catalase (CAT), and suppression of gamma-glutamyl transpeptidase (GGT) activity, and induction of peroxisome proliferation relative to mussels from the non urban reference sites. Significant correlations were observed between tissue concentrations of PAHs and PCBs (measures of anthropogenic exposure) and NFR, DTD, and CAT enzymatic activities and peroxisome proliferation indicating a possible causal relationship between contaminant exposure and these biological alterations in mussels. These results on specific enzymes and our previous results on cytochemical measurement of lysosomal responses (Krishnakumar *et al.* 1994) demonstrate that, when combined with automated image analysis, these parameters have the potential to be used as sensitive, accurate and rapid measures for assessing contaminant exposure and subcellular responses in mussels and potentially in other bivalves.

The ability to accurately interpret the response of the cytochemical bioindicators in relation to contaminant exposure and the utility of these bioindicators is related, in part, to the influence of physiological changes (e.g. gonadal recrudescence) and environmental parameters (e.g. water temperature) on the response. Thus to assess seasonal changes in cytochemical and cytological activity in digestive cells of *Mytilus edulis*, temporal variation in response to contaminant exposure was examined in mussels sampled monthly from a reference, non-urban site previously studied (Coupeville) and a polluted, urban site (Seacrest, Elliott Bay), also previously studied in Puget Sound. Measurements included size, weight, condition index, gonadal condition, and several of the most promising cytological bioindicators of contaminant exposure. The cytological bioindicators measured were: lysosomal membrane stability, NADPH-ferrihemoprotein reductase activity, lipofuscin deposition, and accumulation of lysosomal and cytoplasmic unsaturated neutral lipids. In addition, the presence or absence of hemic neoplasia as a potential confounding factor in the bioindicator response to contaminant exposure was also evaluated. Consistent

depression in lysosomal stability, enhanced NADPH-ferrihemoprotein reductase activity, enhanced lipofuscin deposition and increased accumulation of lysosomal and cytoplasmic unsaturated neutral lipids in the digestive cells were observed in the digestive gland of mussels from the urban-associated site when compared to mussels taken from the non-urban site. These alterations were observed despite changes in condition and gonadal development related to the annual variation in reproductive condition. The results of this study suggest that response of the measured cytological parameters to contaminant exposure is not substantially affected by gonadal development or alterations in physiological condition.

As noted in our studies with fish, the effective use of bioindicators in monitoring studies requires information on sensitivity and specificity of the bioindicator to contaminant exposure. An important component in the validation of a bioindicator is the determination of exposure-response relationships. Therefore, initial time- and dose-response studies for the cytological (lysosomal and enzymatic) markers were conducted using reconstituted mixtures of model contaminants administered via the diet. The feeding trial demonstrated that this is a feasible approach to administer contaminants to bivalves in an environmentally realistic manner. Mussels fed with an agar-acacia gum mixture containing model PAHs (a mixture of phenanthrene, fluoranthene, and benzo(a)pyrene) for 30 days acquired body burdens of these contaminants which were comparable to concentrations observed in indigenous populations of mussels from urban environments. The cytological parameters measured were the same ones assessed in the field studies and included lysosomal membrane stability, lipofuscin and neutral lipid content in the digestive cells, and NADPH dependent ferrihemoprotein reductase, NADH dependent DT-diaphorase, catalase, and gamma-glutamyl transpeptidase activity. After 30 days of exposure, mussels receiving either PAHs or PCBs in their diet showed decreased lysosomal labilization period, increased digestive cell lipofuscin content, increased NFR activity, and suppressed GGT activity compared to the untreated control mussels and to mussels administered the carrier (corn oil) in their diet. These alterations were similar to changes observed for indigenous mussels from urban environments known to have higher body burdens of these contaminants compared to mussels from minimally-contaminated environments. These data provide the first supportive evidence that the bioindicator response in field-exposed mussels are associated with contaminant exposure.

Our initial studies of mussels in Puget Sound showed that mussels were generally smaller in size and that somatic tissue weight relative to shell length was lower in animals from urban-associated sites compared to mussels from minimally-contaminated sites. Subsequently, age-length and length-weight relationships, age structure, and reproductive status (fecundity, egg size) were assessed in *Mytilus edulis* from six sites in central Puget Sound and one site in the relatively pristine area of the proposed Northwest Straits National Marine Sanctuary. These field studies were conducted to include a more expansive study of mussels from 7 sites including a site far removed from the immediate influence of urbanization in the Puget Sound region rather than the comparison of these parameters in indigenous mussels from 2 urban and 2 minimally contaminated populations, as originally proposed, and in lieu of the *in situ* studies proposed for FY94. The *in situ* studies will be carried out in FY95.

Mussels from urban-associated sites (areas with elevated sediment concentrations of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and toxic and essential metals) exhibited high tissue burdens of these contaminants. Age-length relationships showed that the population-related growth of mussels from urban-associated areas was lower than in mussels from minimally-contaminated environments. Moreover, comparison of the age-structure of mussel populations showed that for mussels of comparable size from urban and non urban sites, the urban mussels were consistently older and the mean

age of urban mussel populations was higher than that of reference mussel populations. In addition, allometric relationships showed that young mussels (<10mm) from urban sites had lower tissue mass than mussels of comparable shell size from minimally-contaminated areas. With respect to their reproductive status, overall fecundity in mussels at urban sites was reduced compared to mussels of comparable age from reference sites. The results show that mussels from urban areas of Puget Sound exhibit impaired growth, altered population age-structure, and reproductive impairment as a result of exposure to chemical contaminants. These findings suggest that in mussels, bioindicators may be diagnostic of population-related biological effects of chemical contaminant on invertebrates; however, this must be verified with additional laboratory and field studies.

Current Approach FY95

The primary focus will be the determination of time- and dose-response relationships to assess the sensitivity of the most promising cytochemical bioindicators in mussels exposed to complex mixtures of contaminants present in the natural environment. These studies will involve exposure to mixtures of model compounds, and contaminated sediments and their organic-solvent extracts. Recent studies on mussels have also identified several potential new bioindicators of exposure that are specific to chlorinated compounds including glutathione (GSH) and glutamyl-cysteine synthetase (g-GCS) activity in the gills and digestive cells. In an attempt to improve the suite of bioindicators that have different specificities for assessing exposure to the complex mixtures of contaminants in the environment, studies will be initiated with invertebrates on these new bioindicators. Additional studies will concentrate on the relationship between the development of hemic neoplasia, a disease endemic to mussels from the sites in Puget Sound, WA, contaminant exposure, and bioindicator response. The development of hemic neoplasia has been suspected to be associated with contaminant exposure; however, there are few studies that have adequately tested this hypothesis. Moreover, it is important to determine whether the development of this particular disease affects the response of the bioindicators to chemical contaminants. Finally, *in situ* studies will be initiated to validate the suspected alterations in growth rates in mussels identified in the studies on population structure in field-exposed mussels. These studies will serve to strengthen the linkages between chemical contaminant exposure, bioindicator changes and causal relationships to serious biological effects that reflect on population characteristics.

Specific areas of study are as follows:

Initiate time- and dose-response studies for cytological (lysosomal and enzymatic) markers using reconstituted mixtures of model contaminants and sediment extracts administered via the diet: The effective use of bioindicators in marine environmental monitoring programs requires that the bioindicator has the necessary sensitivity and specificity to chemical contaminant exposure. Thus, an important component in the process of validating a bioindicator for effective use in biomonitoring projects is the determination of dose- and time-responsiveness to contaminant exposure. Additionally, the use of complex mixtures of chemical contaminants is needed in validating bioindicators, since coastal waters near urban/industrialized areas are contaminated with a wide range of anthropogenic chemicals (e.g., Varanasi et al. 1989a), and thus the potential exists for interactions among contaminants to influence the response of the bioindicators. Results from such validation studies will provide the foundation to better interpret results from field studies using bioindicators. A study patterned after the studies reported in the progress-to-date section for fish will be pursued, except that the studies with *M. edulis* will involve complex mixtures of model compounds, as well as contaminated sediments and their organic-solvent extracts. Feeding mussels a diet containing contaminants in a micro-encapsulated form will be used as previously described. Cytological bioindicators of

contaminant exposure in mussels that will be assessed include lysosomal labilization period, lipofuscin and neutral lipid content of the digestive cells, and NFR and GGT activity in the digestive gland, which we have shown in previous studies to exhibit the greatest potential as bioindicators.

Bioindicator development of exposure to chlorinated compounds: Very recently we have initiated studies, conducted by a post-doctoral associate, to specifically evaluate cellular changes in *M. edulis* from exposure to chlorinated compounds. Recent studies have shown that mussels have the ability to extensively biotransform some chlorinated aromatic compounds via glutathione conjugation (Inouye 1994). For biomonitoring studies, alternative bioindicators may provide the only means of detecting the presence of some chlorinated xenobiotics, as rapid biotransformation via glutathione conjugation may cause some chemicals to pass undetected with conventional protocols for chemical analysis of tissue.

Previous studies (Inouye 1994) have shown that during exposure of mussels to selected chlorinated compounds no significant changes were observed in tissue GSH content, but activity of glutathione transferase, which facilitates the reaction of GSH with electrophilic substrates, increased ten-fold in gill tissue, indicating its potential use as a bioindicator of exposure to environmental contamination. Although GSH levels did not markedly change during these preliminary studies there appeared to be a slight decrease in total GSH, followed by a return to initial levels during the exposure and monitoring study. It is possible that utilization of GSH in the detoxication process, followed by increased production of GSH, had occurred. Monitoring g-GCS activity, the rate limiting enzyme in GSH synthesis, may provide a better indicator of exposure than GSH levels alone, which is also indicated by our recent studies with fish.

To further assess the potential of GSH transferase or g-GCS induction as reliable bioindicators in *M. edulis,* the response to model contaminants including the time to maximal induction and the duration of the response must be determined. In addition, natural variations in the enzyme activity should be investigated, as seasonal fluctuations in glutathione transferases of *Mytilus* may be large enough to pose a potential problem for their use as bioindicators of exposure.

The studies in FY95 would determine the feasibility of utilizing induction of glutathione transferases and g-GCS as bioindicators of exposure in mussels in a series of four experiments: 1) laboratory exposures of mussels to model chlorinated compound and a metal, 2) laboratory exposures of mussels to contaminated sediments, 3) comparison of mussels collected from contaminated and reference sites, and 4) determination of seasonal variability. A parallel experiment utilizing sediments and /or sediment extracts from contaminated and reference sites in Puget Sound will also be conducted, as well as analyses of mussels sampled from an urban and a reference site in Puget Sound to assess the potential of glutathione transferase and g-GCS activities to serve as bioindicators of contaminant exposure and provide information on contaminant specificity in the response.

In situ studies: In previous studies, we have observed that mussels from urban-associated areas showed alterations in bioindicators when compared to mussels from reference environments. Concomitant with these bioindicator responses, we observed that resident mussels from contaminated environments were typically smaller than mussels from reference environments. The implications of these findings are that mussels from urban-associated environments have impaired growth or that bigger mussels were disappearing from populations in the urban-associated environments. To assess whether altered growth rate in indigenous populations of mussels is a factor, *in situ* exposures of caged mussels will be conducted. We will also determine difference in growth and bioaccumulation

potential of intertidally and subtidally deployed mussels to evaluate the potential of mussels deployed in cages to accurately reflect the impact of environmental contaminants on indigenous populations. The results of these experiments will aid in substantiating that the observed population level effects are contaminant associated and to aid in further assessing the potential of the bioindicators in diagnosing population level impacts.

Association between development of hemic neoplasia and contaminant exposure in mussels: Recent studies (Elston et al. 1992) have provided conflicting results on the role of chemical contaminant exposure in the etiology of hemocytic neoplasia in bivalve organisms, including *M. edulis*. Some laboratory and field research has suggested that exposure of bivalves to chemical contaminants increases the prevalence of hemocytic neoplasia, a disseminated blood cell tumor. Our preliminary observations indicate that there is no observable relationship between the prevalence of hemocytic neoplasia and exposure to chemical contaminants in mussels from a number of sites in Puget Sound, WA., whereas chemical contaminant exposure was associated with alterations in cytochemical bioindicators. Hemocytic neoplasia is a disease indigenous to mussels from Puget Sound, WA. To further assess whether chemical contaminant exposure is an important factor in the development of hemocytic neoplasia, either directly or indirectly as an additional stress factor in natural populations of mussels, a population of *Mytilus edulis* already exhibiting a high prevalence of hemocytic neoplasia will be exposed to contaminants in a long-term laboratory study. The development of this disease in bivalves appears to be associated with a viral vector, thus exacerbation of the prevalence would be expected in a natural population infected with the principal causative agent and subjected to an additional stress imposed by contaminants. If contaminants are associated with the development of hemic neoplasia (not necessarily the cause) it is expected that we should observe an increase in the prevalence of hemic neoplasia in mussel populations already affected by this disease. Adult mussels will be exposed in their diet to either a model mixture of polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs), and changes in the prevalence of hemocytic neoplasia in the laboratory population will be monitored.

The results from the studies outlined above will provide important data on dose-response, and the influence of physiological changes (e.g. seasonal effects), and disease on bioindicator response, as well as the relationship of the response of a suite of bioindicators to effects on population level parameters. These data will provide potential diagnostic tools to be used to assess contaminant induced effects in an important bivalve species which is used extensively in NOAA's and other federal and state environmental monitoring programs.

Element D: Bioeffects Research (to Establish Links Between Contaminant Exposure and Significant Effects)

At present, it is not known to what extent contaminant inputs contribute to population declines and community alterations in the marine environment. Even though the goal of the Toxic Chemical Theme of the Coastal Ocean Program is to determine contaminant-induced effects at the population and community structure level, it is first necessary to establish the link between contaminant exposure and demonstrable effects in individuals. The Bioeffects Research Project was formulated to enhance or establish relationships between environmental contaminant exposure and altered biological processes in marine biota. Funded research is to concentrate on measuring and linking contaminant-induced effects in individuals which may have implications at the population level. Alterations in three critical biological endpoints: i.e., survival, growth, and fecundity, are considered most likely to result in significant population perturbations. Successful measurement of processes and

parameters that establish links between contaminant exposure and these three key biological endpoints, either directly or indirectly, is a basic design requirement for all research projects funded under this element.

Funding for Bioeffects Research proposals under Element D was initiated in FY91. Based on the Toxic Chemical Theme Team's recommendations, selection criteria for the research preproposals were that 1) studies should be limited to species and locales for which there is already ample documented evidence of certain biological effects occurring (i.e., lesions, disease, reproductive impairment, reduced growth), and 2) studies should be limited to species and locales for which there is a reasonable suspicion (but not necessarily a firm link) that the observed effects are related to contaminant exposure. This element is designed to embrace interdisciplinary studies by researchers from NOAA and academic institutions or other government agencies on a variety of vertebrate and invertebrate species at different stages of their development and from a variety of geographic locations.

In the first year of this program element, a total of 50 proposals were received; they originated from 18 different states and included numerous NOAA/university collaborations. Following a two-step peer-review process (involving scientists both within and outside of NOAA), five proposals representing a broad range of species, locales, types of contaminants, and types of biological effects were recommended for funding to the Toxic Chemical Theme Program Management Committee (PMC). Funding decisions were made based on: (a) the overall scientific quality of the research proposed; (b) the relevance of the research to the stated objective of the element; and to a lesser extent on (c) availability of matching funds. In October 1991, following approval by the PMC, COP FY91 funds totaling $329,591.00 were issued to the Principal Investigators (PIs) of the five top proposals through use of Sea Grant's funding mechanism. While each funded project is expected to be of 2-3 years duration, funding awards were only made for the first year of each study (Oct. 1991-Sept. 1992) with support for subsequent years depending upon both the availability of funds and progress in the research.

Accordingly, in February, 1993 the Bioeffects Research Project Manager sent letters to all PIs requesting a 1.5 year progress report. Each report was reviewed by the Project Manager, as well as an independent peer, to assess whether progress-to-date had been satisfactory. In addition, scientific guidance was sought from the Toxic Chemical Theme's Technical Advisory Committee (TAC). On August 26-27, 1993 the Toxic Chemical Theme's second TAC meeting was held in Seattle, WA during which time the Bioeffects Research Project Manager formally reported on the status of each project. The TAC strongly and unanimously supported renewing funding for all Element D projects. Level funding for each project was renewed for the period of Oct. 1993-Sept. 1994.

Research highlights for each project for the first 3 years are presented below:

Project 1. PIs: Drs. John J. Stegeman & Michael J. Moore
Woods Hole Oceanographic Institution
Woods Hole, MA
"Cell Proliferation: A Potential Marker for the Biological Effect of Chronic Exposure to Epigenetic Carcinogens in Coastal Benthic Finfish and Shellfish"

The investigators are developing an immunochemical assay to identify proliferative lesions in fish and shellfish via detection of tritiated bromodeoxyuridine (BrdU) incorporation in the nuclei of paraffin-embedded histological sections. BrdU injection allows the PIs to label many dividing tissues at once in a non-invasive way. Through this

method they intend to be able to establish which lesion types represent proliferative lesions. Proliferative lesions have been linked in experimental models to neoplasms. Species to be studied are: winter flounder, mummichog, and soft shell clam.

Progress at 3 years: Experiments and data evaluation have progressed as planned. No interim FY94 PRogress Report was requested as Final Reports are due in October 1994.

Project 2. PI: Dr. Peter Thomas
The University of Texas at Austin
Port Aransas, TX
"Field and Laboratory Evaluation of Endocrine Indices of Reproductive Dysfunction in Atlantic Croaker"

The objective of this study is to conduct field evaluations of various indices of reproductive impairment in female Atlantic croaker at 2 reference sites (Galveston Bay) and 2 contaminated sites (Houston Ship Channel). The indices include: ovarian function, spawning success, and reproductive endocrine status. The investigator intends to relate the findings from reproductive indices studies to levels of chemicals in tissues, bile FACs, and cellular alterations (in collaboration with Michael Moore, the PI listed above). A laboratory study will also be conducted in which endocrine indices of reproductive impairment will be evaluated in relation to: (1) salinity; and (2) exposure to a model mixture of organic and metal contaminants characteristic of the region.

Progress at 3 years: Experiments and data evaluation have progressed as planned. No interim FY94 PRogress Report was requested as Final Reports are due in October 1994.

Project 3. PI: Dr. Richard Peterson
University of Wisconsin
Madison, WI
"Ecological Risk Assessment of Complex Mixtures of Polybrominated Aromatic Hydrocarbons in Feral Fish Eggs"

This project is examining whether exposure to brominated dioxin-like compounds cause early life stage mortality in rainbow trout, and if so, determine the site specific risk to trout eggs from exposure to complex mixtures of these congeners. Fertilized rainbow trout eggs will be injected with graded doses of selected TCDD-like PBB congeners and assessed for toxicity. The investigator proposes to examine mixtures of PCB and PBB congeners to assess interactive effects on fish egg toxicity. The studies with PBBs could potentially be even more valuable than studies with PCBs because PBBs are still being manufactured. Significant findings from these studies could lead to important alterations in regulatory measures.

Progress at 3 years: Experiments and data evaluation have progressed as planned. No interim FY94 PRogress Report was requested as Final Reports are due in October 1994.

Project 4. PIs: Dr. G. T. Chandler
University of South Carolina
Columbia, SC
-and-
Drs. Geoffrey I. Scott & Michael H. Fulton
S.E. Fisheries Science Center

Charleston, SC
"The Acute Toxicity and Bioaccumulation of Azinphos-methyl in Benthic Copepods and the Development of a Model for the Trophic Transfer of Non-persistent Pesticides to Recreationally Important Finfish Species"

This study represents a NMFS/university collaboration whose main objective is to determine acute and chronic, lethal and sublethal (reproductive impairment) effects of a non-persistent organophosphorous pesticide on single species populations of two important copepods. These copepods represent major components of the food webs of estuarine ecosystems in the Carolinas. Azinphos-methyl (AMP) is used on golf courses and has been related to fish kills in the South Carolina area. The PIs will determine the bioaccumulation rates of AMP by the two copepods cultured on contaminated sediments, and the metabolic fate of AMP in these species using radiolabelled tracers. Additionally, they will attempt to follow the trophic transfer of AMP from sediment to copepod to juvenile spot, a benthic feeding fish. Finally, they will measure the effect of AMP exposure in the spot by determining the degree inhibition of acetylcholinesterase (AchE) activity in the fish.

Progress at 3 years: Experiments and data evaluation have progressed as planned. No interim FY94 PRogress Report was requested as Final Reports are due in October 1994.

Project 5. PIs: Ms. Dianne Black
U.S. Environmental Protection Agency
Narragansett, RI
-and-
Dr. Anne McElroy
University of Massachusetts at Boston
Boston, MA
"PCB Effects on Fish Reproduction: Characterization of the Relationship between Endocrine Function and Reproductive Success"

This project represents a university/EPA collaboration to study reproductive effects in the mummichog by conducting laboratory exposures to mixtures of PCB congeners characteristic of New Bedford Harbor, and to establish dose-response relationships for those effects. All reproductive indices will be correlated to levels of PCBs in liver, brain and ovary. The investigators will also conduct field surveys along a known PCB gradient in New Bedford Harbor to identify endocrine indices that can be used as bioindicators of reproductive effects in both mummichog and winter flounder.

Progress at 3 years: Experiments and data evaluation have progressed as planned. No interim FY94 PRogress Report was requested as Final Reports are due in October 1994.

Current Approach (FY95)

Each of the projects funded under the initial Request For Proposals for Element D will be completed by September 30, 1994 and a final report will be submitted in October 1994. Funds for Element D for FY95 were not allocated, thus a new Request for Proposals was not issued.

PRODUCT DEVELOPMENT AND EVALUATION

Element A--The bioeffects surveys are composed of a number of separate studies documenting various aspects of the magnitude and extent of contaminant bioeffects in each of the areas of concern. A number of reports and articles describing the results of these individual studies will be published, primarily in NOAA technical memoranda and peer-reviewed journals. Prior to implementing a study in a new area, an assessment of existing information will generally be performed to determine the relative scales of chemical contamination and to establish a basis for further studies. At the completion of the survey in an area of concern, one or more NOAA Technical Memoranda will also be published focusing on that area and summarizing the results and conclusions from the studies conducted there and assessing our knowledge concerning magnitude and extent of the biological effects due to toxic contaminants. These summary reports will undergo thorough peer review by reviewers both internal and external to NOAA. This reporting process is exemplified by reports and publications from three study areas: San Francisco Bay (Long et al. 1990, Spies et al 1990, Long and Markel 1992); Long Island Sound (Gronlund et al. 1991, Nelson et al. 1991, Perry et al. 1991, Turgeon and O'Connor 1991); and Tampa Bay (Long et al., 1991; Long et al., 1994). Additional reports are currently under development for Long Island Sound, Tampa Bay and the Hudson-Raritan Estuary. Synthesis reports assessing the national extent of environmental degradation due to toxics, or comparing major regions, will be published after several of the primary regions of concern have been surveyed.

Element B--The research on the factors controlling the uptake and bioaccumulation of contaminants will be conducted as a series of research projects, and these will lead primarily to publication of peer-reviewed scientific articles and reports. Periodic summary reports will be prepared on the status of the research being conducted in this area along with recommended priorities for future directions.

Element C-- The bioindicator research will result in the development and evaluation of new and improved bioindicators for assessing contaminant exposure and associated effects on individual organisms and on populations of living marine resources in U.S. waters. Peer-reviewed NOAA reports and scientific articles in peer-review journals documenting the development of these indicators, specifying how they should be measured, and evaluating their utility will be produced.

Element D-- The research on the links between exposure and effects will be conducted as a number of separate research projects, and these will lead to the publication of peer-reviewed scientific articles and reports.

DATA MANAGEMENT

Element A--The intensive bioeffects surveys data are collected on fish and invertebrate reproductive properties, prevalence of lesions and DNA adducts in fish, a battery of sublethal biomarker assays in molluscs, acute and sublethal sediment toxicity, along with biochemical indicators of stress and other bioindicators, as well as on chemical contaminant levels in tissues and sediments. The principal investigator for each one of the studies conducted as part of these surveys will be the primary contact for these data. The data will be available within two years of the completion of the field work and will be maintained by the principal investigator. The data will also be included in the final reports from the studies. After submission of these final reports, the NOS/ORCA Coastal Monitoring and Bioeffects Assessment (CMBA) Division will maintain copies and make them available on request.

Element B--The data collected from the studies to determine the effects of environmental and biological factors on bioaccumulation will be a mixture of laboratory and field results relating such factors as salinity, temperature, and reproductive parameters with concentrations of chemical contaminants in tissues. Different components of the study will be conducted by different principal investigators, who will be the primary contacts for these data. These data will be available within one year of the completion of the studies and will be maintained by the principal investigator. The data will be included in the reports from this work and copies of these reports will be available on request from the principal investigators and from the NOS/ORCA Coastal Monitoring and Bioeffects Assessment (CMBA) Division.

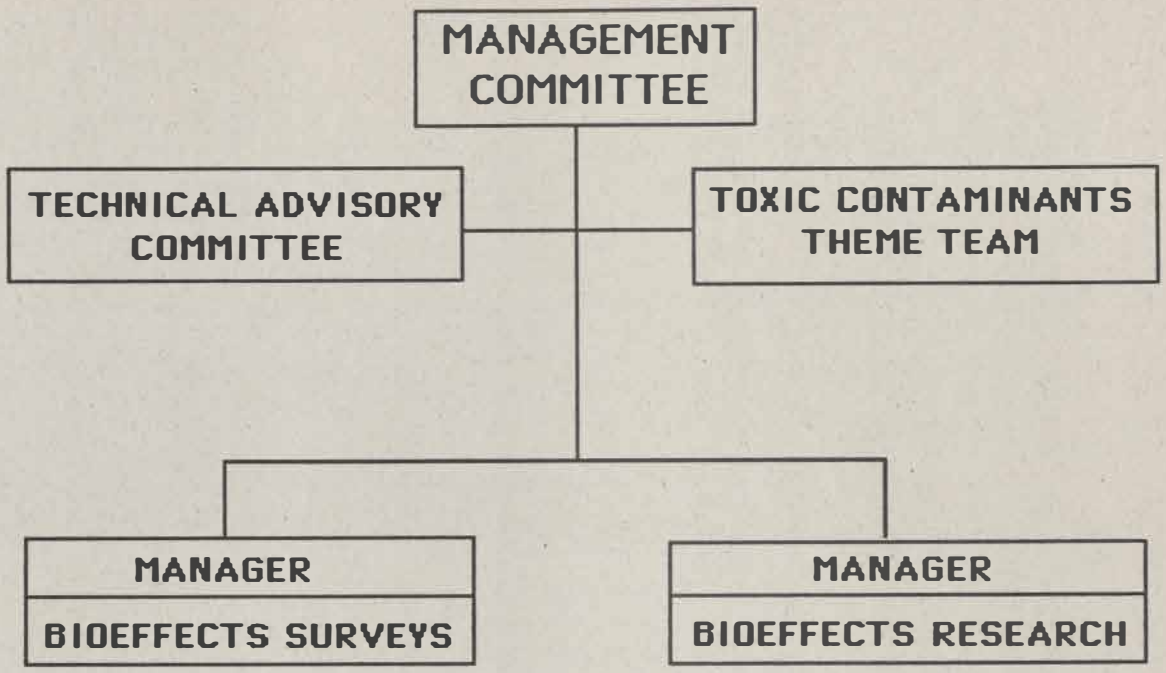
Element C--The data collected from the studies to develop new and improve existing bioindicators are primarily laboratory results measuring such properties as the levels of hepatic aryl hydrocarbon hydroxylase activity, DNA-xenobiotic adducts in livers, and fluorescent aromatic compounds in bile from fish (including English sole, starry flounder, winter flounder, Atlantic croaker, and white croaker) exposed to contaminants extracted from contaminated sediments. The principal investigator (Environmental Conservation Division, NWFSC) for this study will be the primary contact for these data. These data will be available within one year of the completion of the dose-effect laboratory studies and will be maintained by the principal investigator. The data will be included in the reports from this work and copies of these reports will be available on request from the principal investigator.

Element D--The research data collected to establish links between exposure and effects are defined in the individual proposals for work in this area. Funded principal investigators will be required to submit their data in a final report within one year of completion of their studies and copies of these final reports will be available from the Environmental Conservation Division, NWFSC and from the NOS, CMBA Division.

Data management in all the studies outlined in this plan will be handled as an integral part of the studies and will not be funded separately. The number of data points gathered by any one study will be relatively few and will measure a number of properties; further the properties measured will differ among the studies. Thus, it is more efficient to integrate data management into the individual studies rather than to have it handled as a separate component across the theme area.

PROGRAM MANAGEMENT

The final management responsibility for planning and carrying out the Toxic Chemical Contaminants Theme program is jointly with the Assistant Administrator for Ocean Services and Coastal Zone Management and the Assistant Administrator for the National Marine Fisheries Service. To carry out these responsibilities, the management structure in the following diagram has been established.



The composition and responsibilities of the components in this structure are:

Management Committee--This three-person Program Management Committee (PMC) is composed of the cochairs of the Theme Team, one from NMFS and one from NOS, plus a non-NOAA representative who is an expert in the area of contamination of the marine environment by toxic chemicals. The Management Committee is responsible for overall management of the Toxic Chemical Contaminants Program including: (1) setting and revising guidelines for the general direction of the Program, (2) developing and submitting to the Coastal Ocean Program Office the long-term and the annual implementation plans for the work in this Program, (3) obtaining scientific review of studies proposed in the Program, (4) making decisions concerning funding for the studies conducted by the Program, (5) providing broad oversight of the funded studies to assure satisfactory progress, and (6) carrying out periodic reviews of overall program progress. The Management Committee reports to the Assistant Administrator for the National Marine Fisheries Service and the Assistant Administrator for Ocean Services and Coastal Zone Management as well as to NOAA's Coastal Council and provides the interface for the Toxic Chemical Contaminants Theme of the COP with the Council.

Toxic Chemical Contaminants Theme Team--This team is composed of NOAA scientists and scientific managers who are actively involved in programs related to toxic chemical contaminants. They are selected by and represent their NOAA line offices on the team. The primary function of the team is to provide advice and guidance to the Management Committee. It assists in the long-term planning for the program and in providing recommendations on the specific types of studies that should be conducted. The team members provide input to the Program regarding the interests of their Line Office and its scientists in participating in the Toxics Program and also provide information about the Program to interested persons within their offices.

Technical Advisory Committee--Along with the three-person Program Management Committee (PMC), the Technical Advisory Committee (TAC) serves as a major source of program guidance and planning for the Toxics Theme. TAC provides assistance, as needed, to enable the PMC to carry out its duties with regard to program planning, implementation, and evaluation. TAC is composed of five scientists/science managers from outside of NOAA who are experts in diverse fields related to the overall issue of chemical contamination of the aquatic environment (e.g., aquatic toxicology, ecology, toxicokinetics, histopathology, environmental chemistry), and who have the broad-based knowledge and vision needed to respond to the following TAC directives. The Committee chairperson, Dr. Donald Crosby, is also the non-NOAA member of the PMC. The four other TAC members were selected by the PMC in consultation with the Director of the Coastal Ocean Program Office.

TAC Members:

Dr. Donald Crosby (Chair)	University of California, Davis
Dr. David Hinton	University of California, Davis
Dr. Margaret James	University of Florida
Dr. Guri Roesijadi	University of Maryland
Dr. Philippe Ross	The Citadel

The primary functions of the Technical Advisory Committee are:

- to review and provide advice on enhancing the overall scientific quality of COP's Toxics Program;
- to offer ongoing guidance to the PMC on the Toxics Program's present and future direction;
- to identify for the PMC priority research areas to be addressed, including new issues which may emerge over time, and to recommend a level of effort for each;
- to advise the PMC as to suggested adjustments in future Implementation Plans in response to changing needs;
- to evaluate the scientific quality of individual studies (both extramural and in-house NOAA projects) as they are being conducted;
- to evaluate the appropriateness of mechanisms for extramural funding and the balance between internal and external efforts.
- to evaluate the scientific merit of studies to be conducted in-house by NOAA scientists, and the PMC will not approve funding for any studies judged by TAC to be of poor scientific quality.

The first two meetings of the Committee took place in Seattle June 11-12, 1992, and August 26-27, 1993, respectively. The TAC members received detailed briefings from PMC members and project leaders on the objectives and status of activities under each of the Theme Elements. It is anticipated that the Committee will continue to meet with the Program Managers once a year to review the Program's overall objectives as they evolve over time, and to suggest mid-course corrections when warranted. Additional meetings will be called if special needs arise. TAC members will also be asked to review the various scientific progress reports (see Milestones Chart) produced by each program element.

Towards the end of FY93, a Toxics Theme Symposium was organized as a special session ("Linkages between contaminants and biological effects") at the 14th Annual Meeting of the Society for Environmental Toxicology and Chemistry (SETAC) in Houston, 14-18 November 1993. This special session included participation by the Program Management Committee, the Toxics Theme Project Managers, and all Principal Investigators funded under Element D. At the Symposium, the COP-funded researchers

presented and discussed their data. Most members of the Technical Advisory Committee attended, and had their first opportunity to review the results to date with the outside investigators in person.

Bioeffects Surveys and Bioeffects Research Projects--The Toxic Chemical Contaminants Program is divided into two major projects that are managed separately. One project encompasses the Bioeffects Surveys (Element A) and any work formally undertaken on Uptake/Bioaccumula-tion (Element B) of the Program, while the second includes Bioindicator Development (Element C) and Bioeffects Research (Element D). Each of the projects is directed by a project manager selected by the Management Committee. Projects may be redefined, added, or subtracted in future years as deemed appropriate by the Management Committee in consulta-tion with the Theme Team and the Coastal Ocean Program Office. The project managers have responsibility for detailed management of their projects. They develop guidelines and specifications for studies to be conducted in their project, obtain proposals for carrying them out, and provide recommendations to the Management Team on which proposals to fund and the levels of funding required. They assure peer-review of all proposed research studies, including at least two reviews from outside of NOAA for each proposal. They provide support to the Technical Advisory Committee in its assessment of the scientific quality of all studies conduc-ted in-house by NOAA. They oversee evaluation and review of the studies in their project and provide assessment to the Management Team and Coastal Council on these matters as appropriate.

The procedures used to plan and implement the studies in the each of the four elements are as follows:

Element A (Bioeffects Surveys): After initial discussions among the Management Committee, Technical Advisory Committee, and Project Managers to identify target areas for study, the first step in planning a survey is generally to develop a synthesis of existing information on the occurrence and distribution of toxic contaminants and associated biological effects in the survey area. A survey proposed to be conducted in that area is then planned based on this summary and in close consultation with scientists and resource managers who have experience in the specific geographical area. In the sediment quality surveys, each survey region is stratified into roughly equal size areas and sampling locations are randomly chosen within each area. In the surveys of fish and bivalve bioeffects, samples are collected from discrete points that are chosen to represent conditions in major areas of each study region. The plan for each area is sent to scientific and resource management experts in the geographical area under consideration for their review and is revised based on this review. Once a year, the Technical Advisory Committee will review the overall scientific competence and relevancy of the studies carried out in this element.

Element B (Bioaccumulation Research): The program of laboratory and field experiments to study the physical, chemical, and biological factors determining uptake and bioaccumulation of toxic materials will be planned initially by scientists within NOAA, in consultation with academic scientists working in this same area of research. All proposed studies will be reviewed by the Technical Advisory Committee and through a peer-review process The Technical Advisory Committee will periodically review the overall scientific competency and relevancy of these studies.

Element C (Bioeffects Indicator Development): To take advantage of NOAA's extensive experience and interest in development of bioindicators, the studies in this element are carried out by scientists from within NOAA, although subcontracting to other organizations is encouraged as appropriate. All proposed studies are reviewed both

through peer-review (at least two outside NOAA reviewers) and by the Technical Advisory Committee.

Element D (Bioeffects Research): The research studies concerning linkages between contaminant exposure and bioeffects are carried out through a competitive proposal procedure. An RFP detailing the scientific area of interest and the criteria by which proposals will be judged is developed and distributed. The proposals received are peer-reviewed (by at least two outside NOAA reviewers) and the Management Team makes the final selections based on the peer-review and the recommendations of the Project Manager.

REVIEW

Proposals for research studies to be conducted in this theme area are peer-reviewed, including at least two reviews from outside NOAA. The peer reviewers are asked to evaluate the proposals for intrinsic technical merit relative to the following concerns: (1) applicability of the proposed study toward meeting the theme and project objectives, (2) use of the best available data and information for planning and conducting the study, (3) appropriateness and scientific validity of proposed research design and testing methodologies, and (4) background, experience, and scientific competence of the proposed investigators to carry out the proposed study. For the parts of the Program where substantial numbers of proposals are expected a Proposal Review Panel is established comprised of distin-guished scientists external to the Program. These panels evaluate the scientific merit of the proposed research and advise the Project Manager and the Management Committee on the merits of and modifications recommended for the submitted proposals. In FY92 such a panel was established to review the proposals received to carry out studies under Element D. The Project Managers make recommendations on funding decisions in their respective projects to the Management Committee based on the results of reviews such as these, and on the cost effectiveness of the proposed studies. Proposals with cost sharing, matching funds, or other mechanisms to increase cost effectiveness in the use of the Coastal Ocean Program funds are especially encouraged.

In addition to the peer review of proposals, the program in this theme area will be reviewed periodically by a panel of outside experts to evaluate the scientific competence and relevance of the work being conducted. These reviews will be held approximately biennially and will be organized by the Management Team in conjunction with the Technical Advisory Committee.

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PRODUCTS TO DATE

Many publications and reports have been generated during FY's 91-94 under the Toxic Contaminants Theme of the Coastal Ocean Program.

Element A: BIOEFFECTS SURVEYS

The following publications and reports, generated during 1991-94 with partial support under the Toxics Theme of the Coastal Ocean Program, are on file at NOAA's Office of Ocean Resources Conservation and Assessment, Bioeffects Assessment Branch, 6001 Executive Blvd, Rockville, MD 20852. Except for contractor reports which are in limited supply, they are available upon request.

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PROGRAM IMPACT ON REGIONAL COASTAL WATER QUALITY MANAGEMENT

The information generated by the Intensive Bioeffects Surveys in specific areas is of considerable interest to, and has been used extensively by regional managers engaged in water quality management and coastal zone planning. As evidenced in the foregoing list of technical presentations, NOAA personnel are directly involved in regional workgroups and are regularly consulted for briefings on ongoing studies and advice on additional research needs. The planning for Intensive Bioeffects Surveys is carried out cooperatively with related State and regional Programs. For example, Dr. Andrew Robertson is a member of the Toxics Management Work Group of the New York-New Jersy Harbor Estuary Program. The results of the COP bioeffects surveys in this area are the best available data on contaminant effects and are being used extensively in developing assessment and regulatory activities. Dr. Douglas Wolfe briefed this group in 1993 at a well-attended public meeting on the results of the COP bioeffects surveys in the Hudson-Raritan Estaury and discussed their implications for environmental management. During the same year, Dr. Robertson and Edward Long also briefed the Technical Advisory Committee of the Tampa Bay Estuary Program and participated in planning discussions with Florida state officials to identify research needs in Tampa Bay. Edward Long is working with officials of the states of Florida, Washington, and California to identify appropriate information and approaches for development of effects-based sediment quality criteria. Information generated by this project on the distribution of contaminants and associated biological effects has also been used by the U.S. Environmental Protection Agency: (a) to identify sampling strategies for comprehensive monitoring under the Estuaries component of the Environmental Monitoring and Assessment Program (EMAP-E), (b) to determine the impact of revised testing protocols on the Army Corps of Engineers dredging efforts, and (c) to evaluate the potential significance of proposed sediment quality criteria on present practice in the dredged materials management program.

Element C: BIOINDICATOR DEVELOPMENT

The following publications and reports, generated during 1991-94 with partial support under the Toxics Theme of the Coastal Ocean Program, are on file at the Environmental Conservation Division of NOAA's NMFS Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA.

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Element D: BIOEFFECTS RESEARCH: EXTRAMURAL PROJECTS

The following publications and reports, generated during 1992-93 with partial support under the Toxics Theme of the Coastal Ocean Program, are available from the authors. Copies are also on file at the Environmental Conservation Division of NOAA's NMFS Northwest Fisheries Science Center, 2725 Montlake Blvd. E., Seattle, WA.

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FY94 MILESTONES (PLANNED VS ACHIEVED)

Element A (Bioeffects Surveys)	Planned Completion Date	Actual Outcome
A. LONG ISLAND SOUND		
1. Complete draft report on previous results	Jun 94	Aug94
B. BOSTON HARBOR		
1. Complete draft report on previous results	Aug 94	Deferred
C. SOUTHERN CALIFORNIA		
1. Complete bivalve biomarker (contractor) report	Jan 94	Mar 94
2. Initiate year 3 sampling	Apr 94	Apr 94
3. Complete fish biomarker (contractor) report	May 94	Delayed
6. Complete draft report on year 2 studies	Jun 94	Delayed
D. TAMPA BAY		
1. Draft report on fish bioeffects	Nov 93	Sep 94
2. Final report on sediment toxicity & chemistry	Dec 93	Aug 94
3. Draft report on oyster bioeffects studies	Apr 94	Sep 94
E. HUDSON-RARITAN ESTUARY		
1. Complete report on sublethal sediment toxicity	Nov 93	(Dec 94)
2. Complete Phase II sediment toxicity/chemistry	Dec 93	(Dec 94)
3. Draft report on sediment toxicity & chemistry	May 94	(Dec 94)
F. Northwest Florida Estuaries		
1. Bivalve Biomarker Workshop/study plan	Apr 94	Mar 94
2. Implement sediment toxicicty survey (Phase II)	Jul 94	Jul 94
3. Preliminary Phase I sedtox/chemistry report	Jul 94	Jun 94
4. Preliminary fish bioeffects report	Aug 94	Sep 94
G. COASTAL SOUTH CAROLINA/GEORGIA		
1. Implement sediment toxicity survey (Phase II)	Jul 94	Aug 94
2. Complete Phase 1 sedtox-chemistry report	Aug 94	Delayed
3. Preliminary fish bioeffects report	Aug 94	Sep 94
H. BISCAYNE BAY		
1. Develop and implement Study Plan	Apr 94	(Nov 94)

Element B (Bioaccumulation Research)	Planned Completion Date	Actual Outcome
No activities planned		

Element C (Bioindicator Development)	Planned Completion Date	Actual Outcome
A. Present research findings at SETAC '93 COP session	Nov 93	Nov 93
B. Bioindicators: Fish		
1. Complete evaluation of porphyrin profies as a biomarker of exposure to heavy metals	Mar 94	June 94

FY94 MILESTONES (PLANNED VS ACHIEVED)

2. Complete laboratory studies of dose response of biomarker of oxidative stress (glutathione) in winter flounder exposed to contaminants	Jan 94	Mar 94
3. Complete field test of the biomarker of oxidative stress	Jun 94	
4. Continue development and validation of biomarkers of immune function	Sept 94	Sept 94
5. Continue development of biomarker of structural genetic damage (single strand breaks in DNA)	Sept 94	Sept 94
6. Conduct dose response study in black croaker for the biomarkers of genetic damage and P450 induction and linkage to contaminant exposure	Sept 94	Revised
7. Complete evaluation of biomarker of oxidative DNA damage (8-hydroxydeoxyguanosine)	Sept 94	Ongoing
C. Bioindicators: Invertebtates (bivalves)		
1. Complete evaluation of annual cycle of lysomal responses and enzymatic activity (cytochemical biomarkers) in digestigve cells of *M. edulis* from contaminanted and reference sites	Mar 94	April 94
2. Initiate evaluation of cytochemical biomarkers in oysters	Jun 94	Deferred
3. Continue field studies assessing the relationship between altered frowth and contaminant exposure in *M. edulis*	Sept 94	Sept 94
4. Complete initial dose response studies for cytochemical biomarkers in digestive cells of *M. edulis*	Sept 94	June 94
Element D (Bioeffects Research)		
1. Presentation of research findings at special SETAC Plenary Session	Nov 93	Nov 93
2. Develop RFP for new proposals-FY95	Oct 94	Deferred
3. Receive final reports of studies funded during the first three years of COP	Oct 94	(Oct 94)
All Elements		
1. Organize Special SETAC '93 Plenary Session on research from the COP Toxics Theme	Nov 93	Nov 93

FY95 MILESTONES

Element A (Bioeffects Surveys)	Target Date
A. Long Island Sound	
1. Complete summary of bioeffects of contaminants	Aug 95
B. BOSTON HARBOR	
1. Complete draft report on previous results	Mar 95
C. SOUTHERN CALIFORNIA	
1. Complete year 1 sed-tox survey report	Nov 94
2. Complete fish biomarker report	Nov 94
3. Draft bivalve biomarker report	Nov 94
4. Draft year 2 sed-tox survey report	May 95
5. Initiate year 2 bivalve biomarker survey	Nov 94
D. TAMPA BAY	
1. Complete report on oyster bioeffects studies	Mar 95
E. HUDSON-RARITAN ESTUARY	
1. Complete draft report on sed-tox surveys	Jan 95
F. NORTHWEST FLORIDA	
1. Complete draft report on sed-tox surveys	Jul 95
2. Complete planned field program in this region	Aug 95
G. COASTAL SOUTH CAROLINA/GEORGIA ESTUARIES	
1. Complete planned field program in this region	Aug 95
2. Complete draft of sed-tox survey report	Sep 95
H. BISCAYNE BAY	
1. Develop plan and MOU for phase 1 sed-tox survey.	Nov 94
2. Complete phase 1 of sed-tox survey	Jan 95
3. Complete phase 2 of sed-tox survey	Aug 95
4. Initiate bivalve biomarker survey	Jan 95

Element B (Bioaccumulation Research)	Completion date
(No milestones planned in FY95)	--

Element C (Bioindicator Development)	Completion date
Objective 1- New Bioindicators: Fish	
1 Complete manuscript describing the usefulness of the mitogen assay as a bioindicator of exposure in English sole.	Dec. 94
2. Initiate studies with red drum examining their mitogenic response after exposure to contaminants	Feb. 95
3. Complete preliminary studies describing macrophage function as a bioindicator of exposure in English sole.	Mar. 95
4. Initiate disease challenge studies with English sole and red drum exposed to contaminants.	April 95
5. Determine optimum conditions for the ELISPOT assay for the examination of the antibody response in English sole.	Sept. 95

6.	Initiate studies examining biochemical characteristics of a specific English sole and winter flounder antibody.	Sept. 95
7.	Complete field evaluation of macrophage function as a bioindicator of contaminant exposure.	Sept. 95
8.	Complete 8-oxo-dG method evaluation/improvement and if results are positive, examine oxidative DNA damage in fish (red drum, English sole) exposed to sediment extracts.	Sept. 95
9.	Complete dose-response studies of bioindicator of oxidative stress (glutathione) in red drum, a target species in Element A.	Sept. 95
10.	Complete evaluation of a g-GCS mRNA cDNA probe as a bioindicator of oxidative stress.	Sept. 95
11.	Complete manuscript descibing the use of image analysis to quantitate cellular CYP1A expression in liver tissue.	Sept. 95
12.	Using several species, complete evaluation of PCNA labeling to objectively quantify cell proliferation in hepatic lesions.	Sept. 95
13.	Complete initial dose-response studies of DNA damage in fish and bivalves using the "comet assay".	Sept. 95

Objective 2 - Existing Bioindicators: Fish

1.	Complete manuscript on bioindicator dose-response studies with English sole and winter flounder.	Sept. 95
2.	Complete dose-response studies of bioindicator of exposure (biliary FACs, hepatic DNA adducts and CYP1A activity) in red drum.	Sept. 95

Objective 3 - New Bioindicators: Invertebrates

1.	Initiate evaluation of cytochemical bioindicators in mussels exposed to chlorinated compounds.	Oct. 94
2.	Initiate time- and dose-response studies of cytochemical bioindicators in mussels exposed to model compounds and sediment extracts.	Jan. 95
3.	Complete in situ field studies assessing the relationship between growth and contaminant exposure in *M. edulis*.	Sept. 95
4.	Complete assessment of association between development of hemic neoplasia and contaminant expsoure in *M. edulis*.	Jan. 95

Element D (Bioeffects Research)*	**Completion date**
A. Receive final reports of studies funded during FY91-93	Oct 94

*On November 17, 1993, the Coastal Ocean Program deferred further funding for Element D.

PROGRAM BUDGET

PROGRAM ELEMENT	(Dollars in thousands)		
	FY95	FY96	FY97
Element A--Bioeffects Surveys	750	400	
Element B--Uptake/Bioaccumulation Research	0	0	
Element C--Bioindicator Development	400	0	
Element D--Effects Research	0	0	
TOTALS	1,150	400	

PROGRAM BUDGET (continued)

ANTICIPATED FUNDING
INTENSIVE REGIONAL BIOEFFECTS SURVEYS, FY 95-96

	FY95	FY96	FY97
Western Florida Panhandle			
Sediment Toxicity	$ 0K	$0K	
Sediment Chemistry	$ 0K	$0K	
Fish Exposure & Bioeffects	$ 90K	$0K	
Bivalve Exposure & Bioeffects	$ 50K	$0K	
Coastal South Carolina			
Sediment Toxicity	$ 0K	$0K	
Sediment Chemistry	$ 50K	$0K	
Fish Exposure & Bioeffects	$ 90K	$0K	
Bivalve Exposure & Bioeffects	$120K	$0K	
Biscayne Bay			
Fish Exposure/Bioeffects	$100K	$100K	
Bivalve Exposure/Bioeffects	$ 75K	$ 75K	
Sediment Toxicity	$175K	$125K	
Sediment Chemistry	$ 0K	$100K	
TOTALS	$750K	$400K	

PROGRAM BUDGET (continued)

PLANNED FY95 OBLIGATIONS, BY FISCAL QUARTER

	Qtr. 1	Qtr. 2	Qtr. 3	Qtr. 4
Element A--Bioeffects Surveys				
NW Florida fish bioeffects (NMFS/NWFSC)			$ 90K	
NW Florida bivalve bioeffects (U. S. EPA)		$ 50K		
So. Car./GA sediment chemistry (NMFS-SEFSC)	$50K			
So. Car./GA fish bioeffects (NMFS-NWFSC)		$ 90K		
So. Car./GA bivalve bioeffects (U. S. EPA)			$120K	
Biscayne Bay fish bioeffects (NMFS-NWFSC)		$100K		
Biscayne Bay bivalve bioeffects (U. S. EPA)			$ 75K	
Biscayne Bay sediment toxicity (TBD)		$175K		
Element B--Bioaccumulation Research				
(no activities in FY 94)				
Element C--Bioindicator Development				
NMFS costs (salaries, expendables)	$75K	$120K	$120K	$85K
Element D--Effects Research*				
NMFS costs (salaries, expendables)*				
Grants*				

PROGRAM BUDGET (continued)

Element A--Bioeffects Surveys

COP OBLIGATIONS, FY94
IMPLEMENTATION PLAN versus ACTUAL

	Planned	Actual
Sediment Toxicity in W. Florida So. Car/Ga., S. Florida		
• SAIC	$115K	$100.5K
• U. S. NBS	$ 96K	$ 96K
• NMFS-SEFSC	$ 52K	$ 52K
• FDEP	$ 30K	$ 25K
• TAMU/GERG	$ 48K	$ 0K
W. Florida sediment chemistry		
• Battelle	$ 0K	$ 49K
Cytochrome P-450 assays of sediments		
• Columbia Aquatic Sci.	$ 9K	$10K
Microtox mutatox assays of sediments		
• U. S. National Biological Survey	$ 0K	$5.5K
Fish biomarkers in w. Florida, So. Car/Ga., S. Florida		
• NMFS-NWFSC	$280K	$280K
Bivalve biomarkers and chemistry in W. Florida and S. Florida	$120K	$120K
Travel, shipping costs	$ 0K	$ 12K
TOTAL	$750K	$750K

INTERAGENCY COOPERATION IN THE SUPPORT OF ELEMENT A

The Intensive Bioeffects Surveys project, supported primarily by the Toxics Theme of the Coastal Ocean Program, is a collaborative effort with two main-line components of NOAA (NOS and NMFS). The surveys have been developed and implemented with the cooperation of several different federal and state agencies, which make direct management use of the research results from the program. These agencies have also made direct contributions of staff time and financial support to the program. The combined effort (tabulated below for FY94) represents a cumulative support level approximately twice the level of COP support. The other agency support for FY95 is projected at about the same level as in FY94.

INTENSIVE BIOEFFECTS SURVEYS
ESTIMATED FY 1994 FUNDING

	So. Cal	NW Florida	S.C./GA. estuaries	Biscayne Bay	HRE	TOTAL
COP	13	311	261	165	---	750
NOS/ORCA	15	35	35	5	50	140
NMFS						
• NWFC-ECD	---	25	25	50		100
• SWFC-BL	---	5	5	---	---	10
• SWFC-CL	---	---	50	---	---	50
State of CA	250	---	---	---	---	250
UC-Long Beach	5	---	---	---	---	5
EPA-EMAP	150	1	1	1		153
EPA-GB		50				50
State of FL	---	75	10	2		87
US NBS	---	45	45		25	115
Dade County	---	---	---	280	---	280
TOTALS	433	547	432	503	75	1990

(TOTAL MATCHING FUNDS: $1,240K)

APPENDIX A

Summary of Progress: Sediment Toxicity Surveys

INTRODUCTION

Sediment quality surveys, thus far, have been initiated or completed in the embayments listed in Table 1. The sizes of many of the embayments have been determined and they range from 550 km^2 in the Tampa Bay estuary to 0.3 km^2 in the Tijuana Slough estuary. The sizes of other areas are estimates that are yet to be verified with actual measurements. Based upon these calculations and estimates, approximately 2700 km^2 have been sampled among the study areas. A total of 1458 samples have been collected and tested for toxicity among all of these areas.

In all of the surveys, random-stratified sampling designs were used in the placement of sampling stations. Samples were collected throughout each study area. Samples of the upper 2 cm. of the sediment were retained for toxicity testing using the same procedures in all areas. In the initial surveys (e.g., Tampa Bay, Hudson-Raritan Bay, San Pedro Bay) samples were collected at randomly chosen locations selected to represent conditions in the immediate vicinity. However, beginning with the survey of the Newark Bay area, a probabalistic, random-stratified design was used in which a computer program chose station locations within prescribed boundaries of geographic strata. The data from the two different designs, although not strictly comparable, provide a broad representation of each study area. Also, they can define spatial gradients and patterns in toxicity over relative small spatial scales. In all study areas, sample station selections were not biased toward any known point sources and/or waste sites.

Table 1. Study areas in which sediment toxicity has been quantified, the sizes of study areas, the numbers of samples tested in each area, sampling designs, and status of each survey.

Area	km^2	N	Design	Status
Tampa Bay	550	165	S	C
Hudson-Raritan	350	117	S	C
San Pedro Bay	54	105	S	C
San Francisco Bay	est 300			
• historical		111	B	C
• survey		135	S	C
Newark Bay	13	57	P	C
Charleston	50	63	P	C
Wynyah Bay	est 2	9	P	C
Leadenwah Creek	est 1	9	P	C
Pensacola Bay	273	40+6	P	C
St. Andrew Bay	127	31	P	C
Long Isl. Sound bays	est 20	60	S	C
Boston Harbor	56	55	P	C
Choctawhatchee Bay	est 300	39	P	U
San Diego Bay	39	102	P	U
San Diego River	0.5	2	P	C
Savannah River	est 20	60	P	U
Apalachicola Bay	est 10	9	P	U
St. Simons Sound	est 20	20	P	U
Mission Bay	6	11	P	U
Tijuana Slough	0.3	6	P	U
SoCal small bays	est 10	43	P	U
Biscayne Bay	est 500	205	P	U
Totals	est 2702 km^2	1458 samples		

S = selective
P = probabalistic
B = biased

C = completed
U = underway

In each study area, NOAA/ORCA has collaborated with one or more agencies and worked with one or more different contractors (Table 2). Often, the NOAA COP funds have covered the expense of the sampling collections and toxicity tests, and funds from another agency have covered the costs of chemical analyses. In the surveys of southern California, the state has provide matching funds equal to those provided by the COP. In the surveys of Florida bays, the state of Florida has provided a sampling vessel and crew and paid for chemical analyses. Also, toxicity tests have been performed by several academic institutions with funds provided either by the COP or a state agency. EPA's EMAP staff have provided invaluable assistance in setting up the sampling locations using their probabalistic designs and computer software.

Table 2. Collaborators and contractors that have participated in the Coastal Ocean Program sediment quality surveys.

Collaborators/ partners	Contractors
Florida Dept. of Envir. Protection	Science Applications Itl. Corp.
NMFS-Charleston	Battelle Ocean Sciences
National Biological Survey	Skidaway Inst. of Oceanog.
U.S. EPA Region 2	Texas A&M University
Mass. Bays Program	University of South Carolina
Cal State Water Res. Ctl. Bd	ToxScan, Inc.
Cal. Dept. of Fish & Game	University of Cal. Santa Cruz
Moss Landing Marine Lab.	University of Cal. San Jose
U.S. EPA Region 1	Columbia Aquatic Sciences
Army Corps of Eng-NY District	Parametrix, Inc.
ORCA-HazMat	
U. S. EPA - EMAP	

Following a field trial of the relative attributes of five candidate toxicity tests, a battery of two tests was selected for the synoptic survey of San Francisco Bay. The two tests selected - an elutriate test performed with bivalve embryos and a microbial bioluminescence test - provided little evidence of toxicity in an area previously shown to be toxic. In the Hudson-Raritan estuary survey, a different type of elutriate test was attempted and the marine amphipod survival test was added to the battery of tests. These tests provided much better data, however, the elutriate test misidentified some highly contaminated samples as non-toxic. In the Tampa Bay survey, a toxicity test of the pore waters was used in place of the elutriate test. In most of the subsequent surveys, three tests have been used as the standardized battery of tests: the amphipod survival test, the microbial bioluminescence test, and the pore water test with invertebrate gametes and/or embryos (Table 3).

However, to evaluate the utility and sensitivity of new bioassays, some surveys have included exploratory experimentation with additional tests (Table 3). Tests with polychaetes and sand dollars were with some samples from Tampa Bay and Hudson-Raritan estuary. In Newark Bay, a dioxin equivalency bioassay performed with a cultured cell line was conducted on most samples. In the first year of the coastal South Carolina/Georgia survey, a harpacticoid copepod test was performed to determine toxicity to reproductive success. The Mutatox test, a variant of the Microtox test, was performed in the second phase of the western Florida survey and with some samples from South Carolina. In FY94 a cytochrome P-450 induction assay performed with a rat hepatome cell culture is underway on selected samples from San Diego Bay, Charleston Harbor, and (possibly) Biscayne Bay.

Table 3. Sediment toxicity tests used in the Coastal Ocean Program sediment toxicity surveys.

Region	Elutriate	Amph-pod	Micro-tox	Pore water	Other
SF Bay	X		X		
HRE	X	X	X		
Tampa		X	X	X	polychaete, sand dollar
San Pedro		X		X	
San Diego		X		X	P-450
SoCal estuaries		X[a]		X	benthos
LIS	X	X	X		
Boston		X	X	X	
Newark		X			dioxin equiv.
SC/GA coast		X	X	X	harpacticoid, Mutatox, P-450
western FL.		X	X	X	Mutatox
Biscayne[b]		X	X	X	harpacticoid, P-450

[a] two amphipod species - R. abronius, A. abdita
[b] tests that are planned

In each bay samples were collected throughout the suspected hotspots, dilution zones, and suspected clean or background areas. The survey designs were not focused upon any known point source or other suspected sources. Therefore, the data probably provide a balanced representation of conditions within each bay. Assuming that the data from each bay are representative of conditions within each bay, they can be compared among the bays surveyed thus far.

The amphipod survival tests have been performed with samples from all of the survey areas, except San Francisco Bay (where historical data from these tests performed by other agencies were compiled). Thus far, the incidence of the samples that were toxic to amphipod survival ranges from 0.0% to 84.2% (Table 4). As a basis for comparison, Williams et al. (1985) reported that 39% of the samples from Commencement Bay - a Superfund site - were significantly toxic. Seven bays surveyed in the COP studies exceeded Commencement Bay in the incidence of toxicity to amphipods. The incidence of toxicity, thus far, was highest in the Newark Bay area.

Two species of amphipods have been used in these tests: *Rhepoxynius abronius* in surveys conducted along the Pacific coast, and *Ampelisca abdita* in surveys along the Atlantic and Gulf coasts. *R. abronius* is a native of the Pacific coast and not the other two coasts and *A. abdita* is found along both the Atlantic and Gulf Coast, as well as San Francisco Bay. The two species are generally comparable in sensitivity to toxicants.

The incidence of toxicity in the southeastern bays, such as Tampa Bay and Charleston Harbor, was very low. Generally, most of the study areas in the southeast have not been very toxic to the amphipods. In contrast the northeastern bays and the California bays were relatively toxic as indicated by these tests.

During FY 95, data from San Diego Bay, Newport Bay, coastal estuaries of Southern California, Choctawhatchee Bay, Apalachicola Bay, St. Simons Sound, and Savannah River will be added to the list.

Table 4. Incidence of toxicity in solid-phase amphipod survival tests in each study area.

Region	Toxic/Total	(%)	Species
Newark Bay	48/57	(84.2%)	A. abdita
Long Island Sound bays	50/60	(83.3%)	A. abdita
San Diego Bay	68/102	(66.7%)	R. abronius
Tijuana River Slough	4/6	(66.7%)	R. abronius
San Pedro Bay	61/105	(58.1%)	R. abronius
San Francisco Bay*	56/111	(50.4%)	R. abronius
Hudson-Raritan Estuary	54/117	(46.2%)	A. abdita
Commencement Bay**	18/46	(39.1%)	R. abronius
Boston Harbor	13/55	(23.6%)	A. abdita
Mission Bay	1/11	(9.1%)	R. abronius
Tampa Bay	10/165	(6.1%)	A. abdita
Pensacola Bay	0/40	(0.0%)	A. abdita
St. Andrew Bay	0/31	(0.0%)	A. abdita
Charleston Harbor	0/63	(0.0%)	A. abdita
Winyah Bay	0/9	(0.0%)	A. abdita
Leadenwah Creek	0/9	(0.0%)	A. abdita
San Diego River	0/2	(0.0%)	R. abronius

* compiled from 61 historical studies
** from Williams et al. (1986)

The incidence of samples significantly toxic to microbial bioluminescence is compared among surveyed bays in Table 5. Thus far, the incidence of toxicity ranges from 0.0% to 100%. In St. Andrew Bay none of the samples were toxic to amphipod survival, but all of them were toxic to microbial bioluminescence. Similarly, in several other bays there was an apparent lack of correspondence in these two tests. This disagreement is not unexpected since the two tests differ considerably in the toxicological endpoints recorded, the phase of the sediments tested, and their relative sensitivity. The incidence of toxicity in the Commencement Bay Superfund site was exceeded in two area - St. Andrew Bay and Pensacola Bay. In contrast, none of the samples from San Francisco Bay were toxic in this test.

Table 5. Incidence of toxicity in microbial bioluminescence (Microtox) tests of organic extracts of sediments in each study area.

Region	Toxic/Total	(%)
St. Andrew Bay	31/31	100.0%
Pensacola Bay	32/40	80.0%
Commencement Bay*	29/46	63.0%
Long Island Sound bays	35/60	58.3%
Boston Harbor	31/55	56.4%
Winyah Bay	4/9	44.4%
Hudson-Raritan estuary	47/116	40.5%
Tampa Bay	24/90	26.7%
Leadenwah Creek	1/9	11.1%
Charleston Harbor	6/63	9.5%
San Francisco Bay	0/45	0.0%

* from Williams et al. (1986)

Beginning with the surveys of Tampa Bay and San Pedro Bay, a sublethal test of the pore waters extracted from the sediments was added to the battery of tests. Toxicologists suspect that chemicals in sediments are in dynamic equilibrium between the particulate phase and the dissolved phase. Further, they have evidence that the toxicants in the dissolved phase are responsible for toxicity since they are readily bioavailable. Therefore, a direct approach to testing sediment toxicity could be a method in which the test organisms are exposed directly to the pore waters in which the dissolved phase toxicants are found.

The pore waters are extracted either by gently squeezing the sediment through a filter or by centrifuging the sediments. Three species have been used in the pore water toxicity tests. In the San Pedro Bay survey, sexually mature urchins were not available at the time of the survey. Therefore, red abalone embryos (*Haliotis rufescens*) were used in the tests, since they were readily available, effluent testing protocols existed, and the testing laboratory had excellent experience with the methods. Percent normal morphological development of the abalone embryos was determined in each sample. In San Diego Bay, the red urchin *Strongylocentrotus purpuratus* was used (data not yet available). In all other survey areas, percent fertilization of the eggs of the Gulf coast urchin (*Arbacia punctulata*) was determined in the pore water tests. The relative sensitivities of these three species have not been quantified, but, they are generally accepted as similar.

Thus far, the incidence of toxicity in the tests of undiluted pore waters ranges from 0% to 90% (Table 6). A large majority of the samples from San Pedro Bay, Winyah Bay, and Tampa Bay were significantly toxic in these tests.

Table 6. Incidence of toxicity in undiluted sediment pore waters extracted from sediments in each study area.

Region	Toxic/Total	(%)	Species
San Pedro Bay	89/99	89.9%	H. rufescens
Winyah Bay	8/9	88.9%	A. punctulata
Tampa Bay	130/165	78.8%	A. punctulata
Charleston Harbor	34/63	54.0%	A. punctulata
Pensacola Bay	4/40	10.0%	A. punctulata
St. Andrew Bay	7/31	22.6%	A. punctulata
Leadenwah Creek	0/9	0.0%	A. punctulata

The calculations of the incidence of toxicity provide a basis for comparing the relative quality of the samples collected in each area. However, they do not provide any information on the spatial scales of toxicity. To provide an estimate of the sizes of the toxic areas, the spatial dimensions of the study areas and the sampling zones within each area must be accounted for.

The spatial distribution of the toxicity in each survey area was determined, using an approach similar to that used by EPA's EMAP. The dimensions and size of each sampling zone or stratum was plotted on navigation charts and measured with a planimeter. The toxicity data were weighted according to the size of the stratum in which the station(s) was (were) sampled. The cumulative distribution functions of the toxicity data weighted to the stratum size were prepared. The sizes of the strata in which toxicity results were less than 80% of the respective control value were summed.

Results available thus far from the three toxicity tests performed most often in the surveys are summarized in Table 7. Some tests were not performed in some areas (shown as no data). Also, some data have not been received for areas recently sampled. Additionally, the calculations of spatial extent in some areas recently surveyed have not been calculated. Therefore, the estimates listed in Table 7 are likely to change as new data are included and they should be viewed cautiously as rough estimates.

Based upon the data available thus far, about 12.3% of the cumulative areas surveyed thus far were significantly toxic (<80.0% of control survival) in the amphipod survival tests (Table 7). The samples from the relatively large bays in the southeastern USA were not toxic to the amphipods, thus driving the estimates of spatial extent downward. Also, although 58% of the samples from San Pedro Bay were toxic, they were collected in strata that were relatively small. In Tampa Bay, the largest estuary sampled thus far, only a few samples were toxic and they were collected in small industrial waterways.

Based upon the data available thus far, about 58% of the cumulative areas surveyed were toxic in the tests of 100% pore waters (Table 7). This estimate is influenced largely by the data from Tampa Bay and, to a lesser extent, those from San Pedro Bay. The data from Boston Harbor, San Diego Bay and the Savannah River may drive this estimate upward.

Based upon the data from four survey areas (Table 7), about 32.7% of the cumulative area surveyed was significantly toxic (i.e., results <80% of controls). This estimate was influenced largely by the data from the Hudson-Raritan estuary and Pensacola Bay. The calculations have not been performed for Charleston Harbor, Winyah Bay, St. Andrew Bay, and the Savannah River.

Table 7. Spatial extent of sediment toxicity in each survey area (kilometers^2 and percent of study area) as estimated with each toxicity test.

Survey Area	Total area (km^2)	Amphipod survival	Pore water toxicity	Microbial bioluminescence
Hudson Raritan	350.0	133.3 (38.1%)	nd	136.1 (38.9%)
Boston Harbor	56.1	14.0 (25.0%)	na	11.5 (20.5%)
Charleston Harbor	41.1	0.0	12.5 (30.4%)	na
Newark Bay	12.7	10.8 (85.0%)	nd	nd
San Pedro Bay	53.8	7.8 (14.4%)	52.6 (97.7%)	nd
Tampa Bay	550.0	0.5 (0.08%)	463.6 (84.3%)	0.5 (0.09%)
Winyah Bay	7.3	0.0	3.1 (42.4%)	na
Leadenwah Creek	1.7	0.0	0.0	na
Pensacola Bay	273.6	0.0	5.5 (2.0%)	253.9 (93.2%)
Totals	1346.3	166.4 (12.3%)	537.3 (58.0%)	402.0 (32.7%)

na = data not yet available
nd = no data

TAMPA BAY

During both phases of the Tampa Bay sediment toxicity survey, 165 samples were collected and tested for toxicity. Three samples were collected at each site location. The data were reported by individual station and as the means for each site. Emphasis was placed upon characterizing the suspected pollution gradient in Hillsborough Bay, the most industrialized and urbanized lobe of the estuary.

As expected toxicity was highest in the northwestern portion of Hillsborough Bay and the Port of Tampa and generally diminished down-bay away from these areas. The overall ranks in toxicity based upon the site means and a compilation of the data from all three toxicity tests (Figure 1) illustrates the most obvious toxicity gradient. One site in Ybor Channel in the uppermost reach of the Port of Tampa was significantly toxic relative to controls in all three of the tests. Other sites sampled nearby in northern Hillsborough Bay were significantly toxic in one or two of the tests. Sites sampled in southern Hillsborough, Old Tampa Bay, and middle Tampa Bay were among the least toxic.

Among the three toxicity tests that were performed, the sea urchin fertilization test was the most sensitive. It identified more samples as significantly toxic than the other two tests combined. The test was performed with 100%, 50%, and 25% pore water extracted from the sediments. Figure 2 illustrates the pattern in response of the sea urchin tests to the 100% pore water for the 55 sites (n=3) sampled during both phases 1 and 2 of the survey. The height of the bars indicates the percent fertilization success, i.e., a short bar depicts high toxicity.

Toxicity to the sea urchins was most apparent in Hillsborough Bay and diminished into other adjacent regions of the estuary (Figure 2). Some sites in the western lobe of Old Tampa Bay, in Bayboro Harbor and adjacent yacht basins along the St. Petersburg shoreline, Cockroach Bay, and Anna Maria Sound also were relatively toxic in the 100% pore water tests.

The spatial extent of toxicity was estimated during the analyses of the Tampa Bay data (Tables 8). Each of the 55 sampling strata or zones that were sampled in the estuary during phases 1 and 2 were plotted on a navigation chart. Each sampling site, then, was plotted within its respective stratum. The size of each stratum was

determined with a planimeter. Cumulative distribution functions for the toxicity data were determined based upon the sizes of the strata that were significantly toxic. That is, the spatial size (kilometer^2) of the strata that were toxic in each of the tests were summed. Further, the strata that were toxic in more than one test were identified and the spatial extent of toxicity calculated.

Table 8 summarizes the spatial extent of toxicity estimated with the toxicity tests. The total area represented in the survey was approximately 550 km^2. Over 84% of that area was toxic in the sea urchin tests of 100% pore water. Approximately 10.8% of the area was toxic in the tests of both 100% and 50% pore water. Only about 0.1% of the area was toxic in all tests. This data analysis focuses upon the upper Ybor Channel and the mouth of the Hillsborough River as the most highly toxic areas within the Tampa Bay estuary. This analysis also illustrates the huge difference in the sensitivity of the sea urchin test of pore water relative to that of the other two tests.

Table 8. Estimates of the spatial extent of sediment toxicity in the Tampa Bay estuary, based upon the results of three toxicity tests. Areas were defined as "toxic" when results were less than 80% of the control values.

Toxicity Tests	Area (km^2)	Percent of Area
Sea urchin @100% pore water	463.6	84.3%
Sea urchin @100% and 50% pore water	59.2	10.8%
Sea urchin @100%, 50%, 25% pore water	12.9	2.3%
Sea urchin @100%, 50%, 25% pore water and Microtox	0.6	0.1%
Sea urchin @100%, 50%, 25% pore water, Microtox, and amphipod	0.45	0.08%

The cause(s) of toxicity could not determined with the sampling design and a determination of the causes of toxicity was not an objective at the initiation of the survey. However, chemical analyses of the solid-phase sediment were conducted on most of the samples in phases 1 and 2. The correlations between toxicity data and

chemical concentrations were determined and they showed that several trace metals, numerous polynuclear aromatic hydrocarbons, and, most notably, several chlorinated hydrocarbons were highly correlated with toxicity.

Following the correlation analyses, the concentrations of the chemicals most correlated with toxicity were compared with the concentrations published in several different publications as screening level, toxicity thresholds, or effects-based criteria. The concentrations of nine chemicals or chemical classes stood out as sufficiently elevated to contribute significantly to the toxicity in the samples. The nine toxicants were: lead, zinc, pyrene, high molecular weight PAH, phenanthrene, dibenzo(a,h)anthracene, total PCBs, total DDTs, and endrin. In addition, approximately 14 of the 165 samples had sufficiently high ammonia concentrations in the pore water to cause zero fertilization success among the sea urchins exposed to the pore waters.

Table 9 lists the nine toxicants and toxicant classes most highly associated with the three tests of toxicity. The data are expressed as toxicity units, calculated as the products of dividing the average chemical concentration in the significantly toxic samples by the respective guideline value. For example, the average concentration of lead in the samples that were significantly toxic to amphipod survival exceeded the guideline value for lead by a factor of 3.55. Among the nine chemicals, the concentrations of PCBs, DDTs, and endrin were most elevated in the toxic samples and were sufficiently high to contribute significantly to the observed toxicity.

Table 9. Toxicity unit concentrations for those substances in which the average concentrations in the significantly toxic samples equalled or exceeded the respective guideline values*.

	Amphipod survival	Sea urchin fertilization	Microbial bioluminescence
Lead[a]	3.55	<1.0	<1.0
Zinc[a]	1.13	<1.0	<1.0
Pyrene[a]	1.51	1.01	1.60
HPAH[a]	1.95	1.27	2.05
Phenanthrene[a]	<1.0	<1.0	1.04
Dibenzo(a,h)anthracene[a]	1.0	<1.0	1.08
Total PCBs[a]	17.73	8.21	19.71
Total DDTs[a]	12.96	4.13	14.43
Endrin (ug/goc)[b]	3.0	<1.0	3.81

* Average concentrations in significantly toxic samples divided by the respective SQC, ERM, EC50, or LC50 values.

[a] Based upon the ERM values (dry wt.) of Long et al. (in press).

[b] Based upon the proposed National sediment quality criterion (SQC)of U. S. EPA (1991e).

The results of the Tampa Bay survey have been published as NOAA Technical Memorandum NOS OMA 78. Additionally, at least two journal articles are anticipated based upon the results of this survey.

HUDSON-RARITAN ESTUARY

Chemical analyses of selected samples collected throughout the study area during Phase 1 have been completed. Toxicity tests of samples collected in Newark Bay and vicinity during Phase 2 have been completed and chemical analyses of those samples are completed. Dioxin equivalency bioassays of each sample from Phase 2 have not been completed. Analysis and interpretation of the data from both phases is underway and a draft technical memorandum is targeted for completion in December, 1994.

In Phase 1 of the survey, the study area covered approximately 350 km^2. Each sample was tested with four test end-points: amphipod survival, bivalve embryo survival, bivalve embryo morphological development, and microbial bioluminescence. These four tests indicated that approximately 133 km^2 (38.1%), 87.4 km^2 (25.0%), 103.8 km^2 (29.7%), and 136.1 km^2 (38.9%) of the study area was significantly toxic (Table 7).

During Phase 2 of this survey, 57 samples were collected in Newark Bay, the lower Passaic and Hackensack rivers, upper Arthur Kill, and upper New York Harbor (Figure 3). Each sample was subjected to an amphipod survival toxicity test. This test indicated that 48 of the 57 samples were significantly different from controls. Toxicity was pervasive throughout the Phase 2 study area (Figure 4).

In Figure 3, stations in which amphipod survival was significantly reduced are shown with open circles. Stations in which amphipod survival was significantly reduced and in which survival was less than 80% of the control response are shown with closed squares. Most of the samples caused at least a 20% reduction in amphipod survival. The sediments from stations 26 and 47 caused 0% survival; a highly unusual result in these tests. The sample from station 57 in the upper New York harbor was not toxic. Sediments from this station were not toxic, also, during the Phase 1 testing. The samples from the lower Passaic River generally were more toxic than those from the lower Hackensack River. Samples collected near the Diamond Alkali Superfund site (stations 6, 7, and 8) were consistently toxic.

The Newark Bay survey area covered approximately 12.7 km^2. Approximately 85% of the area (10.8 km^2) was significantly toxic in the amphipod tests (Table 7).

Chemical analyses of 20 of the samples has been completed and the data indicate strong negative correlations between the concentrations of most analytes and amphipod survival. Several trace metals, all PCBs, most pesticides, and dioxin isomers were negatively correlated with amphipod survival. It is apparent that all of these toxicants co-occurred and co-varied with each other.

EPA Region 2 performed analyses of 2,3,7,8-TCDD in samples not analyzed by NOAA. EPA has forwarded the data to NOAA/ORCA for inclusion in the regional report. The concentrations of 2,3,7,8-TCDD in all 57 samples are plotted in Figure 4, based upon a compilation of the NOAA data and the EPA data. None of the concentrations exceeded 1 ng/g dry wt., however, several approached 500 pg/g dry wt. As expected the samples from the lower Passaic River had the highest concentrations of dioxins. Samples from stations 7 and 8 nearest the Diamond Alkali site had the highest concentrations. These concentrations were similar to those observed in this area in previous surveys performed by other agencies. Dioxin concentrations decreased downstream into Newark Bay, except, curiously, at station 26 of Elizabeth Port. Dioxin concentrations in the lower Hackensack River were among the lowest observed.

BOSTON HARBOR

Sediment samples were collected throughout most of Boston Harbor, including the Inner Harbor, Northwest Harbor, Central Harbor, and Southeast Bay (Figure 5). In addition, three samples were collected outside the Harbor in Massachusetts Bay as reference samples. The results from the three toxicity tests showed overlapping, but different spatial patterns in toxicity. The amphipod survival test indicated that at least one sample from each of the sampling regions was toxic. The incidence of toxic samples was highest in the Inner Harbor and in Northwest Bay, both located nearest the city of Boston. One sample from the Massachusetts Bay area was, surprisingly, toxic in this test.

The survey covered approximately 56 km^2. Approximately 25% of the area (14.0 km^2) was significantly toxic to the amphipods and approximately 20.5% of the area (11.5 km^2) was toxic to microbial bioluminescence (Table 7).

Chemical analyses of the samples were performed by Texas A&M University and the data delivered to NOAA/ORCA. Some samples had very high concentrations of ammonia in the toxicity test chambers. The relationships between toxicity and chemical concentrations will be determined when data analyses are initiated during FY95.

SAN PEDRO BAY

In the first year of the southern California survey, 102 samples were collected in clusters of three each at 34 sites (Figure 6). Samples were collected in the inner reaches of the Los Angeles and Long Beach Harbors. Also, they were collected in San Pedro Bay seaward to the perimeter breakwater. Additionally, samples were collected beyond the breakwater in the Pacific Ocean. Finally, Alamitos Bay and Anaheim Bay/Huntington Harbor were sampled as a part of this survey.

Figure 6 illustrates the distribution of individual samples that were significantly toxic and those that were not toxic to amphipod survival. Toxicity was most prevalent among the samples from the Los Angeles/Long Beach harbors, Alamitos Bay, and Huntington Harbor. Toxicity generally diminished seaward into San Pedro Bay, and, furthermore, did not occur in samples collected in the Pacific Ocean. The abalone embryo development tests showed a similar pattern in toxicity.

The study area encompassed approximately 53.8 km2. The amphipod survival test indicated that approximately 14.4% of the area (14.4 km^2) were significantly toxic (Table 7). The abalone embryo development test indicated that approximately 97.7% (52.6 km^2), 76.7% (41.3 km^2), and 27.0% (14.5 km^2) were significantly toxic in tests of100%, 50%, and 25% pore water.

Chemical analyses of the samples have been completed and the state of California has produced several iterations of draft technical reports. A technical report is expected to be published in October or November, 1994.

SAN DIEGO BAY

In the second year of the southern California survey, sediment samples were collected throughout San Diego Bay and the adjoining San Diego River, Mission Bay, and Tijuana Slough estuary (Figure 7). The data from the amphipod survival indicate that toxicity was widespread throughout San Diego Bay (Figure 7). Amphipod survival was significantly reduced in samples collected in much of the southern portion of the bay, among the piers of the Naval Station, in the Naval carrier base, and in the smaller harbors and yacht basins. Also, four of the six samples collected in the Tijuana Slough estuary were significantly toxic. In contrast, neither of the two samples from the San Diego River were toxic and only one of the 10 samples from Mission Bay were toxic in this test. Also, most of the samples taken from the San Diego Bay channel and near the mouth of the bay were not toxic.

The data from the sea urchin fertilization tests of the pore waters are not yet available. Some preliminary data from these tests, however, indicate that toxicity was probably pervasive throughout the study area. The survey covered 39 km^2 in San Diego Bay, 6 km^2 in Mission Bay, 0.5 km^2 in San Diego River, and 0.3 km^2 in the Tijuana Slough estuary. The percentages of these areas that were toxic have not yet been calculated. The chemistry data have not yet been delivered to NOAA/ORCA.

WESTERN FLORIDA

In the western Florida panhandle, four bays have been sampled in the sediment quality surveys: Pensacola Bay and St. Andrew Bay in 1993; and Choctawhatchee Bay and Apalachicola Bay in 1994. The toxicity data from the 1993 surveys have been delivered to NOAA/ORCA. The chemical data from Pensacola Bay have been delivered to NOAA/ORCA by the state of Florida. Analyses of the samples from St. Andrew Bay will be initiated early in FY95. The data from the samples collected in 1994 are not yet available.

None of the samples collected in either Pensacola Bay or St. Andrew Bay were significantly toxic in the amphipod survival tests. Only 4 of 40 samples from Pensacola Bay and 7 of 31 samples from St. Andrew Bay were significantly toxic in the sea urchin fertilization tests of pore waters. In contrast, 32 of 40 samples from Pensacola Bay and all 31 samples from St. Andrew Bay were toxic in the microbial bioluminescence tests.

In Pensacola Bay the samples that were toxic in the sea urchin tests of 100% pore water were collected mainly in the bayous adjoining the central basin of the bay. Most of the samples from Bayou Chico, an industrialized harbor located west of Pensacola, were toxic (Figure 8). Also, samples from Bayou Texar, surrounded by residential neighborhoods, and from Bayou Grande, bordering a major military base, were toxic. Two of the samples from the municipal harbor were toxic in these tests. Sediments from stations 3, 14, and 31 were toxic in the sea urchin fertilization tests, but, were not toxic in the morphological development tests. Very few of the samples from the central basin of the bay were toxic. The tests of morphological development were considerably more sensitive than those of fertilization success, as indicated by a higher percent incidence of toxicity.

In the neighboring St. Andrew Bay, toxicity, again, was restricted mainly to a small bayou adjoining the central basin of the bay (Figure 9). Many of the samples from Watson's Bayou, located east of Panama City and which has a mixture of industrial and residential uses, were toxic in the tests of fertilization success in 100% pore waters. In addition, two samples from north bay, a relatively rural area, were toxic. The spatial pattern in toxicity indicated with the tests of fertilization success was similar to that indicated with the tests of morphological development (Figure 10).

Of the 273 km^2 sampled in Pensacola Bay, about 2.0% was toxic in either of the sea urchin tests, whereas about 93% was toxic in the microbial bioluminescence tests (Table 7). The St. Andrew Bay survey area covered about 127 km^2. The spatial extent of toxicity has been calculated thus far.

SOUTH CAROLINA/GEORGIA

In the surveys of sediment toxicity in the coastal bays of South Carolina/Georgia, samples were collected in 1993 in Charleston Harbor, Leadenwah Creek (a tributary of North Edisto Bay that is periodically contaminated with pesticides), and Winyah Bay. In 1994 additional samples were collected in 1994 in St. Simons Sound at Brunswick, Georgia and the lower Savannah River.

The results of the toxicity tests of the 1993 samples are available and the chemical analyses of the samples are underway. The toxicity tests of the 1994 samples are underway. Chemical analyses of the samples collected in 1994 are planned for FY95.

None of the samples collected in Charleston Harbor, Winyah Bay, or Leadenwah Creek were significantly toxic in the amphipod survival tests. In Charleston Harbor, 9.5% of the samples were toxic in the microbial bioluminescence tests and 54% of the samples were toxic in the sea urchin fertilization tests of 100% pore water. In Winyah Bay 44% of the samples were toxic in the microbial bioluminescence tests and 89% were toxic in the sea urchin tests. In Leadenwah Creek 1 of 9 (11%) of the samples was toxic in the microbial bioluminescence tests and none of the samples was toxic in the sea urchin tests.

The spatial distribution of the samples from Charleston Harbor that were toxic in the microbial bioluminescence tests is illustrated in Figure 11. Most of the samples that were toxic in the Microtox tests were collected in the Cooper River inland of Charleston. Also, one sample each from the Ashley River and the Wando River were toxic. In the Winyah Bay area, the Microtox tests indicated that samples from the Sampit River near Georgetown were toxic, along with two of the samples from the upper bay (Figure 12).

The survey of Charleston Harbor covered approximately 41 km^2 (Table 7). Based upon the sea urchin tests of 100% pore water, about 12.5 km^2 (30%) of the area was significantly toxic. Of the 7.3 km^2 that were surveyed in Winyah Bay, about 3.1 km^2 (42%) of the area was toxic in the 100% pore water tests. None of the samples from the Leadenwah Creek survey area (1.7 km^2) was toxic in this test.

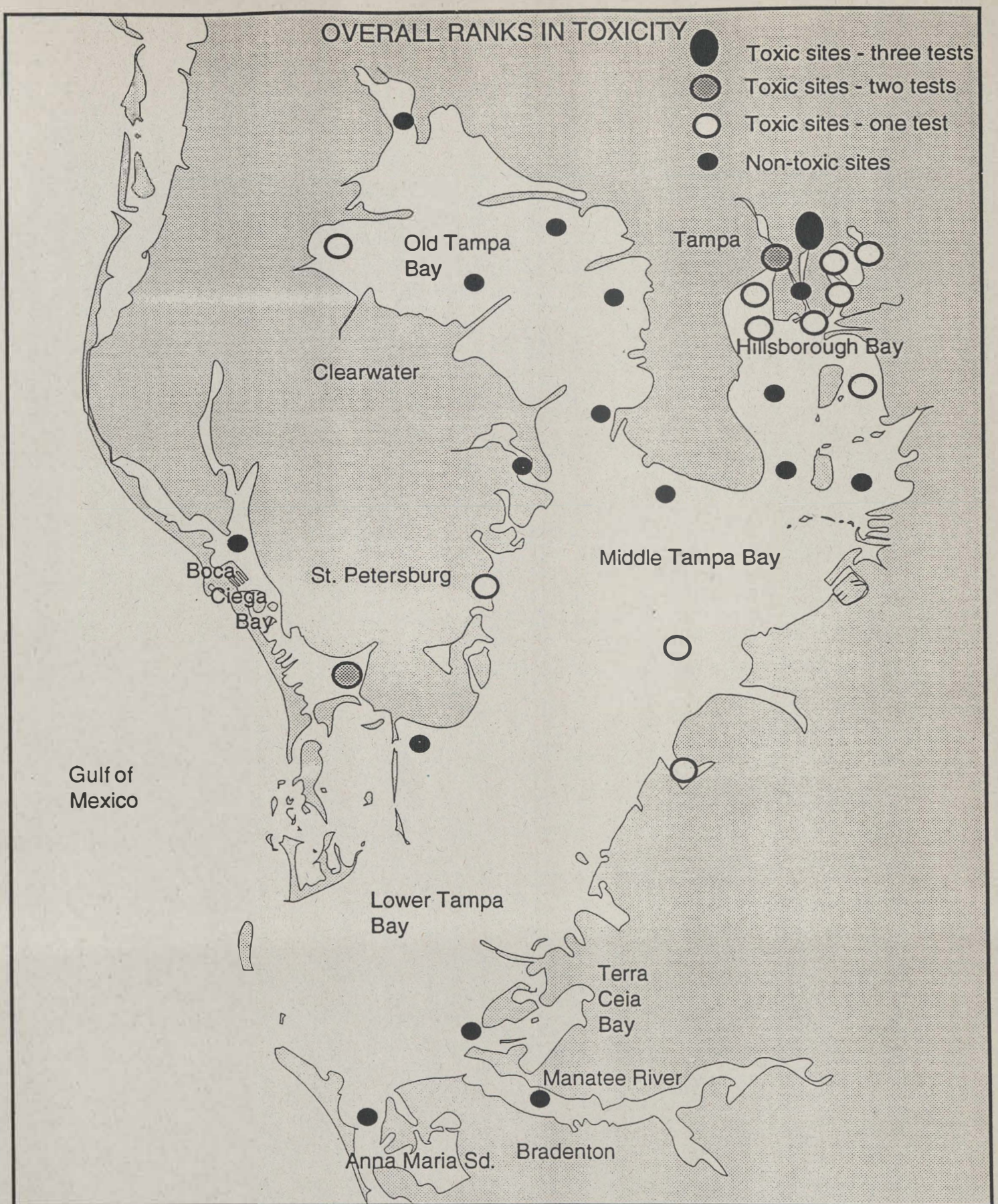


Figure 1. Phase 1 sampling sites in Tampa Bay that were not toxic in any test, or significantly toxic in one, two, or three toxicity tests (amphipod, Microtox, sea urchin @25% pore water).

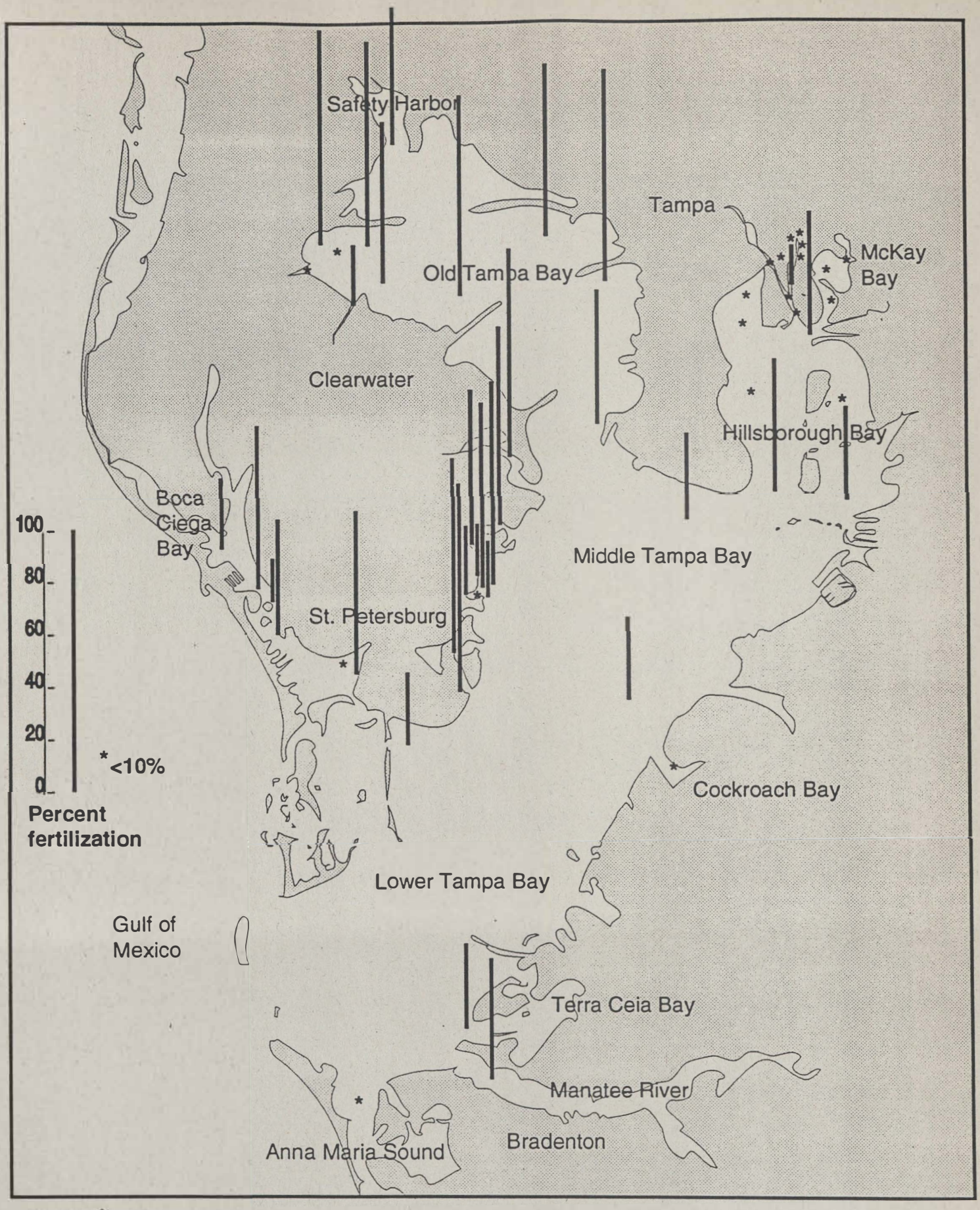


Figure 2. Combined results of Tampa Bay Phase 1 and Phase 2 pore water toxicity tests; average percent fertilization success of sea urchin eggs exposed to 100% pore water from 55 sites.

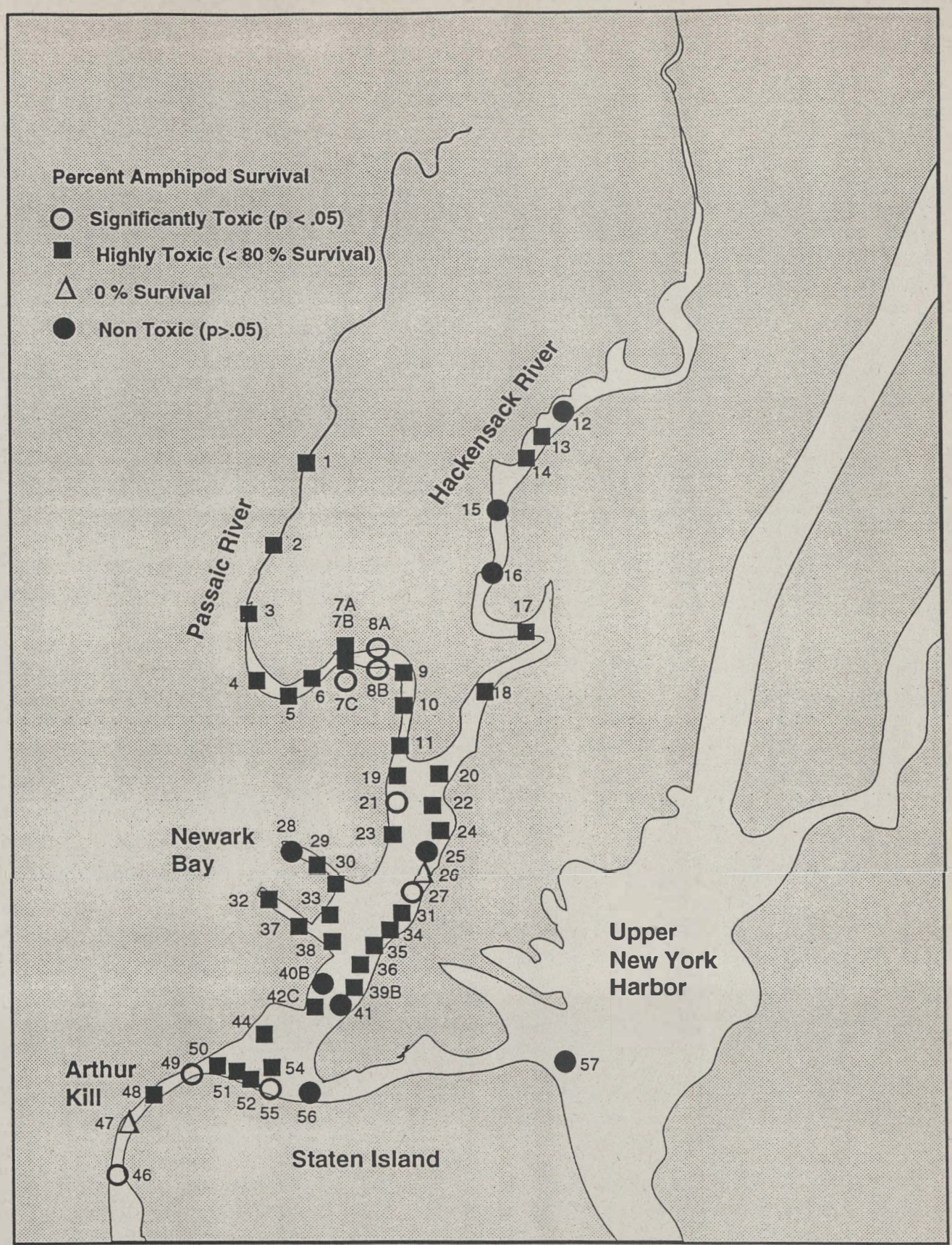


Figure 3. Distribution of stations in Newark Bay and vicinity that were toxic, highly toxic, and non-toxic in amphipod survival tests.

2378-TCDD in Newark Bay Sediments (pg/g)

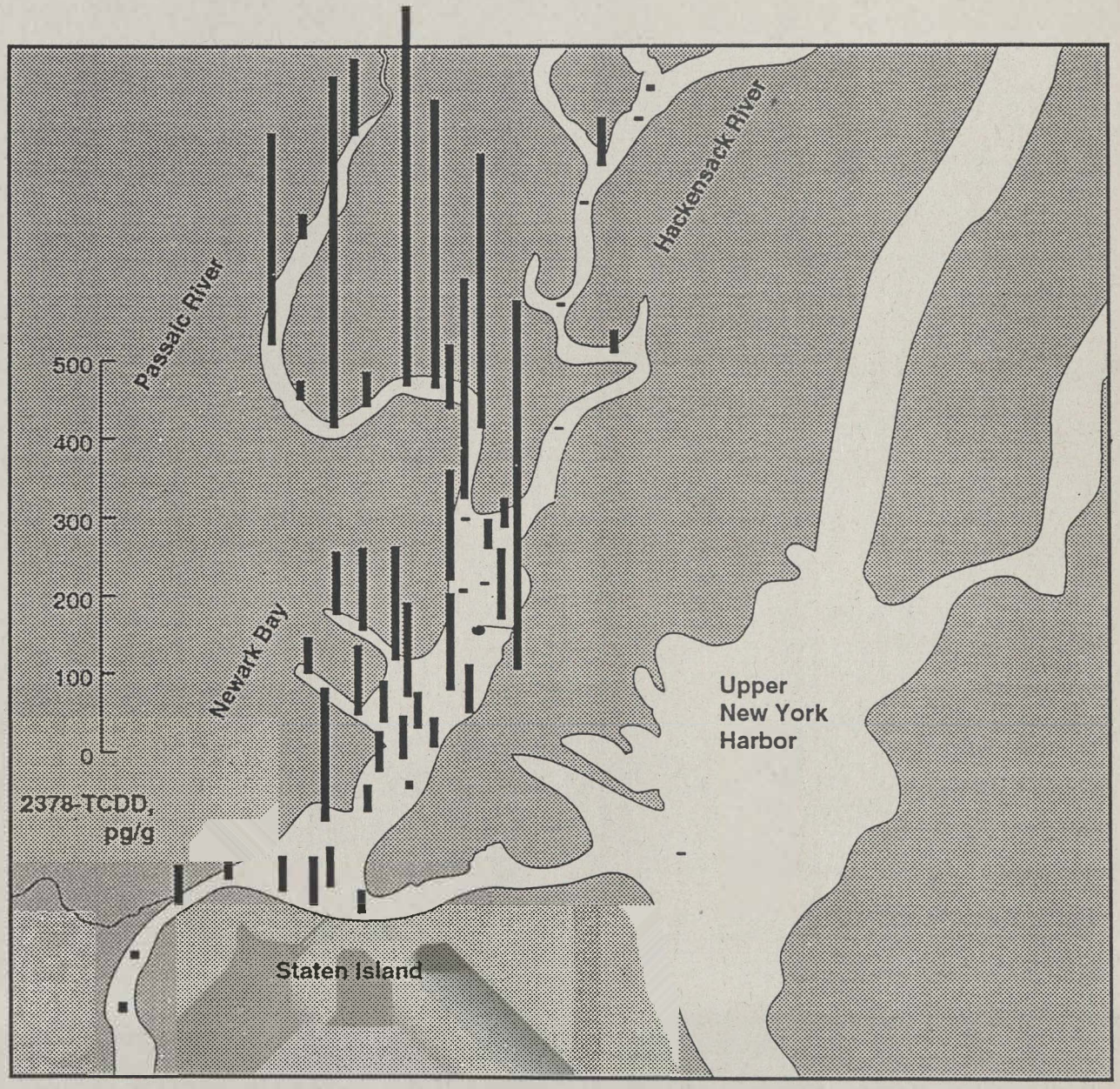


Figure 4. Dioxin (2,3,7,8-TCDD) concentrations (pg/g) in sediments from Newark Bay and vicinity.

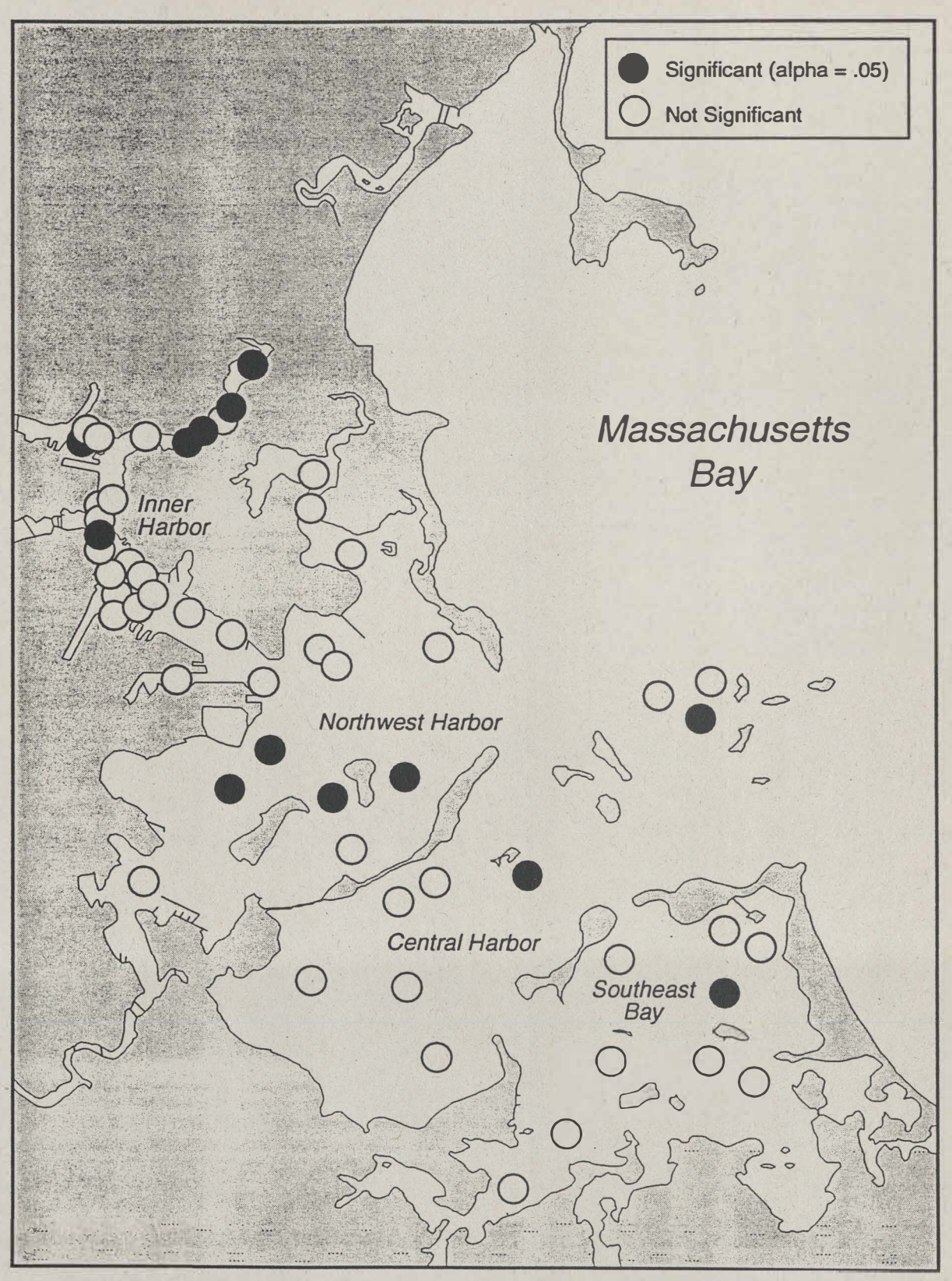
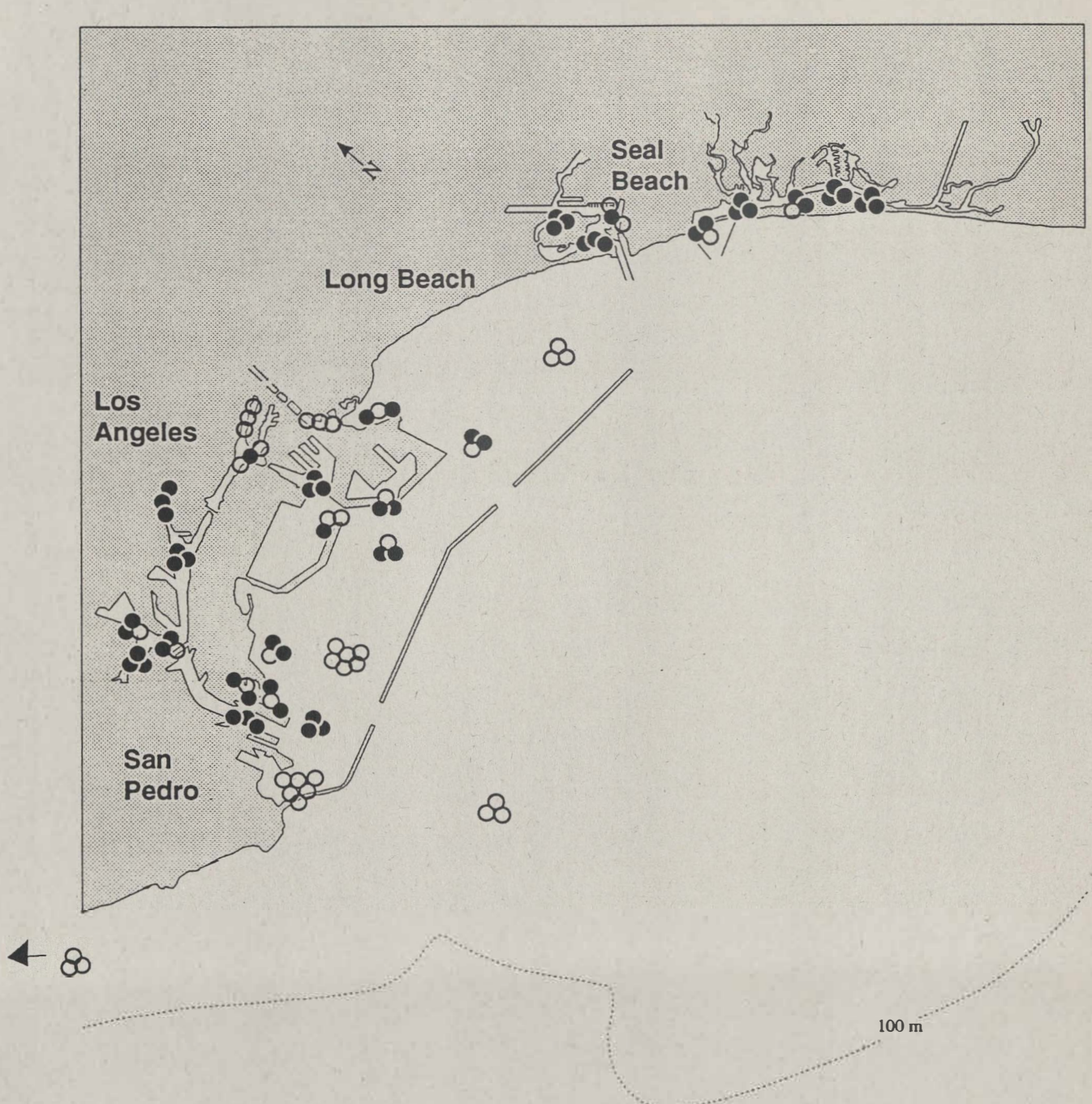


Fig. 5. Distribution of stations in Boston Harbor and vicinity that were toxic, highly toxic, and non-toxic in amphipod survival tests.



Results of toxicity tests of amphipod survival for each station in San Pedro Bay, California.

○ = Not toxic

● = Station mean significantly different from control (alpha<0.05)

Figure 6. Distribution of sediment samples in San Pedro Bay that were either significantly toxic or not toxic to amphipod survival.

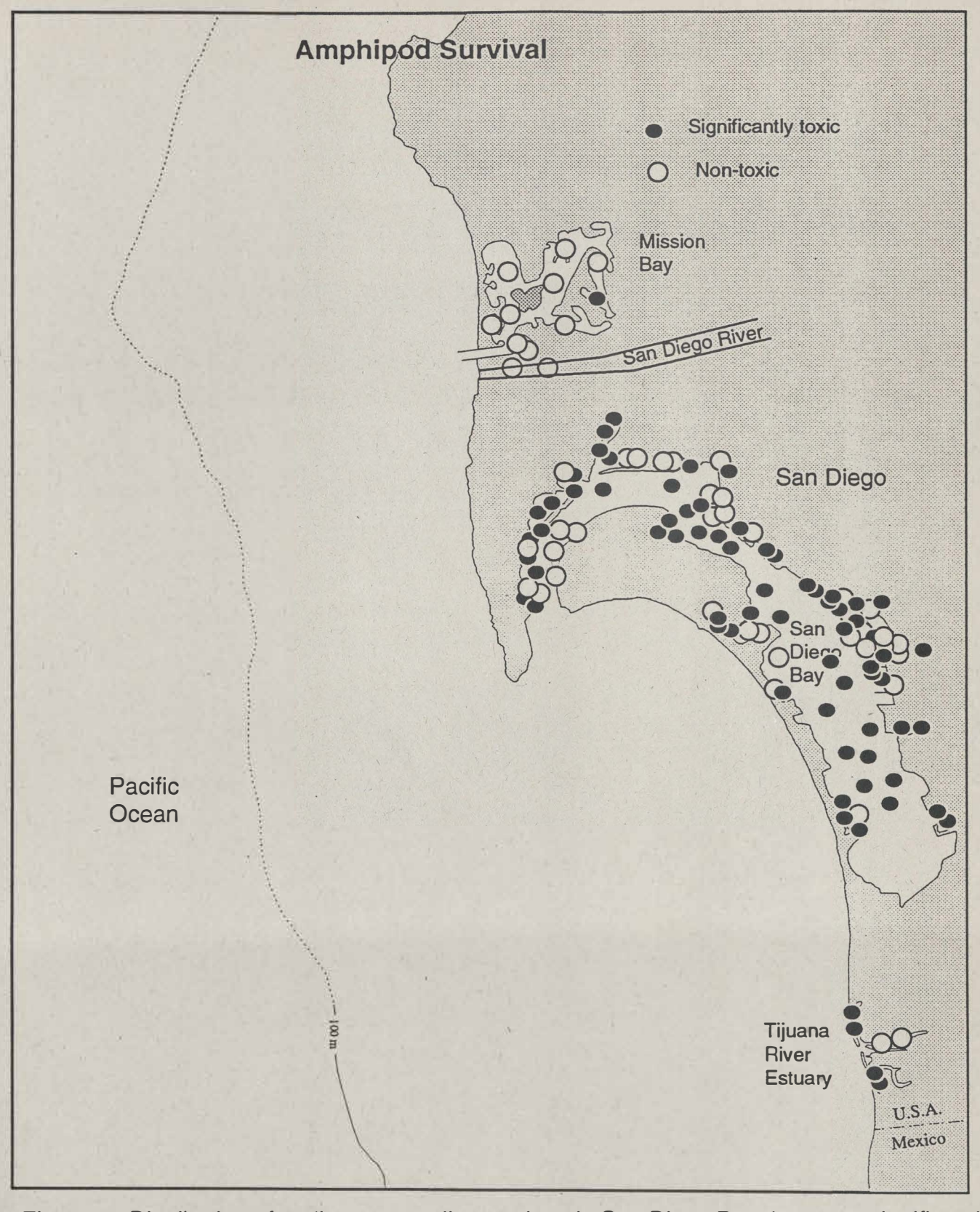


Figure 7. Distribution of sediment sampling stations in San Diego Bay that were significantly toxic or not toxic in amphipod tests.

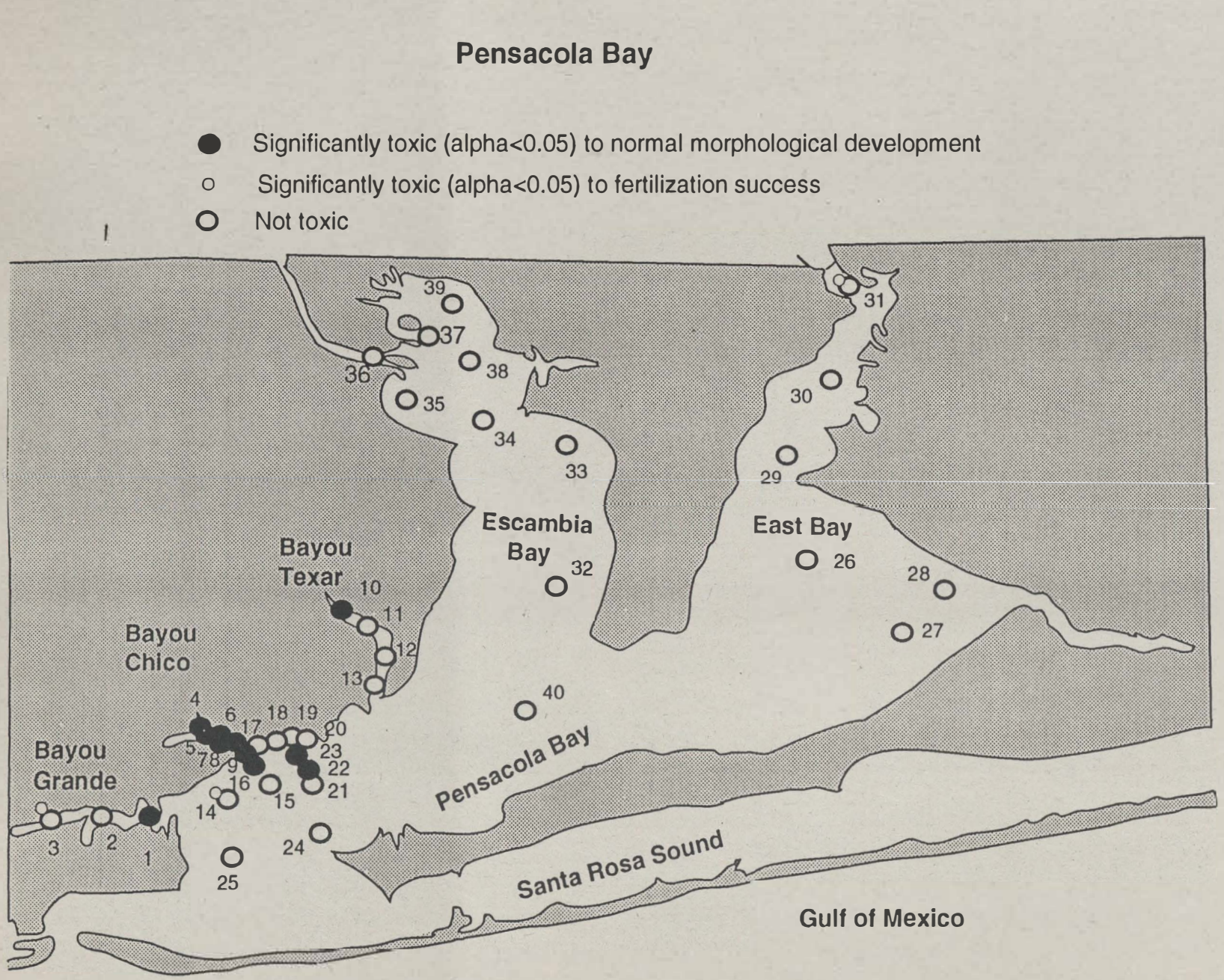


Figure 8. Results of sea urchin toxicity tests of Pensacola Bay 100% sediment pore waters.

100% Pore Water Toxicity to Sea Urchin Fertilization in St. Andrew Bay

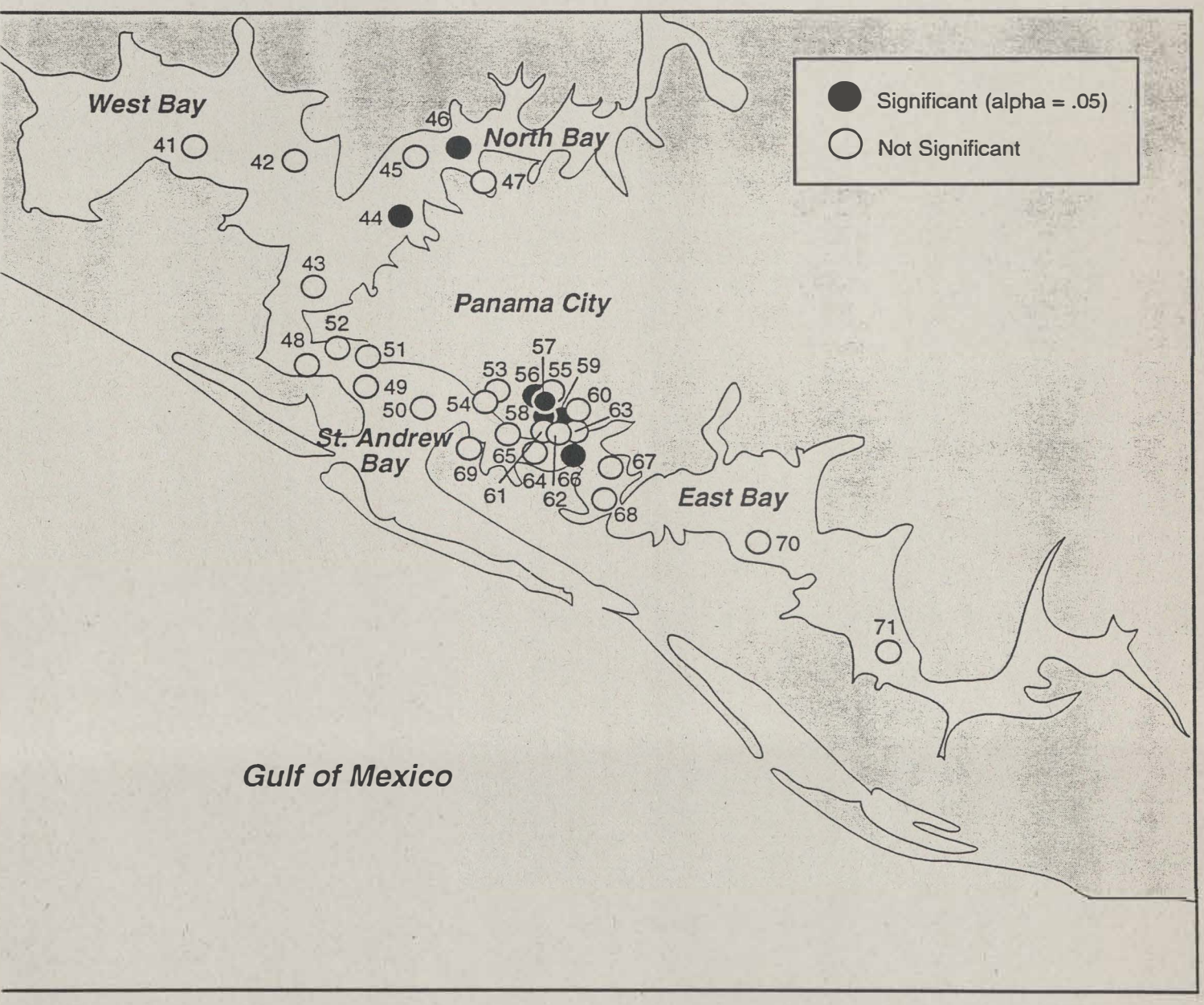


Figure 9. Results of sea urchin tests of fertilization success in 100% pore water from St. Andrew Bay.

100% Pore Water Toxicity to Sea Urchin Development in St. Andrew Bay

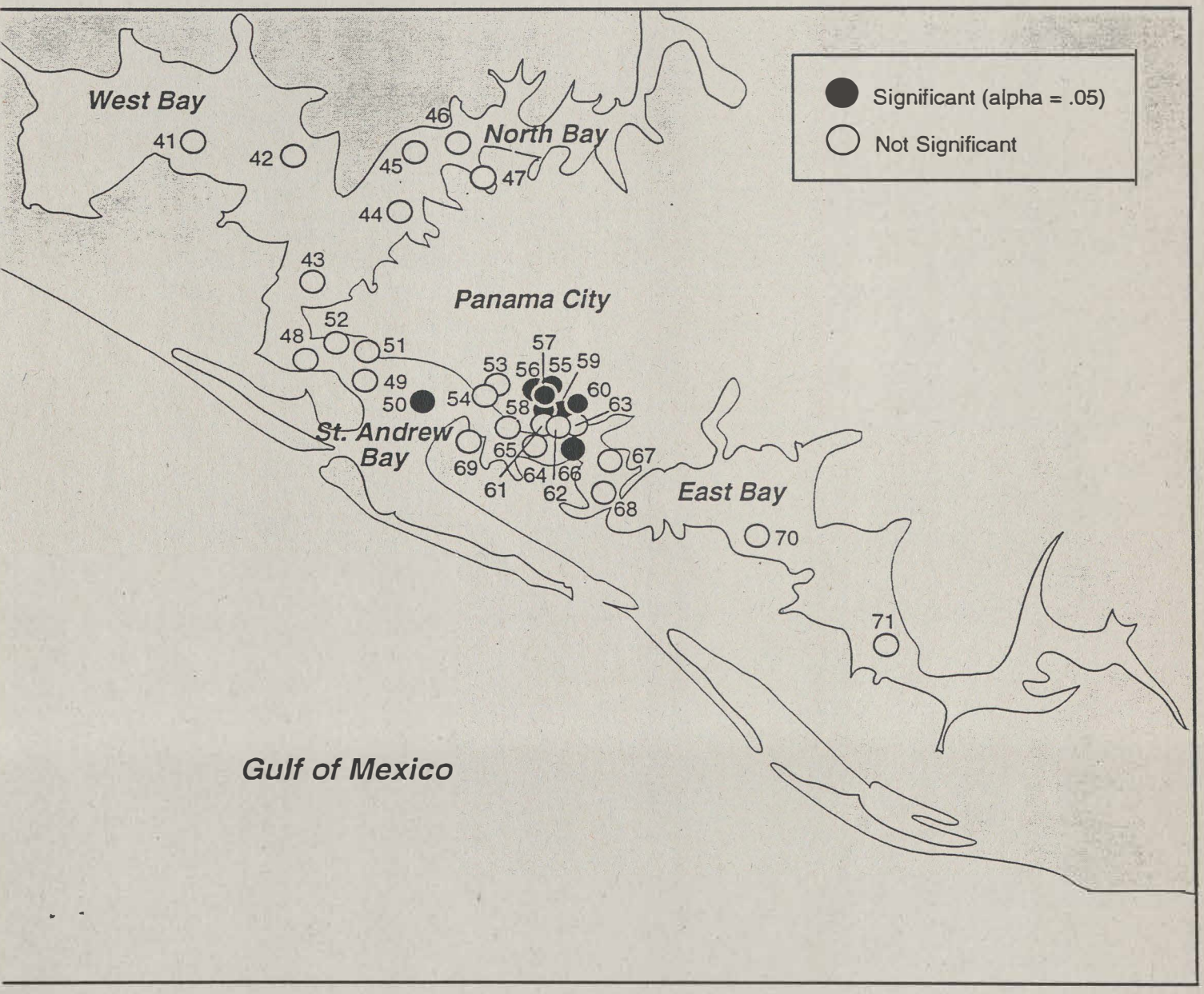


Figure 10. Results of sea urchin tests of morphological development in 100% pore water from St. Andrew Bay

Microtox Bioluminescence Toxicity in Charleston Harbor

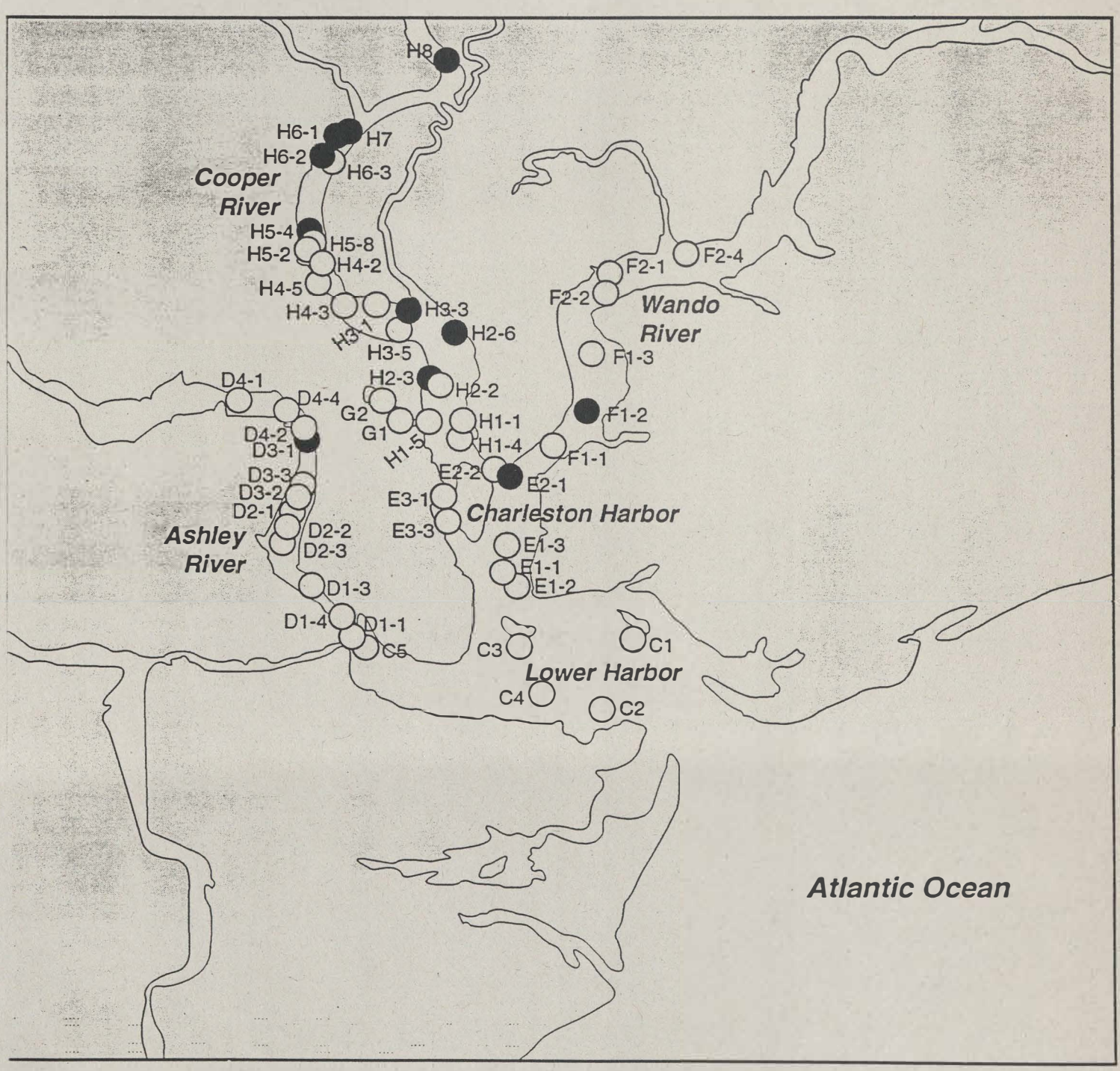


Figure 11. Results of Microtox tests of sediment samples from Charleston Harbor.

Microtox Bioluminescence Toxicity in Winyah Bay

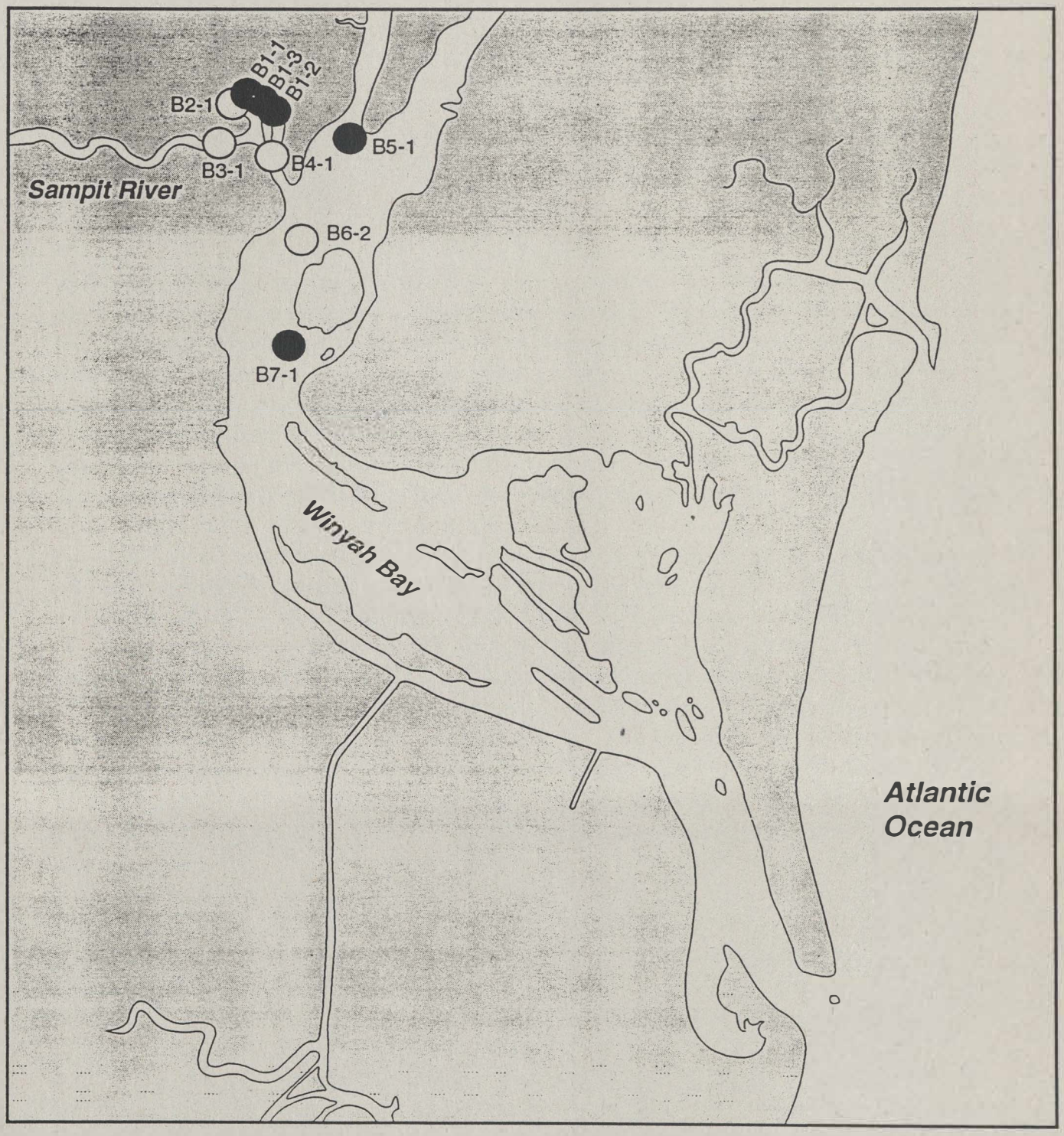


Figure 12. Results of Microtox tests of sediment samples from Winyah Bay.

APPENDIX B

Summary of Progress: Bivalve Biomarker Surveys

Introduction

Biological monitoring systems are needed to identify the presence of potentially toxic chemicals, quantify their presence in animal tissues, and provide meaningful measurements of biological effects. Although there has been extensive use of filter-feeding bivalves to measure bioaccumulation in both laboratory and field studies, synoptic field measurements of exposure and bioeffects have been extremely limited. Bivalves are particularly well-suited to monitoring studies because they are sedentary; their hard shells make them easy to collect, handle, and measure. They also survive well under most environmental conditions and are responsive to the environment at both micro- and macro- geographical scales and at all levels of biological organization. Bivalves have the ability to integrate bioavailable contaminants at concentrations that can be orders of magnitude above those found in other environmental compartments (water or sediment). NOAA has collected bioaccumulation data through the NS&T Program at over 250 coastal areas throughout the U.S. over the last decade and has an extremely powerful tissue chemistry database of historical significance.

During FY94, intensive bioeffects surveys were conducted in Tampa Bay, Florida, and San Diego Bay, California that were intended to complement the sediment surveys being conducted there as part of the COP. These surveys had three primary goals: 1) Evaluate specific areas for exposure to potentially toxic chemicals and resulting adverse biological effects using bivalves; 2) Evaluate the effectiveness of the most promising

bivalve biomarkers in assessing exposure and bioeffects; and 3) Evaluate the relationship between exposure, bioaccumulation, and bioeffects. Other surveys in Massachusetts and Buzzards Bay (MA), independent of the sediment assessment element of COP were also conducted to examine the relationship between the concentration of lipophilic organic contaminants and reproductive effects in bivalves.

It is important to begin with a working definition of biomarkers and to make the distinction between indicators of exposure and indicators of effects. Biomarkers are generally defined as the use of biochemical, physiological, histological, and aberrations in organisms to estimate either exposure to chemicals or resultant effects. Although the use of bivalve biomarkers is not as well developed as in fish, significant progress has been made. Nevertheless, there are few bivalve biomarkers where a clear cause-and-effect relationship has been demonstrated between exposure and biomarker response that would indicate either exposure or resulting bioeffects.

NOAA has pursued the development and testing of bivalve biomarkers during the past two years as part of the COP Program. It has become increasingly clear that biomarker evaluations must include other potential indicators of exposure and bioeffects at higher levels of organization. It is also obvious that bivalves do not always occur naturally along gradients where NOAA trust resources are at risk. Therefore, some method of manipulation or transplantation is necessary. As the thrust of the COP shifts from emphasis on toxics to the effects of natural factors and non-toxic factors induced by man as well as their cumulative effects (eg.; dredging, filling, diking, habitat loss, eutrophication), there will be a need to measure those natural factors as well as effects at higher levels of biological organization. A conceptual approach to monitoring those factors related to adverse biological effects is shown in the Bivalve Monitoring Model in Figure 1. Isolating the relative contribution of those factors could be accomplished by coordinating monitoring and laboratory studies.

The following new elements have been included in the surveys listed above: 1) Use of bivalve transplants to identify status and trends over space and time; 2) Measurements of organismal effects (eg.; growth and reproduction); and 3) Measurements of

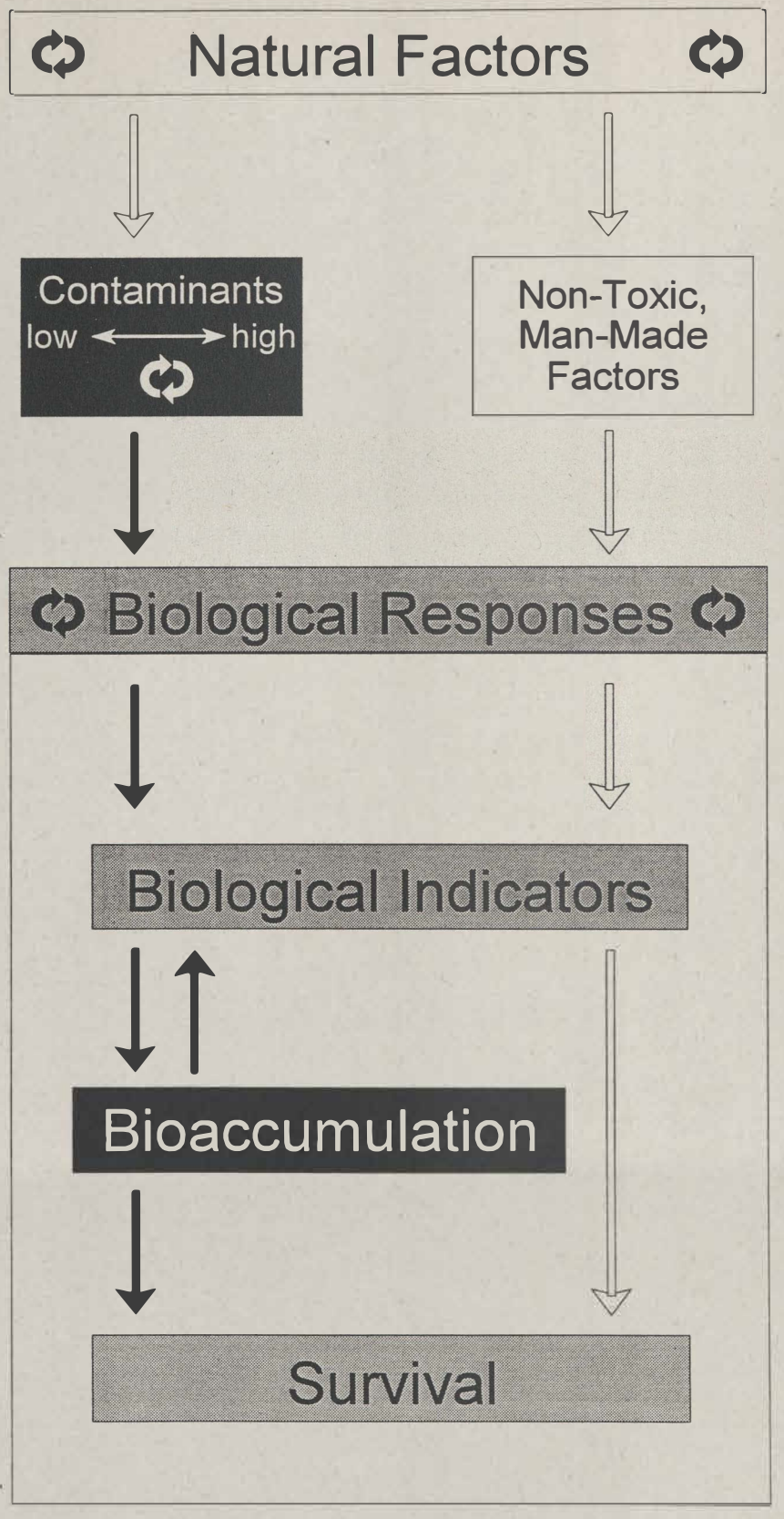


Figure 1. Bivalve monitoring model.

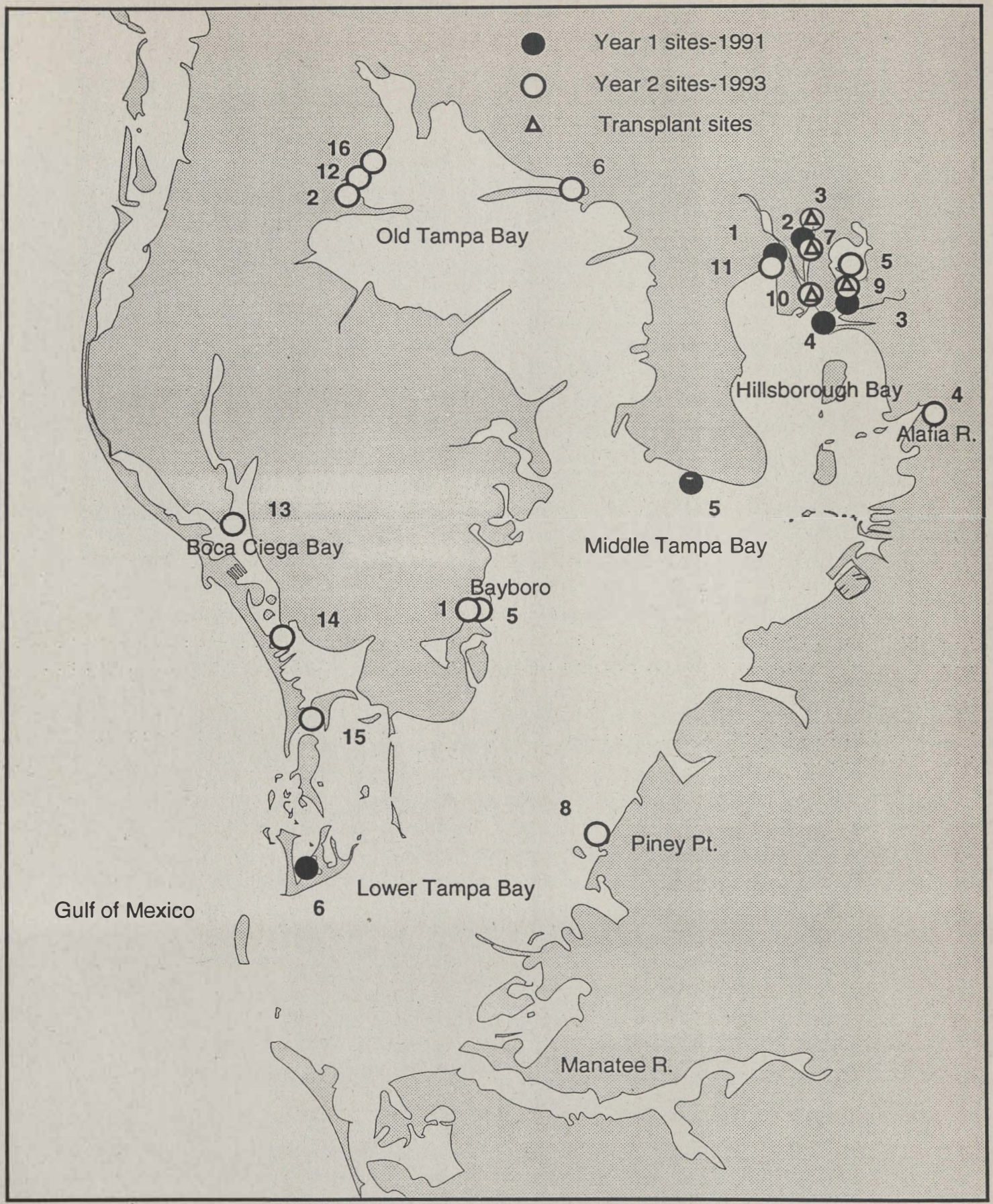


Figure 2. Tampa Bay sites sampled in 1991 and 1993 for oyster biomarkers. Oyster transplant sites where growth was measured are also shown.

natural factors affecting bivalve health (eg.; chlorophyll and temperature).

What follows is a brief summary of progress to date in the three areas that have been studied: Tampa Bay; San Diego Bay and Massachusetts (Massachusetts and Buzzards Bays).

TAMPA BAY

This task, being performed by the U.S. Environmenal Protection Agency Environmental Research Laboratory in Gulf Breeze Florida, is designed to provide a comparative evaluation of candidate measures of bioeffects in oysters (*Crassostrea virginica*) from Tampa Bay, Florida. This progress report primarily includes work from the second year of the biomarker evaluation. Funding for the third year of bivalve biomarker work in Florida on oysters has been obligated but the final work plan has not been finalized. We are waiting for the final report including statistical analyses and subsequent peer review of year 2 results which have not yet been completed.

Site Characterization and Relative Contamination

Site selection for this study was based on availability of oysters, geographical location, and potential differences in contamination. Some regions had only a few locations with oysters and in some locations, oysters were too small to conduct all the biological measurements. Of the available locations in a region with sufficiently large oysters, final sites were selected to provide a gradient of relative contamination based on previous analyses of sediment chemistry. The sites shown in Figure 2 were sampled in 1991 and 1993. Sites were classified prior to collection as follows:

Lightly contaminated sites	2,6,12,16
Moderately contaminated sites	4,8,9,13,14,15
Heavily contaminated sites	1,3,5,7,10,11

Chemical analyses of oyster tissues (Shown in Figures 3,4, and 5) generally confirmed the original site classifications based on sediment contamination. There were no detectable concentrations (MDL = 0.20 ug/g dry wt) of PAH or PCB

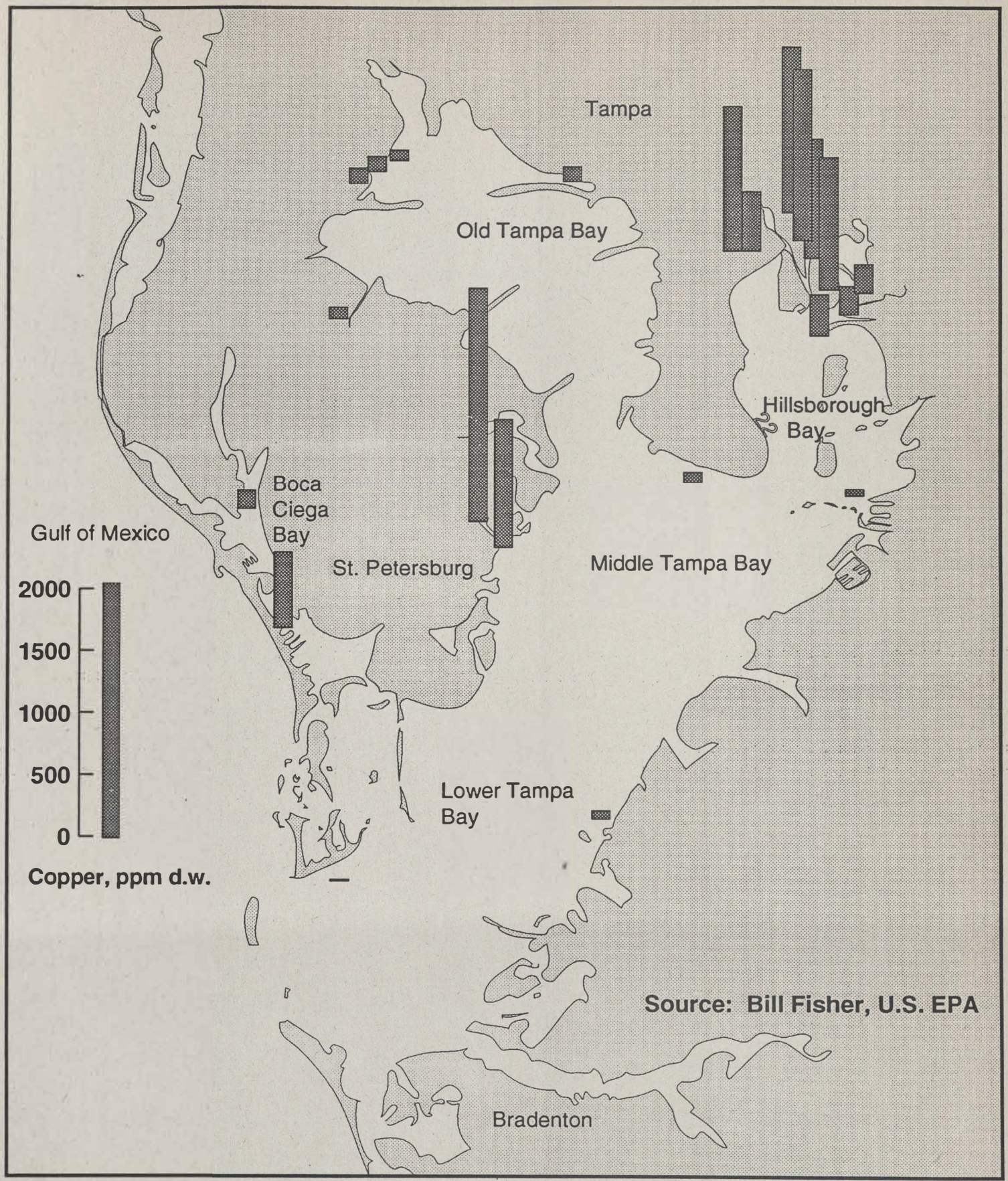


Figure 3. Copper concentrations in resident Tampa Bay oysters.

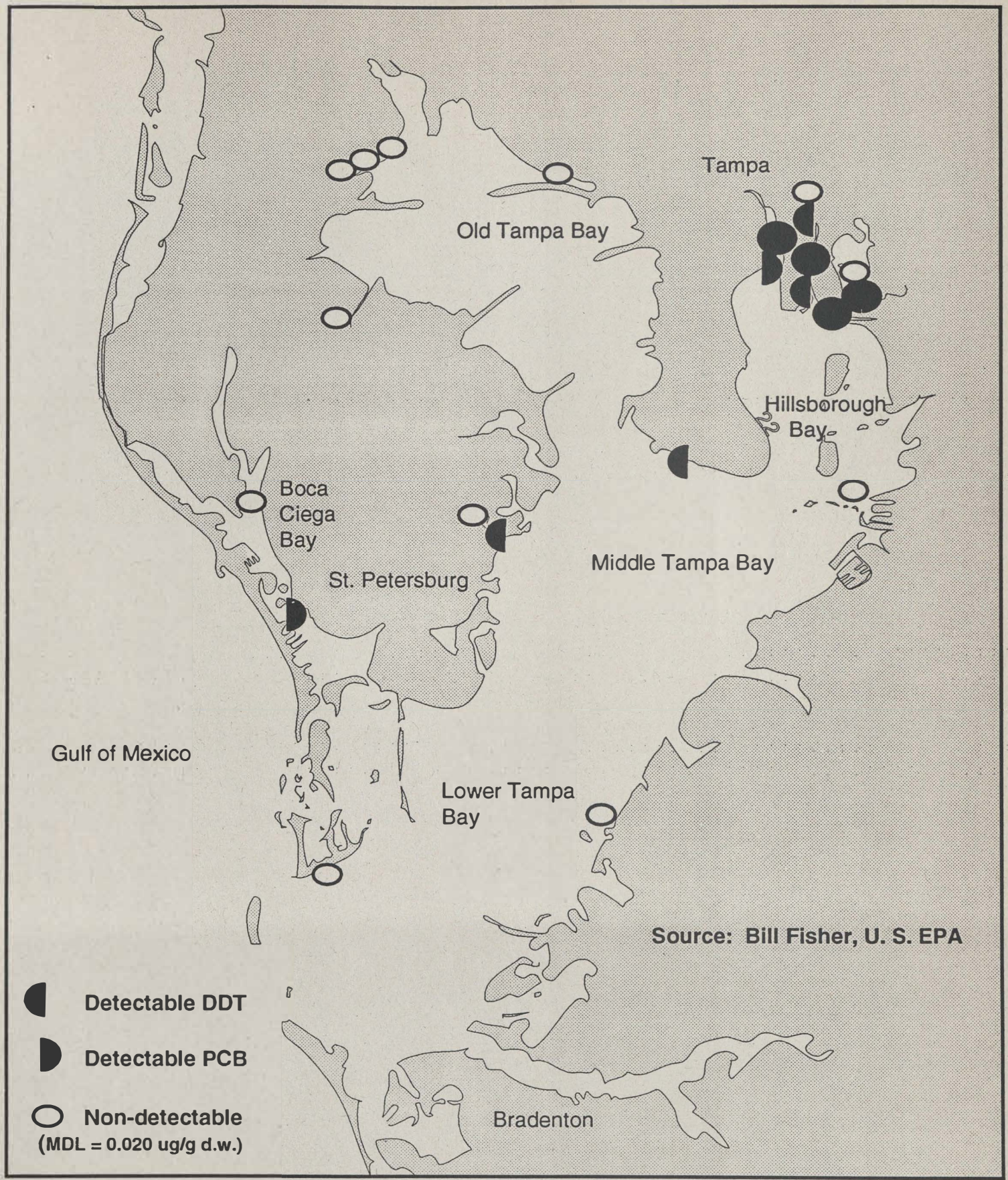


Figure 4. Detectable DDT and PCB in resident Tampa Bay oysters.

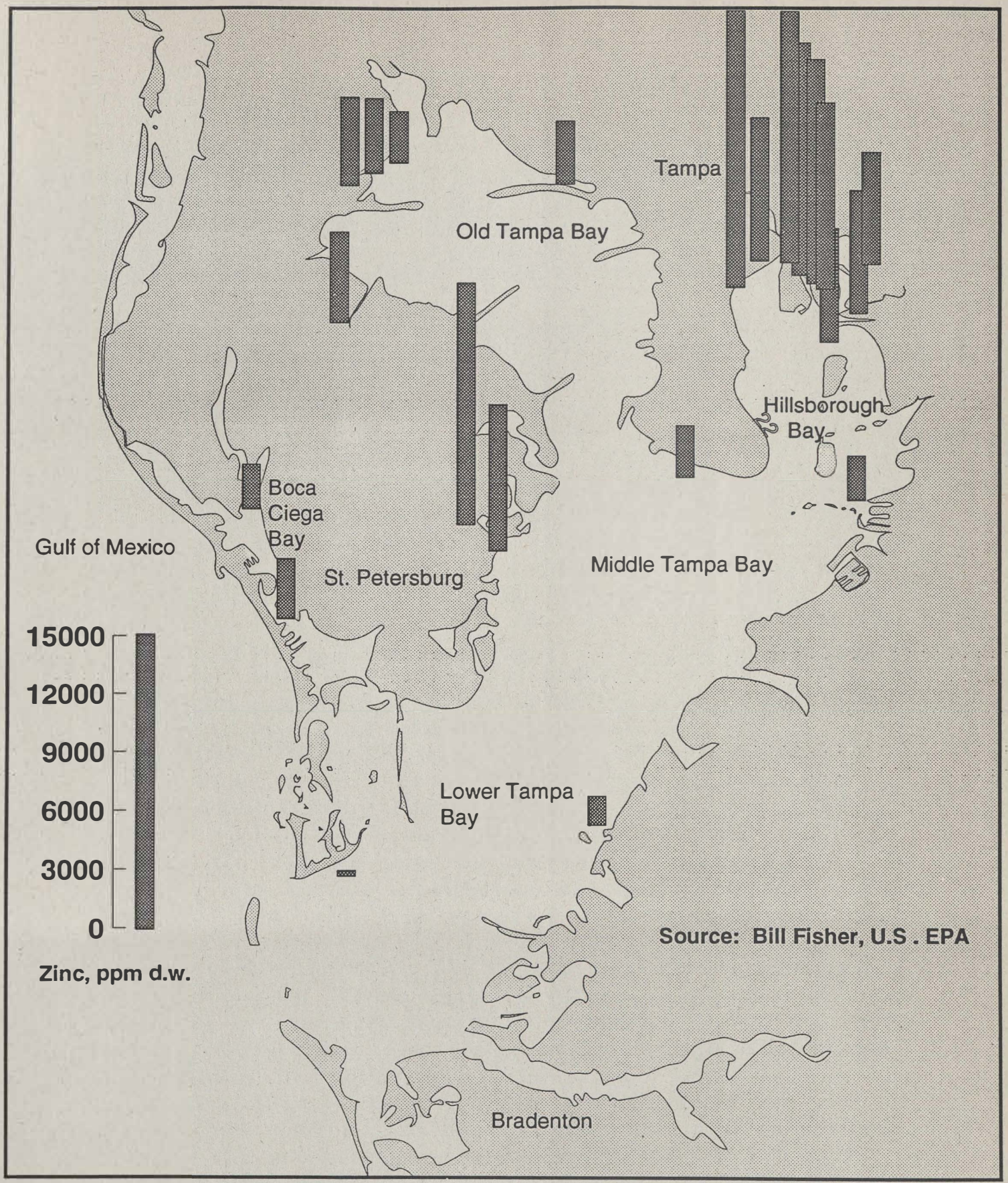


Figure 5. Zinc concentrations in resident Tampa Bay oysters.

compounds at the reference sites. There was one pesticide (t-nonachlor) detected at site 16 but no other pesticides at any other reference site. The reference sites did not have high concentrations of trace metals, but neither were they exceptionally low. Site 2, for example, was ranked second highest for Al and Cr and third for Fe and Ni. Some of the lowest trace metal values were found in the southern half of Hillsborough Bay (site 4).

Sites designated as heavily contaminated were also confirmed by the tissue chemistry data. PAH compounds were detected at site 11, PCB compounds were detected at sites 7 and 11, and the highest concentrations of single or combined pesticides were detected at sites 3,5,7, and 10. Oysters from sites 1,3,5,7, and 10 had the highest tissue trace metal content of all sites. Some "moderate" sites had detectable concentrations of PAH and PCB (site 14) and a single pesticide (site 13). Site 9 was relatively high for Pb and sites 13 and 14 were the highest for As. These data indicate that sites 13 and 14 could be considered the most contaminated and site 4 the least contaminated of the moderate sites.

Considering the tissue chemistry measurements from both year 1 and year 2, chromium, copper, lead, and zinc all had some values that exceed the highest concentrations measured in the NS&T Mussel Watch Program. Copper and lead in particular were reported to be a factor of two above the respective national highs at some sites. Copper in Tampa Bay oyster tissues exceeded the national highs at several sites. Lead exceeded the high at only one site but many others were below the MDL. In addition to exceeding the NS&T high, zinc in Tampa Bay oysters was very high at several other sites as well. Based on these measurements, the extent and magnitude of the highest values relative to NS&T data, and the potential for adverse biological effects in these ranges, copper and zinc appear to be the most significant contaminants in Tampa Bay oysters. Copper and zinc also showed the best relationships with the bivalve biomarkers based on the preliminary statistical analyses. The high detection limits for the organic contaminants precludes a rigorous statistical analysis of the data but the few actual measurements of PAHs and DDT combined with associations among some of the biomarkers suggest that these contaminants could be significant as well.

Biological Measurements

1. PHYSICAL/BIOCHEMICAL/CELLULAR

The physical condition of the oysters varied considerably among the 16 sites. Oyster length and weight were highest at sites 3 and 7 even though these sites were considered highly contaminated. These data suggest that contaminant loading at those sites did not impair the ability of oysters to survive and grow. Shelter from the sun and lower predation may have contributed to the presence of larger animals at these sites, but food availability is probably the most important factor. Oysters were relatively small in size at reference site 6 (light contamination) possibly due to low nutrient and phytoplankton availability. Oysters in Cooper's Bayou appeared to grow over the six-week collection period since sizes at sites 2,12, and 16 increased sequentially. Condition index was generally the reciprocal of the tissue wet:dry weight ratio, meaning that "wet" oysters were associated with poor condition. Oysters from reference sites 2,12, and 16 were in relatively poor condition whereas those from more contaminated sites 3,7,11 and 13 had low wet:dry ratios and better condition.

The majority of oysters at most sites were female during the winter collection. However, oysters from site 8 were mostly neuter, presumably because of parasitic castration caused by a trematode infection of the gonad. Site 9 oysters, for unknown reasons, were predominantly male. Because animals were collected sequentially by station, reproductive changes during the collection period must be considered in any interpretation of other biological results from different collection dates. Stage of development varied significantly among the heavily contaminated sites. Although some comparisons might indicate a site gradient, it is not clear if a contamination gradient also existed along this section.

Histological examination revealed that digestive gland tubules were in better condition in oysters from the heavily contaminated sites than in the control sites and supported the hypothesis that nutrition was more available at those sites. The vesicular connective tissue condition showed limited variability

in oysters at different sites and no consistent pattern in relation to site contamination.

Carbon and nitrogen content of oyster tissues varied by tissue type, site, and date of collection. The seasonal changes found in oyster carbon content appear to modify oyster response to contaminant stress: Slight, but significantly higher concentrations of carbon were found in oysters at contaminated sites during the winter whereas lower concentrations of carbon were found at these sites in the fall. Regardless of collection date, nitrogen content was lower in oysters at contaminated sites, implying higher protein catabolism.

Hemolymph from oysters collected at all sites had, with the exception of low-salinity site 15, relatively constant levels of ions and ionic balance. Of the hemolymph serum enzymes measured, only aspartate aminotransferase (AST) showed promise as a biomarker; the lowest levels of AST were found at sites 1,7, and 10. High iron concentrations were detected in hemolymph serum from site 3, substantiating the high concentrations found in the tissue analyses.

Inducible heat shock proteins (stress-70 and chaperonin-60) were measured on gill and mantle tissues from sites 3,7,9, and 10. Even though stress-70 was highest for site 10 gills, it was lowest in gills at sites 3 and 7 (high contamination) and low in mantles at site 7. Chaperonin-60 was highest for site 9 mantle tissue (the least polluted site) and site 7 gill tissue. It should be remembered however, that stress proteins in the gill represent a more recent accumulation while stress proteins in the mantle represent a more integrated accumulation over time.

2. PARASITES AND DISEASES

Based on the data analyzed to date, there is no obvious relationship between the occurrence of parasites and diseases and any other toxic or natural factor at the sites studied in these surveys.

3. CYTOGENETIC INTEGRITY

Two assays were selected to provide both mutational endpoints and some measure of genetic stability of the population. These

were the Micronucleus Test and DNA Unwinding Assays. Increased frequencies of DNA unwinding and micronuclei formation, when correlated with contaminant loading and other biological endpoints may serve as evidence of structural alteration of genetic material and may reflect potential risk to the population or other sublethal effects.

Assessment of DNA strand breakage exhibited a wide range of values with some of the lowest values at the least contaminated sites (which may also have the least amount of food). In this case, F is the fraction of DNA remaining double stranded after unwinding so a higher number is actually good. Site 9 (reference site) stood out as the most stressful environment for the oysters regardless of the comparison group. Figure 6 summarizes the incidence of micronuclei in Tampa Bay oyster cells. Certain sites (1,15, and 14) that were moderately to heavily contaminated were found to have significantly ($p<0.05$) lower MN vaues than oysters from the reference sites. Site 4 had the highest incidence of MN formation despite its preliminary classification of moderately to lightly contaminated.

4. IMMUNE STATUS

It is assumed that greater numbers of hemocytes in the hemolymph would provide a stronger response to invading microorganisms and/or toxicants. However, increased hemolymph cell density has also been attributed to warmer temperatures. Significantly higher hemocyte densities were found at sites 1,3, and 7, sites identified with the highest levels of contamination. It is possible tht higher cell densities resulted from this contamination, perhaps a requirement for survival in a contaminated environment. Nevertheless, the incidence of hemocytes in Tampa Bay oyster cells showed the best correlations with contamination in oyster tissues of all the biomarkers evaluated in this study. Hemocyte cell densities and rates of hemocyte locomotion are shown in Figure 7. Measurement of locomotory activity may also be a valid reflection. Hemocyte mobility was greatest at sites 1 and 7, which were identified among the most contaminated. Nearly 75% of the hemocytes were mobile at these sites. Site 3, also identified as a relatively contaminated site, ranked third with 65% mobility. The pre-selected reference site 6 showed high

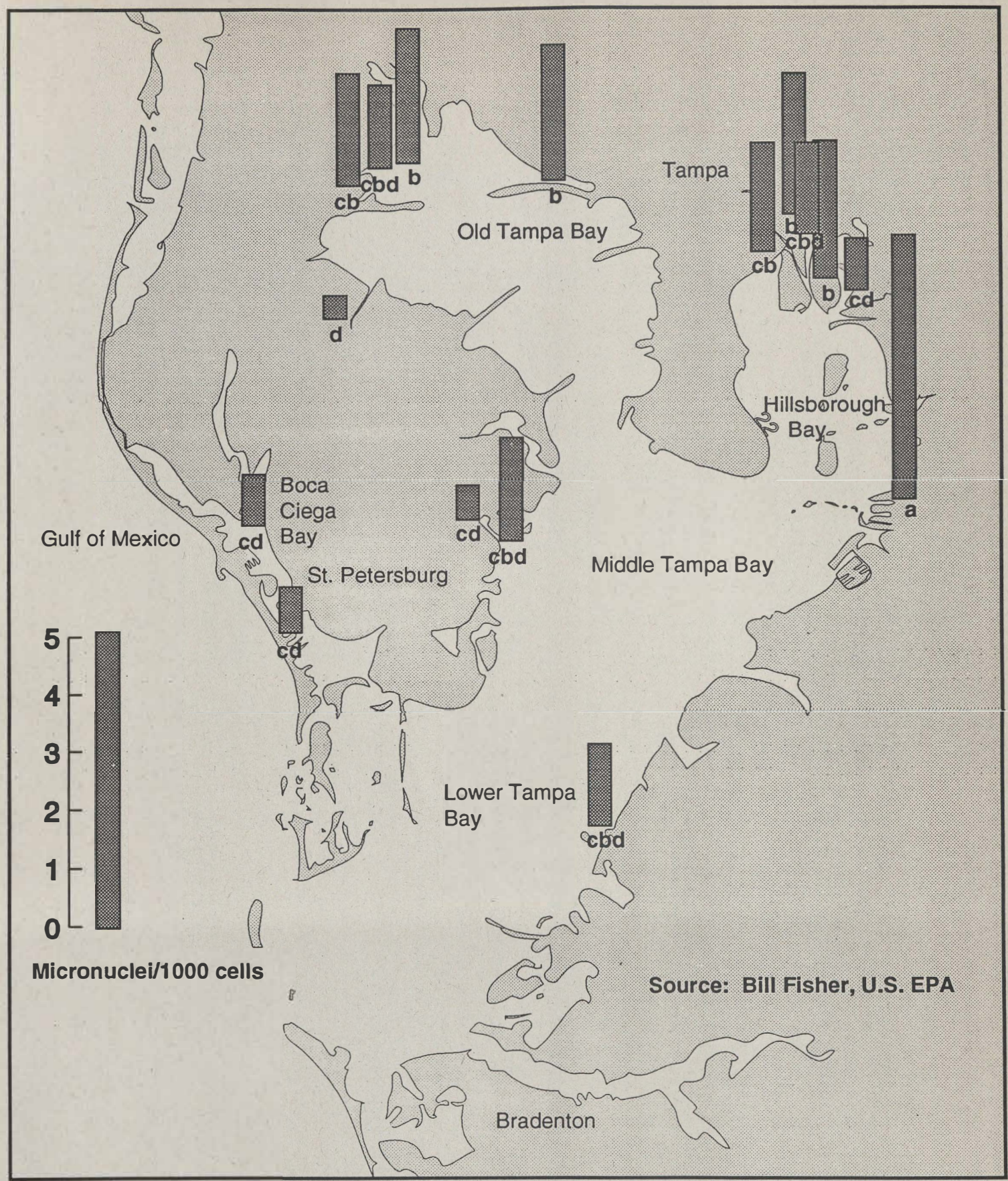


Figure 6. Incidence of micronuclei in Tampa Bay oyster cells. Sampling sites in which the incidence of micronuclei were not significantly different ($p<0.05$) are shown with similar alphabetical designators.

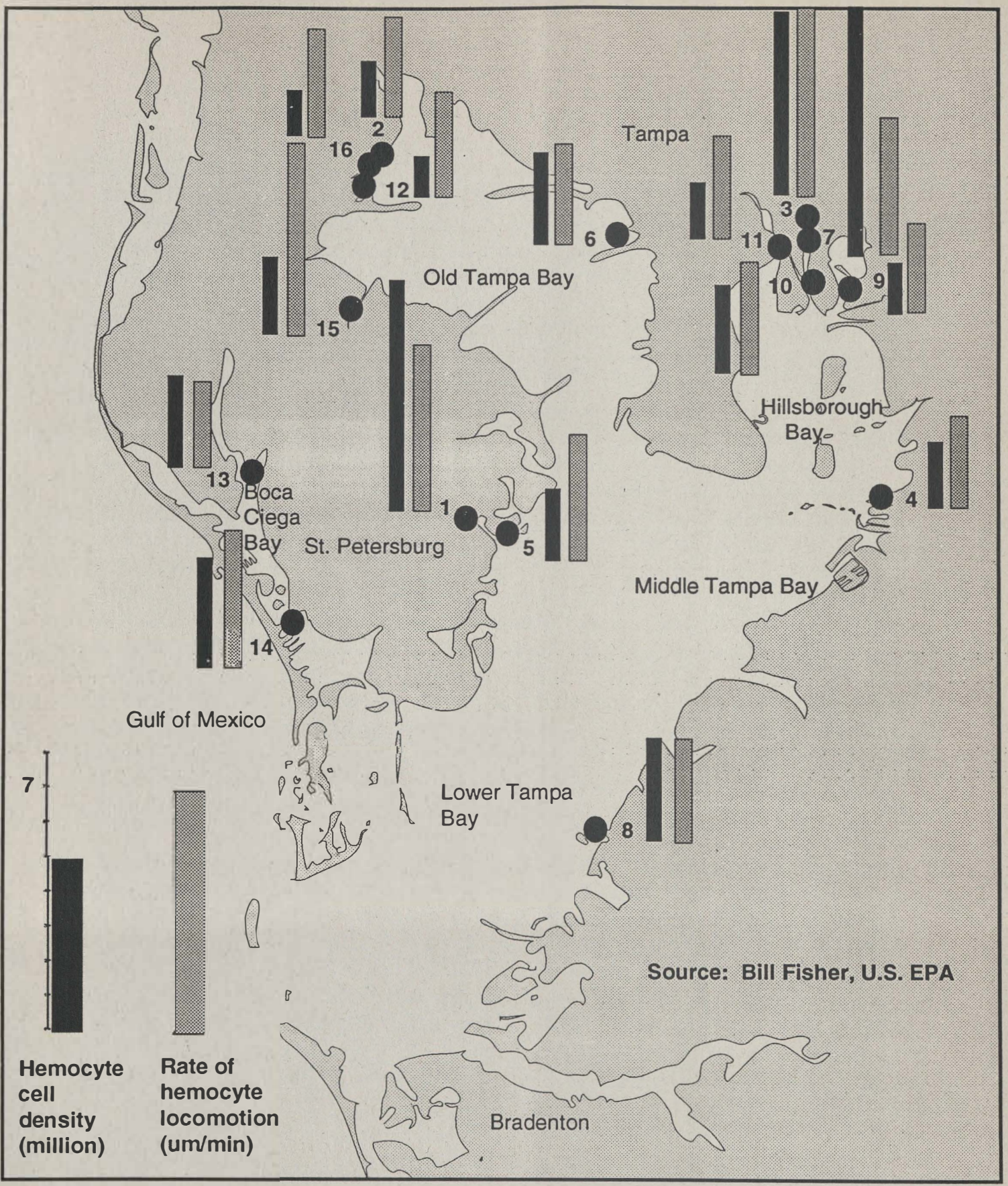


Figure 7. Mean immunological responses of resident Tampa Bay oysters, based upon counts of hemocyte cell densities and rates of hemocyte locomotion.

percentage mobility whereas the reference sites from the same bayou (2,12, and 16) were intermediate in percentage mobility.

The ability of hemocytes to bind or ingest foreign material (phagocytosis) is believed to be the principal means of removing invasive organisms from oyster hemolymph and tissues. Quantifying this potential could be an important measure of their defensive capacity. Sites 1,3,4,8, and 15 ranked highest in particle binding ability as an indication of phagocytosis. With the exception of site 4, each of these sites were associated with some form of contamination. Pre-selected reference sites 12 and 16 showed very low particle binding capacity, even though site 2 was ranked intermediate. It is possible that lower temperatures in February decreased the ability of the hemocytes to bind or was related to reduced stress from temperature. Many of the same sites that were high for other hemocyte activities were high for challenged NBT measurements of superoxide production. Sites 1,3,4,7,8, and 15 were among the top 7 for hemocyte NBT production. Each of these may be related to contamination (1,3,7), parasitism (8), low salinity (15) or progressed gametogenic stage (4). Site 6, a pre-selected reference site, was ranked fourth among these. The other pre-selected reference sites (2,12,16) ranked 9th, 15th, and 16th, respectively, but there were no significant differences among the three sites.

Although hemocytes are considered the most important functional unit in oyster defense, lysozymes within the hemolymph may also play a role. These hydrolytic enzymes can break down invading organisms or alter receptors on foreign particles and attract more hemocytes to the site. Release of enzymes into the hemolymph may be caused by stimulation from foreign material or from stressful conditions. However, cadmium has been reported to inhibit the release of lysozyme from granulocytes into the serum and copper to inhibit the release of acid phosphatase. Sites 3,4,7, and 8 had the highest levels of serum lysozyme. Sites 3 and 7 have been identified as relatively contaminated sites.

Oyster Transplant Studies

The overall objectives of the Bivalve Health Surveys in Tampa Bay were to test and evaluate performance of candidate biomarkers and to identify areas in Tampa Bay where biomarker responses are correlated with environmental contamination. As part of that effort, a small pilot study was conducted to test and evaluate the transplant methodologies previously developed for mussels, with oysters in Tampa Bay. The overall approach was to use synoptic measurements of bioaccumulation and growth to estimate exposure to contamination and associated biological effects. Specific reasons for using transplants include the following: 1) Well-defined exposure period; 2) Similar genetic and environmental history at the beginning of the test across sites; and 3) Unlimited sampling matrix over space and time. These attributes facilitate detecting differences among sites and separating the effects of natural factors from the effects of toxics and other man-induced stresses; particularly when used in concert with laboratory studies.

Small and large juvenile oysters (*Crassostrea virginica*) from a hatchery were transplanted to four sites: site 3 (DETSCO), site 7 (BANANA), site 9 (SEABREEZE), and site 10 (SULFUR). These sites were selected to provide a gradient of contamination in the vicinity of the Ybor Channel in Hillsborough Bay and to supplement and verify biomarker measurements made on resident adult oysters at those sites. Small juvenile oysters were deployed in plastic cages with individual compartments so the same individuals could be measured at the beginning and end of the test for estimates of growth and to increase the statistical power of the test. Large juvenile oysters were held in mesh bags and were used primarily to estimate exposure by measuring bioaccumulation of contaminants in oyster tissues.

Survival was high (>90%), all the cages were retrieved, and there was a statistically significant difference in juvenile oyster growth rates among sites (Figure 8). Adult oyster growth was not as discriminating as juvenile oyster growth. Growth of juvenile oysters by station is shown in Figure 9. The growth differences were as follows: DETSCO>BANANA>SULFUR>SEABREEZE. This pattern was identical for all parameters measured or calculated: final weights, final

	JUVENILES				ADULTS			
	DETSCO	**BANANA**	**SULFUR**	**SEABREEZE**	**DETSCO**	**BANANA**	**SULFUR**	**SEABREEZE**
Initial Weights (grams)	0.722	0.710	0.709	0.704	7.27	7.28	7.40	7.32
	**********				**********			
Initial Lengths (mm)	16.89	16.90	16.95	16.84	41.26	41.30	41.21	40.59
	**********				**********			
Final Weights (grams)	8.00	6.42	4.39	4.05	26.87	24.44	20.46	18.79
	********	********	**********		********	********	**********	
Final Lengths (mm)	34.06	32.26	27.36	26.73	57.01	55.73	52.76	49.20
	********	********	**********		**********		********	********
Weight Growth (grams/wk)	0.565	0.444	0.286	0.257	1.53	1.34	1.02	0.90
	********	********	**********					
Length Growth (mm/wk)	1.34	1.20	0.81	0.76	1.23	1.12	0.86	0.63
	********	********	**********					
Survival	91%	89%	93%	89%	100%	98%	100%	93%

Figure 8. Summary of growth results from Tampa Bay oyster transplant study over a 90-day exposure period. *** = statistically similar stations

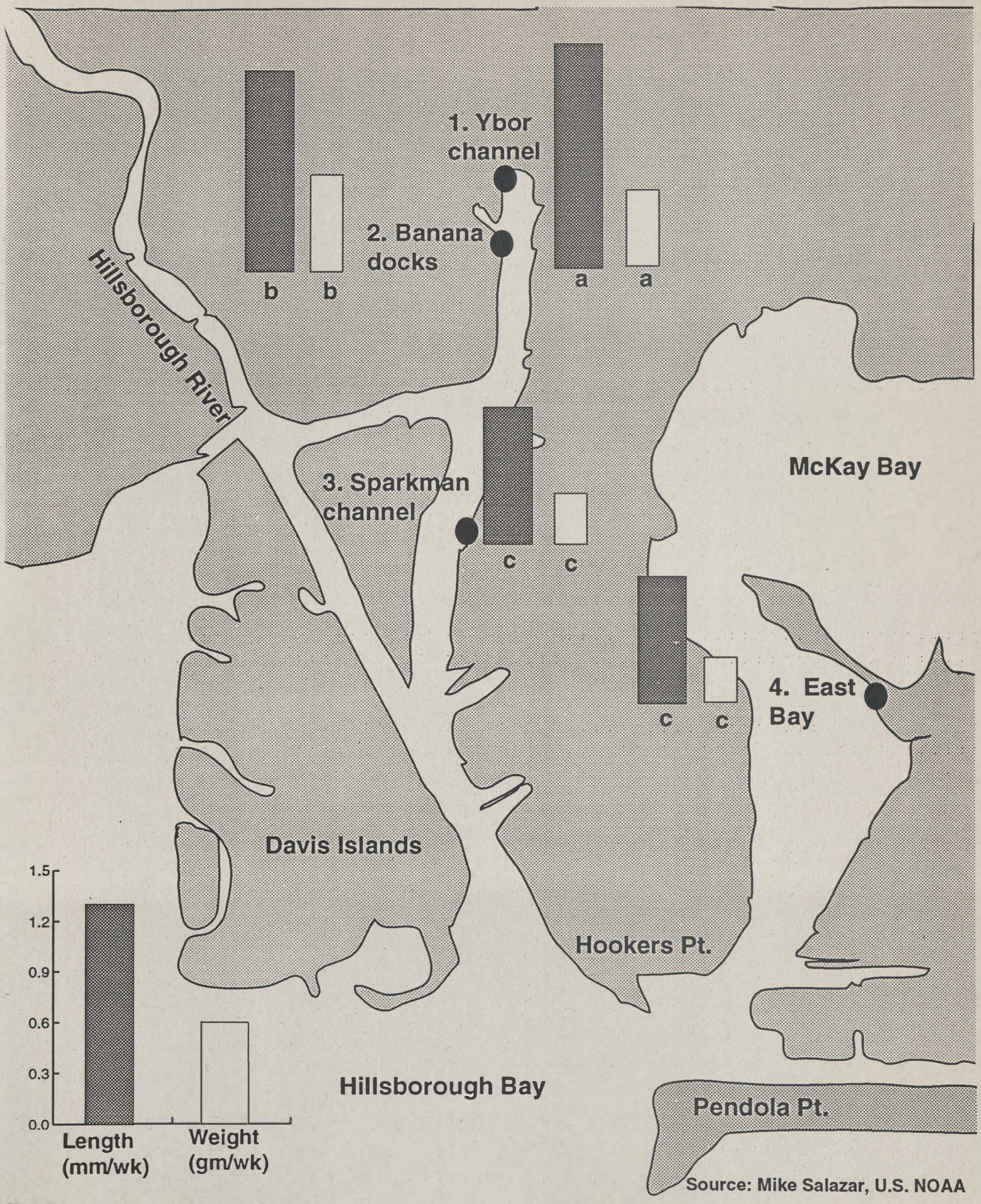


Figure 9. Growth of transplanted juvenile oysters in Tampa Bay.

lengths, weight growth, and length growth. At the highest growth site (site 3), whole animal wet weights increased by a factor of 10 while this increase was only a factor of approximately six at the lowest growth site (site 9). There was a factor of approximately two difference when final weights were compared at these sites. The apparent positive relationship between contamination and growth is surprising, but similar to that observed in the resident adult oyster population. Since the highest juvenile growth rates were found at the most contaminated site, it seems reasonable to conclude that factors other than contamination were controlling oyster growth rates in this study. Since there was some correlation with biomarker responses and inverse correlations with others, it is not clear at this point what growth rates and biomarkers are really measuring relative to toxic and other factors controlling their responses. Neither chemical analyses of transplanted oyster tissues or final statistical analyses of correlations have been completed.

SAN DIEGO BAY

This task, being performed by California State University, Long Beach in cooperation with a Navy Research Lab in San Diego, California, is designed to provide a comparative evaluation of candidate measures of bioeffects in mussels (*Mytilus galloprovincialis*) from San Diego Bay, California. This progress report includes only the first year of field work which has been completed, but not fully analyzed. Although original plans included another southern California bay for year 2 of these studies, funding constraints and a change in thrust from COP for more research and emphasis on cumulative effects suggest that another year in San Diego Bay would be more productive. Additionally, the Navy has funding for bivalve studies which could be supplemented with COP funds in a cooperative effort.

Site Characterization and Relative Contamination

Site selection for this study was based on availability of oysters, geographical location, and potential differences in contamination based on sediment chemistry from concurrent NOAA studies under the COP Program, and previous experience of NOAA and Navy staff in San Diego Bay. A modified pair design consisted of two treatments and one control site in the

north Bay and a similar set in the south Bay for a total of six sites; four treatments and two controls. Mussel transplant sites are shown in Figure 10. The control sites were both on Coronado Island. The North Island Air Station (NIAS) and the Naval Amphibious Base (EOD) have been measured previously and found to have low concentrations of contaminants and used as control sites. In the south Bay, Naval Station San Diego (NAV) has historically had very high concentrations of petroleum hydrocarbons (PAH) and the Paco Terminal (PACO) very high concentrations of copper in sediment. In the north Bay, Commercial Basin (CB) has had very high concentrations of PCBs and other metals while the Shelter Island Yacht Basin (SI), has had among the highest concentrations of tributyltin (TBT) and copper at coastal U.S. sites. The Navy, NOAA, and NMFS staff have monitored fish and bivalves in San Diego Bay for a number of years. The bivalve transplant methods employed here were developed by NOAA staff as part of an organotin research program for the Navy using several of these same sites.

Chemical analyses of mussel tissues generally confirmed the original site characterizations based on sediment contamination and previous studies with one notable exception. Although PACO had extremely high concentrations of copper in sediment, this copper was apparently not bioavailable to mussels in the water column near the surface. As expected, tissue concentrations of copper, tributyltin, and zinc were highest at SI, PCBs were highest at CB, PAHs were highest at NAV, and the control sites were generally the lowest in contaminants associated with the highest toxicity.

Since the San Diego Bay study only utilized six sites, the purpose was not survey-oriented as in Tampa Bay where mapping the extent of contamination and testing biomarkers were emphasized. San Diego was more of an evaluation of biomarkers at specific sites that had previously been reported to be contaminated or uncontaminated and measured with various bivalve indicators. As expected, copper in juvenile mussels transplated to SI was about seven times higher than the NS&T highs reported for Harbor Island in San Diego Bay and the Hudson River Raritan. This concentration of 155 ug/g is about a factor of two above where adverse effects on juvenile mussel growth have been predicted. Only one value for zinc in

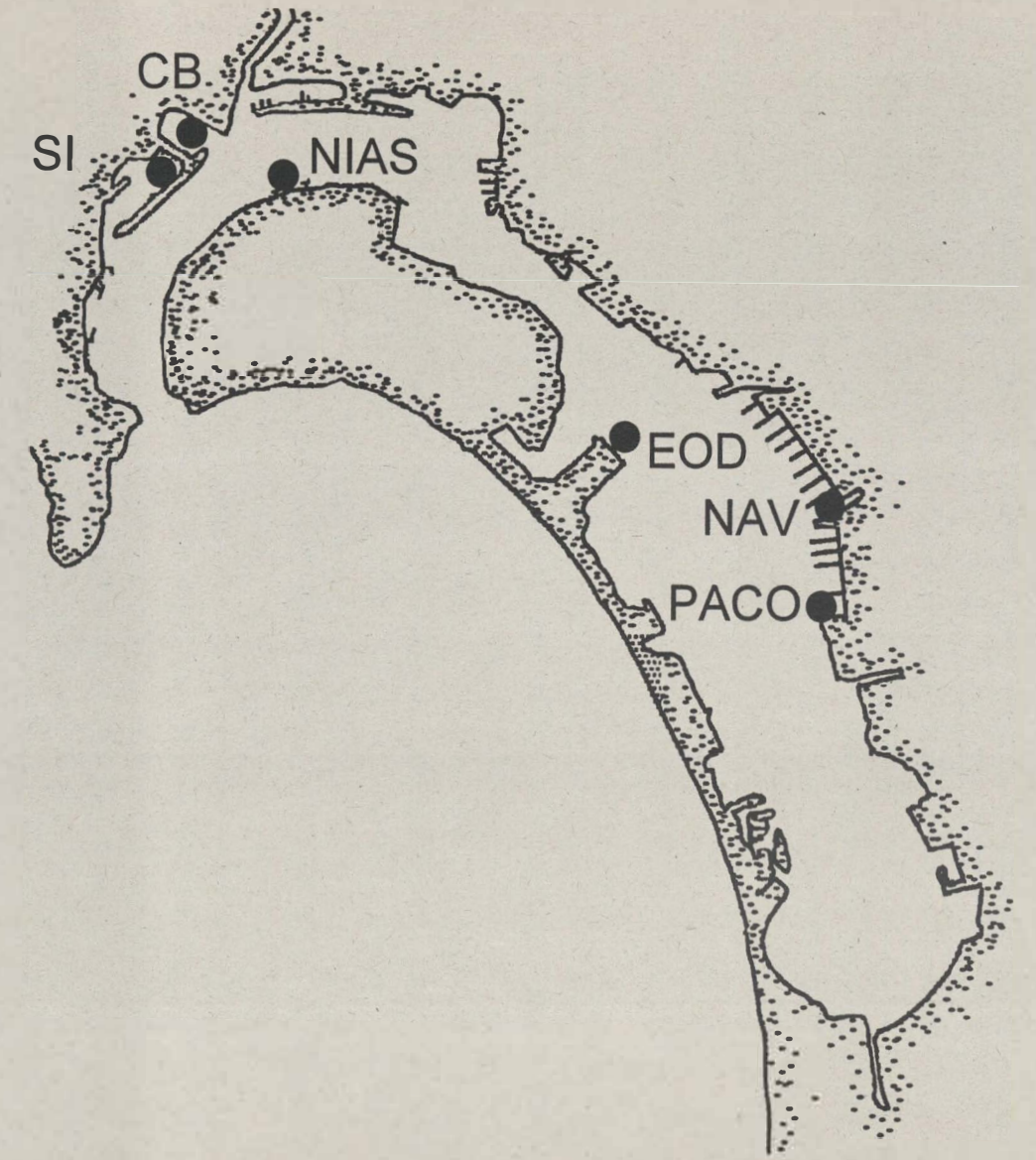


Figure 10. Mussel transplant sites in San Diego Bay.

adult mussel tissues exceeded the national high from NS&T but many others were above the predicted threshold of 150 ug/g. The reported values for TBT in juvenile and adult mussel tissues of 4.0 and 4.7 ug/g are in the zone of possible effects. This most recent survey confirms that the concentration of TBT in mussel tissues at SI has declined by a factor four since the ban on TBT in 1988. Although the concentrations of PAHs in mussel tissues was relatively high, particularly when compared to other San Diego Bay sites in this study, they were well below the national highs reported in NS&T. Nevertheless there appeared to be a strong link between PAHs at NAV and several biomarkers at NAV, including growth rate of adult mussels. Surprisingly, PCBs were also relatively low at CB, although this particular site was removed from the site of highest PCB contamination in Commercial Basin, San Diego Bay.

Biological Measurements

BIOACCUMULATION, GROWTH, & REPRODUCTION

In contrast to the Tampa Bay studies, the San Diego Bay biomarker study was based on the transplant methodology with resident mussels used as a supplement. Every reasonable effort was made to ensure that there were no differences among mussels at test sites in weights or lengths at the beginning of the test. Both juvenile and adult mussels were transplanted at each site. For juveniles, the range in lengths was only about 2.5 mm and the range in weights about 1 g at the beginning of the test. For adults the range in lengths was only about 5 mm and the range in weights about 14 g at the beginning of the test. Any differences were not statistically significant. As in the Tampa Bay study, small juvenile mussels were held in plastic cages with individual compartments so the same individuals could be measured at the beginning and the end of the test for estimates of growth and to increase the statistical power of the test. Another compartmentalized rack held mussels that were sampled at various intervals for the suite of biomarkers. Also, as in the Tampa Bay study, large adult mussels were held in mesh bags and were used primarily to estimate exposure by measuring bioaccumulation of contaminants in oyster tissues.

Juvenile and adult survival was approximately 95% and animals increased in weight and length at all sites; i.e., they grew.

	NI	CB	PAC	EOD	NAV	SI	LAB
Tissue (g)	3.41	3.42	2.14	1.93	1.75	1.78	0.46
Shell (g)	3.28	2.86	2.67	2.69	2.59	1.87	1.03
Whole (g)	9.99	9.00	8.40	8.20	7.81	5.45	2.82
Length (mm)	47.1	45.5	44.2	44.0	43.6	37.4	29.7
Ln. Growth (mm/wk)	2.11	1.60	1.47	1.35	1.31	0.81	0.16
Wt. Growth (mg/wk)	631	553	500	482	451	255	43

Figure 11. Growth of transplanted juvenile mussels in San Diego Bay as estimated by various parameters.

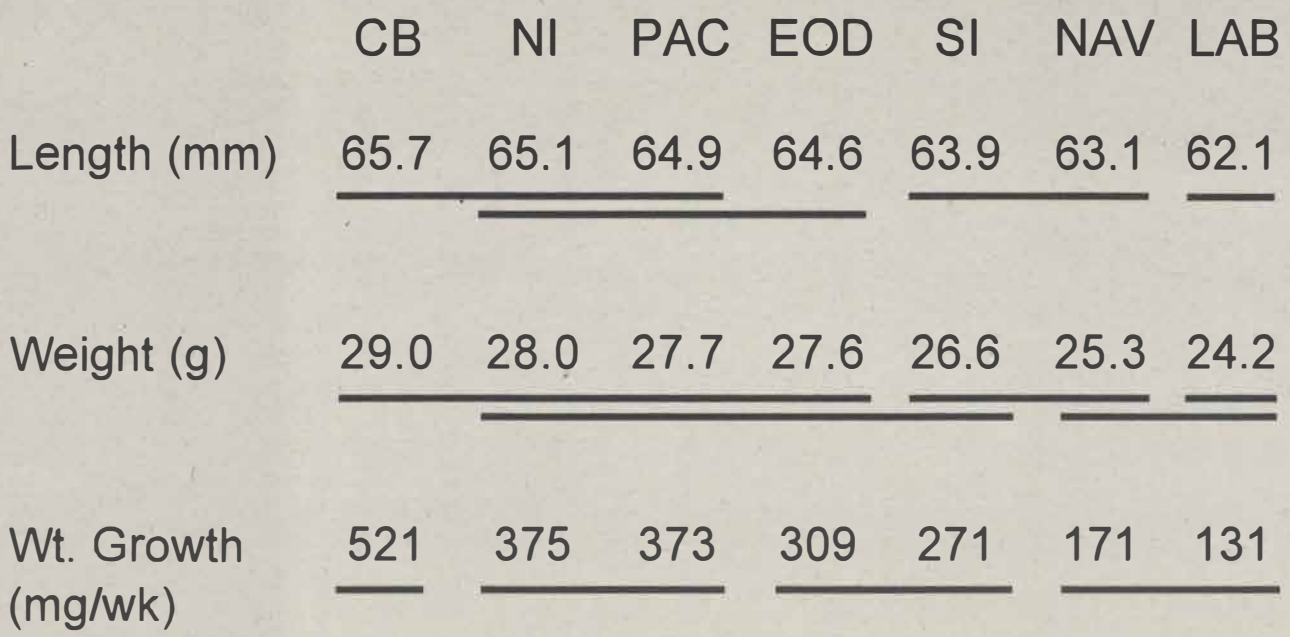


Figure 12. Growth of transplanted adult mussels in San Diego Bay as estimated by various parameters.

There was also a statistically significant difference in juvenile and adult mussel growth among sites as shown in Figure 11 (juveniles) Figure 12 (adults). The growth differences were as follows. For juveniles: NI>CB=PACO=EOD=NAV>SI>LAB. For adults: CB>NI=PACO>EOD=SI>NAV=LAB. At the highest growth rate site for juveniles, whole animal wet weights increased by a factor of 3.3 while this increase was only a factor of 1.3 at the lowest site for growth. By contrast the increase in lab animals was only 0.95. This difference in growth between laboratory and field studies is consistent with previous studies and strongly suggest that growth in the field is seldom achieved in laboratory studies. Increases in weight appeared to be the most discriminating estimator of growth.

As expected, and in contrast to the Tampa Bay studies, there was an inverse correlation between mussel growth rate and contamination at most sites. The lowest growth rates (other than the lab control) were found at the site with the highest concentrations of tissue copper, tributyltin, and zinc as shown in Figures 13, 14, and 15. These results are also consistent with previous work. The highest growth rates were found at the site with the lowest overall concentrations of contaminants. Two notable exceptions to this trend are CB and PACO. Although CB had the highest concentrations of PCBs and relatively high concentrations of other contaminants, growth rates were higher than expected. Although juvenile growth was lower and significantly different than the NI control site, adult growth was significantly higher than the NI control site. It appears that PCBs are not very toxic to mussels and do not have a significant affect on their growth rates at the concentrations measured here. For both juveniles and adults, PACO ranked third in growth rate even though sediments there were highly contaminated with copper. This copper was not bioavailable to mussels in the water column at the surface and other contaminants were not very high.

Another interesting difference between juveniles and adults was found at NAV. For all of the juvenile mussel growth rate studies conducted in San Diego Bay over the last seven years, SI has consistently provided the lowest growth rates of any site tested and significantly different from all other sites. For the adults in this study, NAV was significantly lower than all other sites. It appears that the juvenile mussels are more responsive

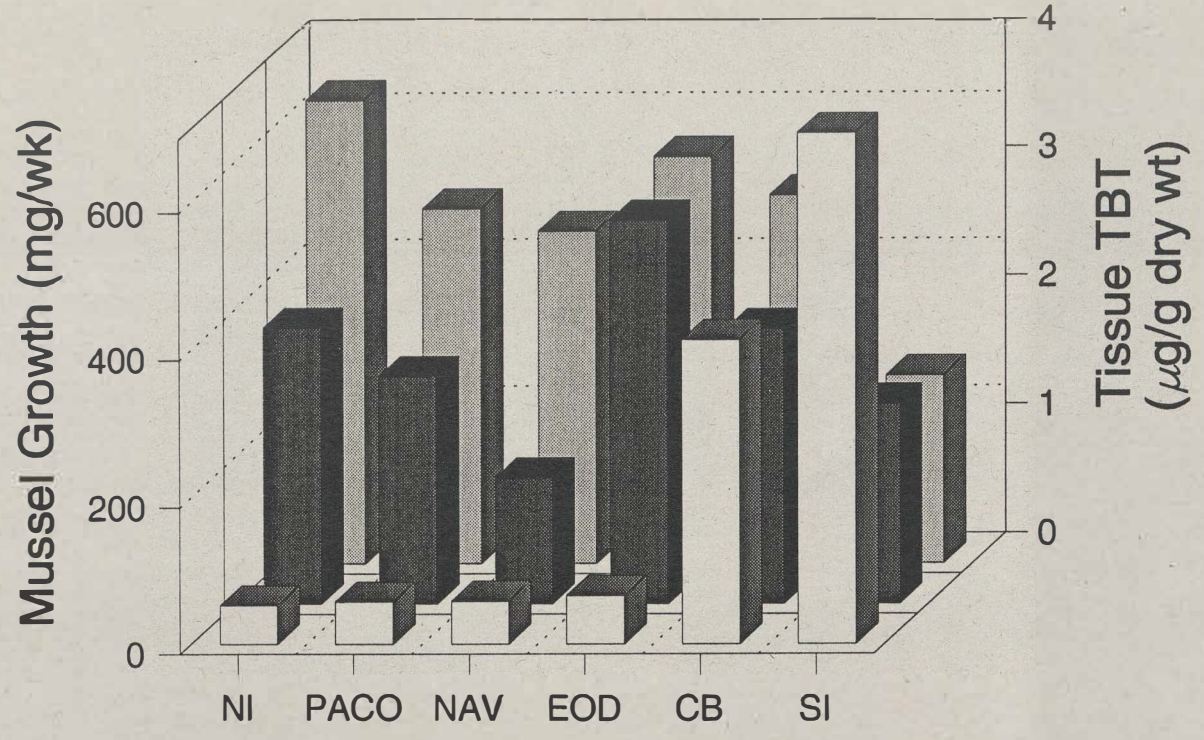


Figure 13. Associations between tissue TBT concentrations and growth rates of juvenile and adult mussels.

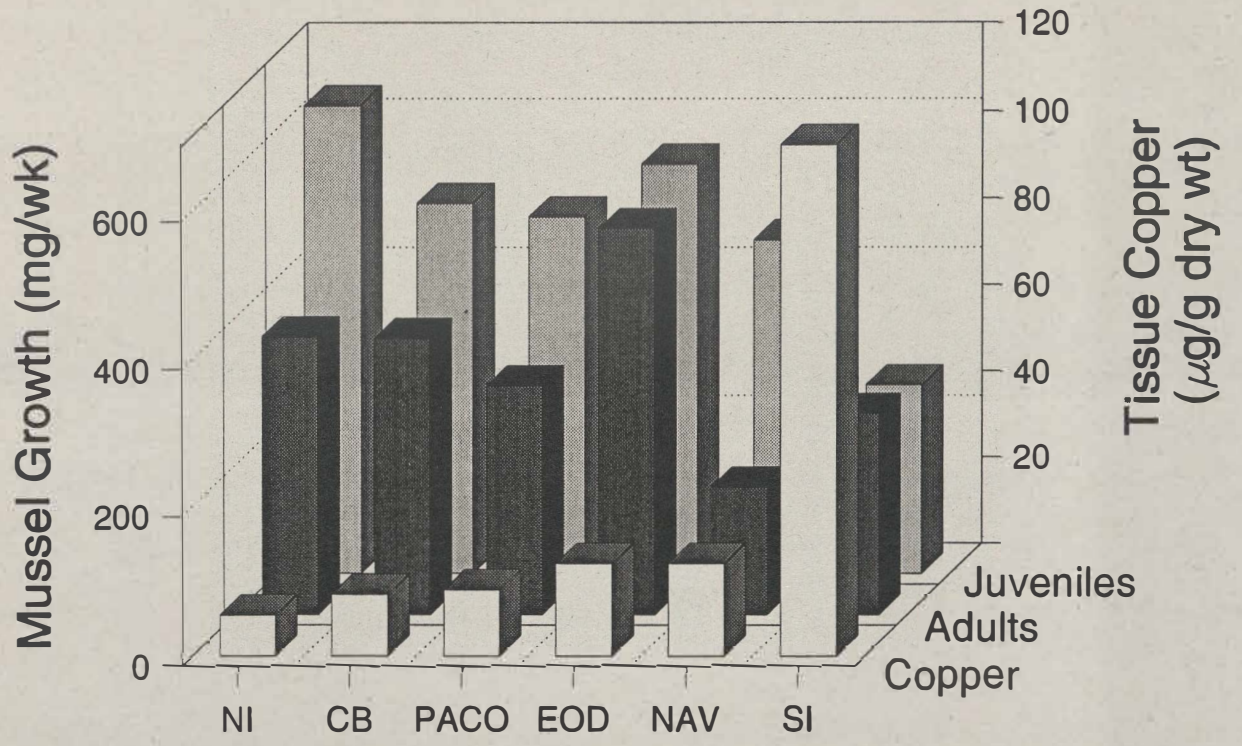


Figure 14. Associations between tissue copper concentrations and growth rates of juvenile and adult mussels.

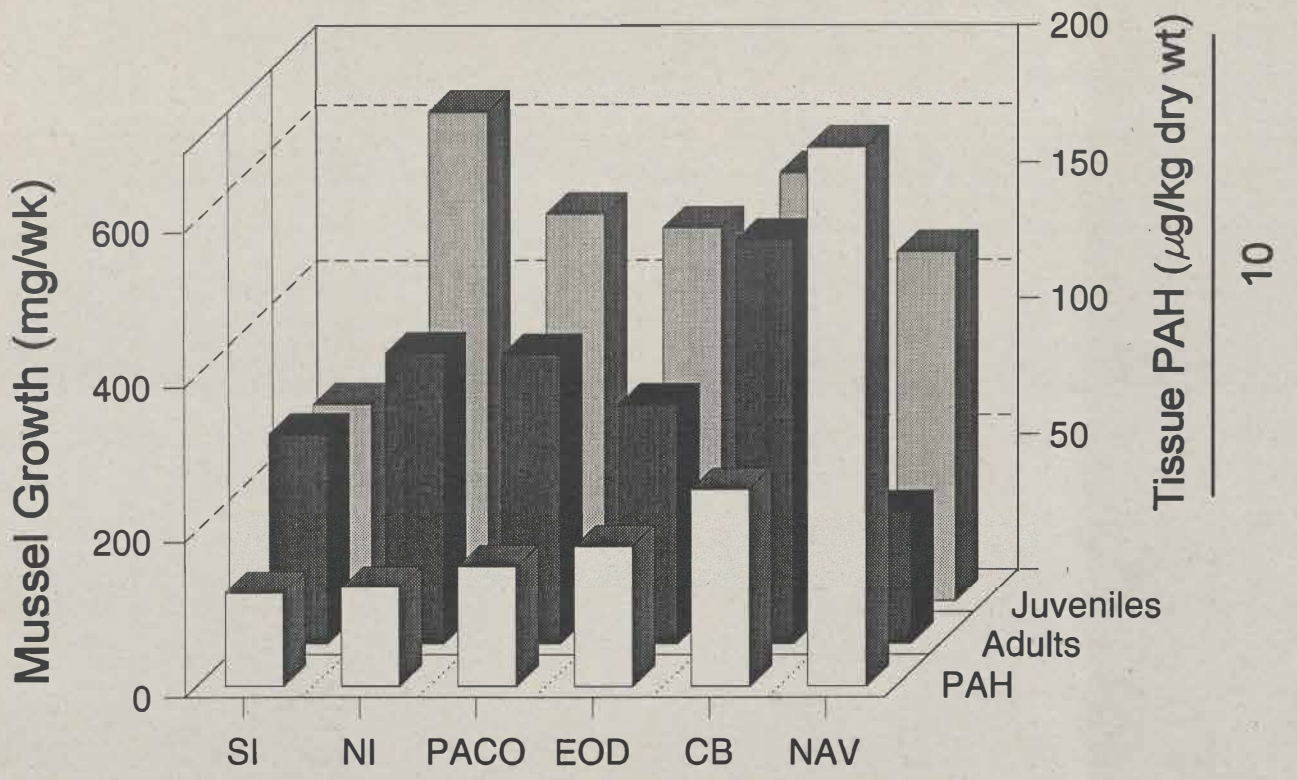


Figure 15. Associations between tissue PAH concentrations and growth rates of juvenile and adult mussels.

to copper, tributyltin, and zinc whereas the adults are more affected by PAHs. This provides additional evidence for using both juveniles and adults in bivalve studies that utilize bioaccumulation and growth.

The statistical analysis of the reproduction data is not yet available and the preliminary analysis did not reveal any obvious trends in the data except that there were differences among sites.

BIOCHEMICAL MARKERS

The best correlation between exposure and mussel growth occurred with the comet assay for DNA unwinding. The results of the comet assay are shown in Fig. 16. The only anomaly occurred at PACO which did not have high concentrations of most contaminants in mussel tissues. Growth rates were relatively high and the DNA unwinding index was the second highest. The two lowest sites in terms of DNA unwinding were the two control sites (NIAS and EOD). The lowest of all were the LAB animals indicating the most stressed. After PACO, SI and NAV were very similar with high DNA unwinding, high tissue chemistry, and the lowest growth rates (other than the lab control). As expected, CB was intermediate in terms of DNA unwinding.

There was also a very good correlation between the P450 measurements, the highest PAH concentrations, and the lowest adult mussel growth rates. As mentioned previously, it appears that adult mussels were more affected by high PAH concentrations than juvenile mussels.

There were differences in accumulation of both stress proteins, HSP-70 and CPN-60, among sites. As with growth and DNA unwinding, juveniles responded differently than adults. The accumulation of HSP-70 and CPN-60 appeared independent of each other and may reflect contaminant-specific responses. There were also differences between mantle and gill tissue which also suggest that the response is contaminant-specific. Preliminary correlation analysis determined that DNA unwinding and stress proteins were positively correlated with each other and negatively correlated with growth. Correlations

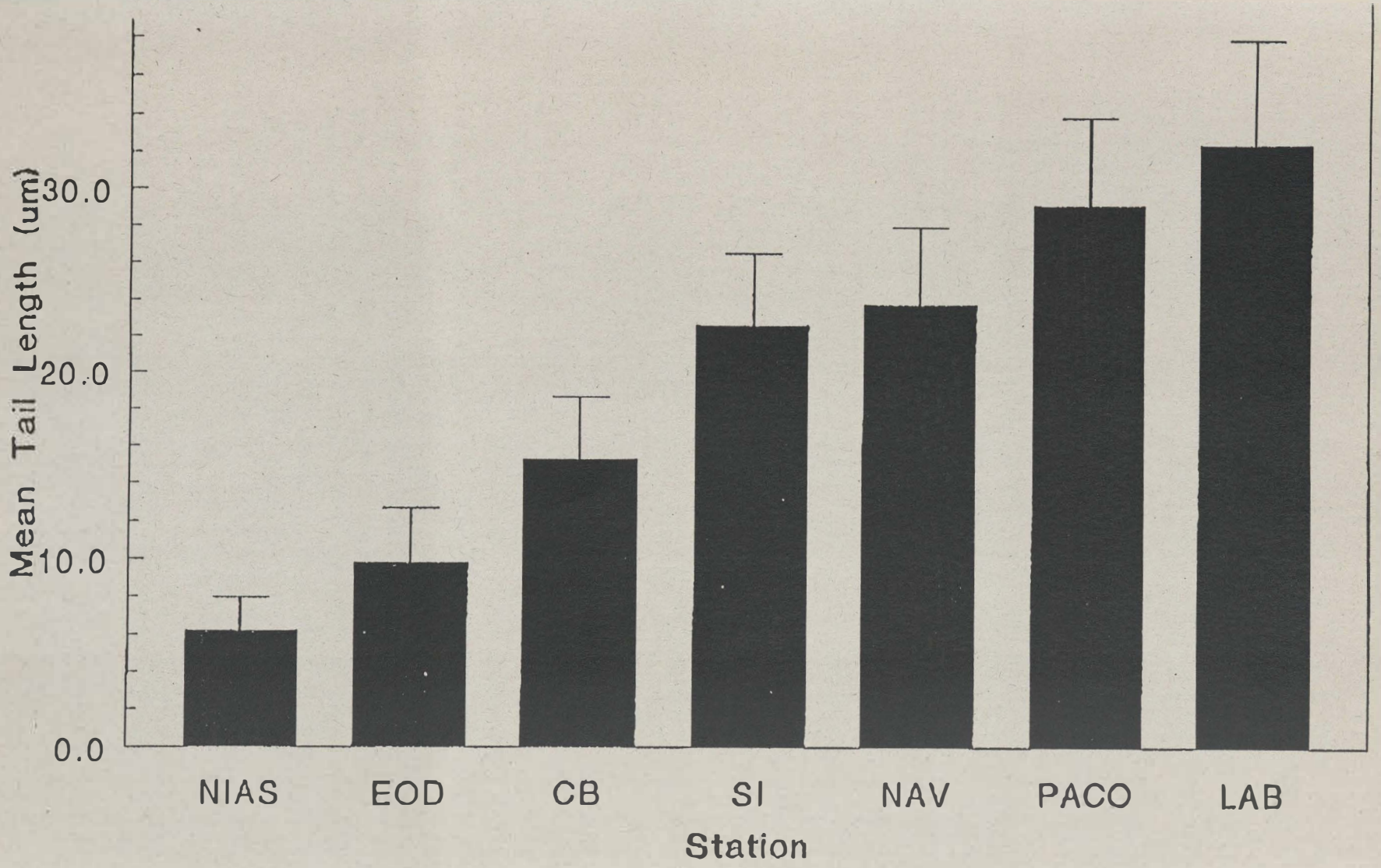


Figure 16. Relative DNA unwinding as measured by the Comet assay.

with reproductive status have yet to be determined. Correlations with bioaccumulation among stations could not be determined because of the lack of replication, however correlations with specific contaminants are being conducted as part of the subsequent project.

MASSACHUSETTS AND BUZZARDS BAYS (MA)

This study, being performed by the Woods Hole Oceanographic Institution (WHOI) is a three-year project designed to examine the relationship between the bioaccumulation of lipophilic organic contaminants and reproductive effects in marine bivalve molluscs. The specific focus is on the following three areas: 1) Defining the mechanisms of reproductive impairment by evaluating the energetic and reproductive consequences of alterations in lipid poos as they relate to gametogenesis and storage metabolism in bivalve molluscs and structural alterations in follicle development; 2) Defining the role of lipid class distributions in influencing accumulation patterns; and 3) applying these data to the design of monitoring programs utilizing sentinel organisms such as *Mytilus edulis* and *Mya arenaria*.

Site Characterization and Relative Contamination

The project focused on both mussels (*Mytilus edulis*) collected at several NS&T Mussel Watch stations in Massachusetts and Buzzards Bays (MA) and soft shell clams (*Mya arenaria*) collected from two sites in Buzzards Bay to assess the effects of lipophilic organic contaminants on reproductive condition. Mussels were collected at NS&T stations in Buzzards Bay and Boston Harbor and had high concentrations of PCBs, PAHs, and other lipophilic contaminants. In addition, mussels from New Bedford Harbor (Buzzards Bay), and uncontaminated sites in Buzzards Bay and Cape Cod Bay were also examined. Soft shell clams were collected from New Bedford Harbor that had high concentrations of PCBs and PAHs. Clams were also collected from an uncontaminated site in Buzzards Bay.

Bioaccumulation and Reproduction

Mytilus populations transplanted to New Bedford Harbor showed reduced reproductive effort, and increased degeneration and premature resorption of oocytes coincident with high body burdens of PCBs and PAHs. Resident populations of *Mytilus* from New Bedford Harbor also showed extensive signs of gonad degeneration. Preliminary investigations of *Mytilus* from Buzzards Bay-Angelica Rock conducted during the summer of 1988 also indicated aberrations in oocyte development. During 1990-1991, however, there were insufficient numbers of sexually mature mussels at the two Buzzards Bay NS&T sites to complete the evaluation of the annual reproductive cycle. Collections of resident populations of *Mytilus* in New Bedford also revealed a marked decrease in adult animals, and those that were collected showed little evidence of developing gonads.

Detailed analysis of the stereology and biochemical composition of gonadal tissues in mussels transplanted to New Bedford Harbor confirm earlier findings of reduced reproductive effort in mussels associated with the accumulation of lipophilic organic contaminants. The percentage of developing follicles in female mussels from New Bedford Harbor transplants is significantly reduced in comparison with control populations. This reduction in developing oocytes is correlated with a significant reduction in the triglyceride content of mantle/gonadal tissues. No aberrations in nutritive cells or connective tissue was noted nor any aberrations in the follicle development of male mussels transplanted to New Bedford Harbor.

Similar analyses have also been completed on resident populations of *Mytilus edulis* in Massachusetts Bay and *Mya arenaria* in Buzzards Bay. Site-specific and species-specific differences in the response to contaminant gradients are apparent and the role of different energetic strategies in influencing reproductive condition of bivalve populations is being investigated further as part of other programs. These data have also been applied to a demographic model evaluating the effects of contaminants and disease status on life history characteristics of bivalve populations.