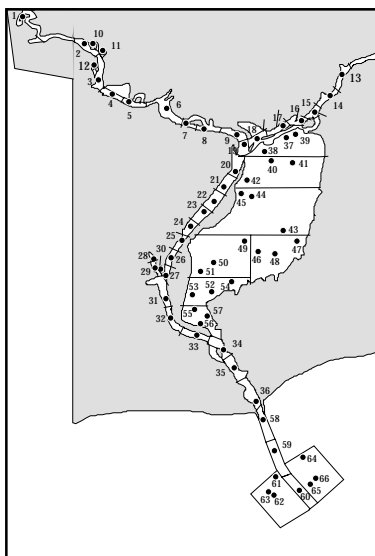
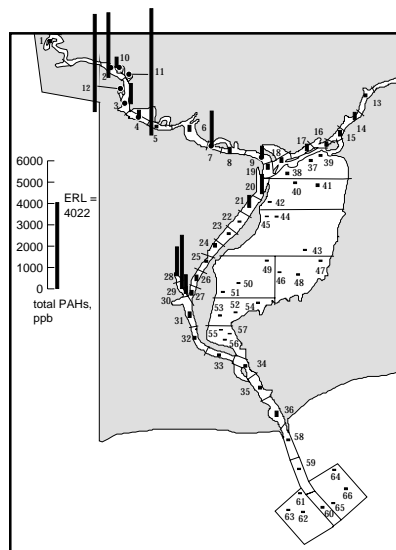
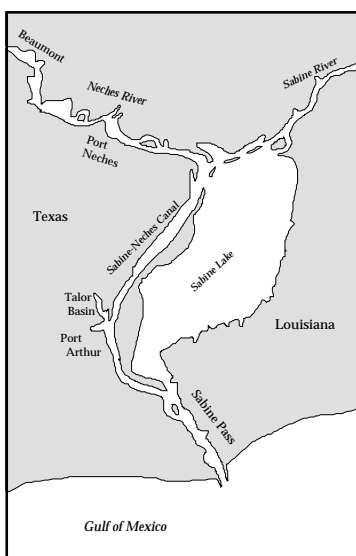


National Status and Trends Program
for Marine Environmental Quality

Survey of Sediment Quality in Sabine Lake, Texas and Vicinity



Silver Spring, Maryland
December 1999

US Department of Commerce

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Center for Coastal Monitoring and Assessment
National Centers for Coastal Ocean Science
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Survey of Sediment Quality in Sabine Lake, Texas and Vicinity

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ABSTRACT

The toxicity of sediments in Sabine Lake, Texas, and adjoining Intracoastal Waterway canals was determined as part of bioeffects assessment studies managed by NOAA's National Status and Trends Program. The objectives of the survey were to determine: (1) the incidence and degree of toxicity of sediments throughout the study area; (2) the spatial patterns (or gradients) in chemical contamination and toxicity, if any, throughout the study area; (3) the spatial extent of chemical contamination and toxicity; and (4) the statistical relationships between measures of toxicity and concentrations of chemicals in the sediments.

Surficial sediment samples were collected during August, 1995 from 66 randomly-chosen locations. Laboratory toxicity tests were performed as indicators of potential ecotoxicological effects in sediments. A battery of tests was performed to generate information from different phases (components) of the sediments. Tests were selected to represent a range in toxicological endpoints from acute to chronic sublethal responses. Toxicological tests were conducted to measure: reduced survival of adult amphipods exposed to solid-phase sediments; impaired fertilization success and abnormal morphological development in gametes and embryos, respectively, of sea urchins exposed to pore waters; reduced metabolic activity of a marine bioluminescent bacteria exposed to organic solvent extracts; and induction of a cytochrome P-450 reporter gene system in exposures to solvent extracts of the sediments.

Chemical analyses were performed on portions of each sample to quantify the concentrations of trace metals, polynuclear aromatic hydrocarbons, and chlorinated organic compounds. Correlation analyses were conducted to determine the relationships between measures of toxicity and concentrations of potentially toxic substances in the samples.

Based upon the compilation of results from chemical analyses and toxicity tests, the quality of sediments in Sabine Lake and vicinity did not appear to be severely degraded. Chemical concentrations rarely exceeded effects-based numerical guidelines, suggesting that toxicant-induced effects would not be expected in most areas. None of the samples was highly toxic in acute amphipod survival tests and a minority (23%) of samples were highly toxic in sublethal urchin fertilization tests. Although toxic responses occurred frequently (94% of samples) in urchin embryo development tests performed with 100% pore waters, toxicity diminished markedly in tests done with diluted pore waters. Microbial bioluminescent activity was not reduced to a great degree (no EC50 <0.06 mg/ml) and cytochrome P-450 activity was not highly induced (6 samples exceeded 37.1 ug/g benzo[a]pyrene equivalents) in tests done with organic solvent extracts. Urchin embryological development was highly correlated with concentrations of ammonia and many trace metals. Cytochrome P-450 induction was highly correlated with concentrations of a number of classes of organic compounds (including the polynuclear aromatic hydrocarbons and chlorinated compounds).

INTRODUCTION

Background.

As a part of the National Status and Trends (NS&T) Program, NOAA conducts assessments of the adverse biological effects of toxic chemicals in selected coastal regions and estuaries. Studies are performed to determine biological effects of toxicants in fishes and bivalve molluscs. This report is one in a series of regional reports on sediment quality. Previous reports have been produced for the Hudson-Raritan estuary, Long Island Sound, Boston Harbor, Tampa Bay, San Diego Bay, San Pedro Bay, southern California estuaries, western Florida panhandle, and South Carolina/Georgia bays and summarized in Long et al. (1996).

Sabine Lake is an inland estuary that straddles the Texas/Louisiana border near Beaumont, Texas. The estuary is adjacent to a portion of the Intracoastal Waterway (ICW), which is composed of several channels of the Neches and Sabine rivers. Freshwater is supplied to the lake by these two rivers. The lake is open to the Gulf of Mexico through Sabine Pass.

Trace metals concentrations in sediments have been reported as relatively low (even depleted relative to other nearby areas) in the Sabine-Neches estuary as compared to other estuaries along the Gulf coast (Ravichandran et al., 1995). However, Harrell and McConnell (1995) reported detectable concentrations of dioxins and furans in the clam *Rangia cuneata* in the Neches River downstream from a pulp mill. There is a huge complex of petroleum – related industries along the Neches and Sabine rivers, particularly many refineries and transshipment docks near Beaumont and Port Arthur. Crude oil and petroleum products are shipped and piped on- and offshore in this area.

Sabine Lake was chosen for this survey based upon the likelihood of chemical contamination within sediments of the area and an interest expressed by the State of Texas. The survey of Sabine Lake was followed in 1996 by a similar survey of Galveston Bay; the results of which are reported in a separate report.

Goals and Objectives.

The overall goal of this study was to provide a characterization of the toxicological condition of sediments in Sabine Lake and surrounding channels, as a measure, or indicator, of adverse biological effects of toxic chemicals. Data from toxicity tests were intended to provide a means of determining whether toxic conditions actually occurred within any of the areas.

Several specific technical objectives were established to serve as guides for the sampling designs and analytical plans. The objectives of the study were to:

- (1) determine the incidence and degree of toxicity of sediments throughout the study area;
- (2) determine the spatial patterns (or gradients) in chemical contamination and toxicity throughout the study area;
- (3) determine the spatial (or surficial) extent of chemical contamination and toxicity as percentage of the total study area; and

(4) determine the statistical relationships between measures of toxicity, concentrations of chemical substances and benthic populations in the sediments.

METHODS

Sampling design.

The study area encompassed all of Sabine Lake, portions of the Sabine River, portions of the Neches River, portions of the Neches-Sabine Canal at the confluence of the two rivers, portions of Sabine Pass channel entrance, and an area in the Gulf of Mexico near the entrance channel (Figure 1). A stratified-random sampling design similar to those used in previous surveys conducted nationwide by NOAA (Long et al., 1996) was applied in Sabine Lake. The study area was subdivided into 22 irregular-shaped strata, designated A through R (Figure 2). Strata established within channels were further subdivided into three sub-strata to improve spatial coverage. Only one location each was sampled within each sub-stratum, whereas three locations were sampled in each of the larger undivided strata (i.e., strata M, R, and P).

This approach provided the least intense sampling effort in areas known or suspected to be relatively homogeneous in sediment type and water depth, and relatively distant from contaminant sources. In contrast, relatively small strata were established in channels and urban harbors nearer suspected sources in which conditions were expected to be heterogeneous or transitional. As a result, sampling effort was more intense in the small strata than in the large strata. Strata R and P were established to document conditions beyond the mouth of the Sabine Pass channel.

This approach combines the strengths of a stratified design with the random-probabilistic selection of sampling locations. Data generated within each stratum or sub-stratum can be attributed to the dimensions of that area. Therefore, these data can be used to estimate the spatial extent of toxicity with a quantifiable degree of confidence (Heimbuch, et al., 1995). Strata boundaries were established to coincide with the dimensions of major basins, bays, inlets, waterways, etc. in which hydrographic, bathymetric and sedimentological conditions were expected to be relatively homogeneous. Sub-stratum boundaries were established at roughly equal intervals along the axes of the channels.

Within the boundaries of each stratum, all possible latitude/longitude intersections had an equal probability of being selected as a sampling location. The locations of individual sampling stations within each stratum were chosen randomly using GINPRO software, developed by NOAA, applied to a digitized navigation chart. Four alternate locations were provided for each station in a numbered sequence. The coordinates for each alternate were provided in tables and were plotted on the appropriate navigation chart. In two cases the coordinates provided were inaccessible (first alternate for station 3 blocked by a dredging operation) or only shell and cobble were present at the location (oyster shells in first alternate for station 57). In those cases, the first station alternate was rejected and the vessel was moved to the second alternate set of coordinates to collect the sample. In both cases, the second location was accessible and sediments were acceptable.

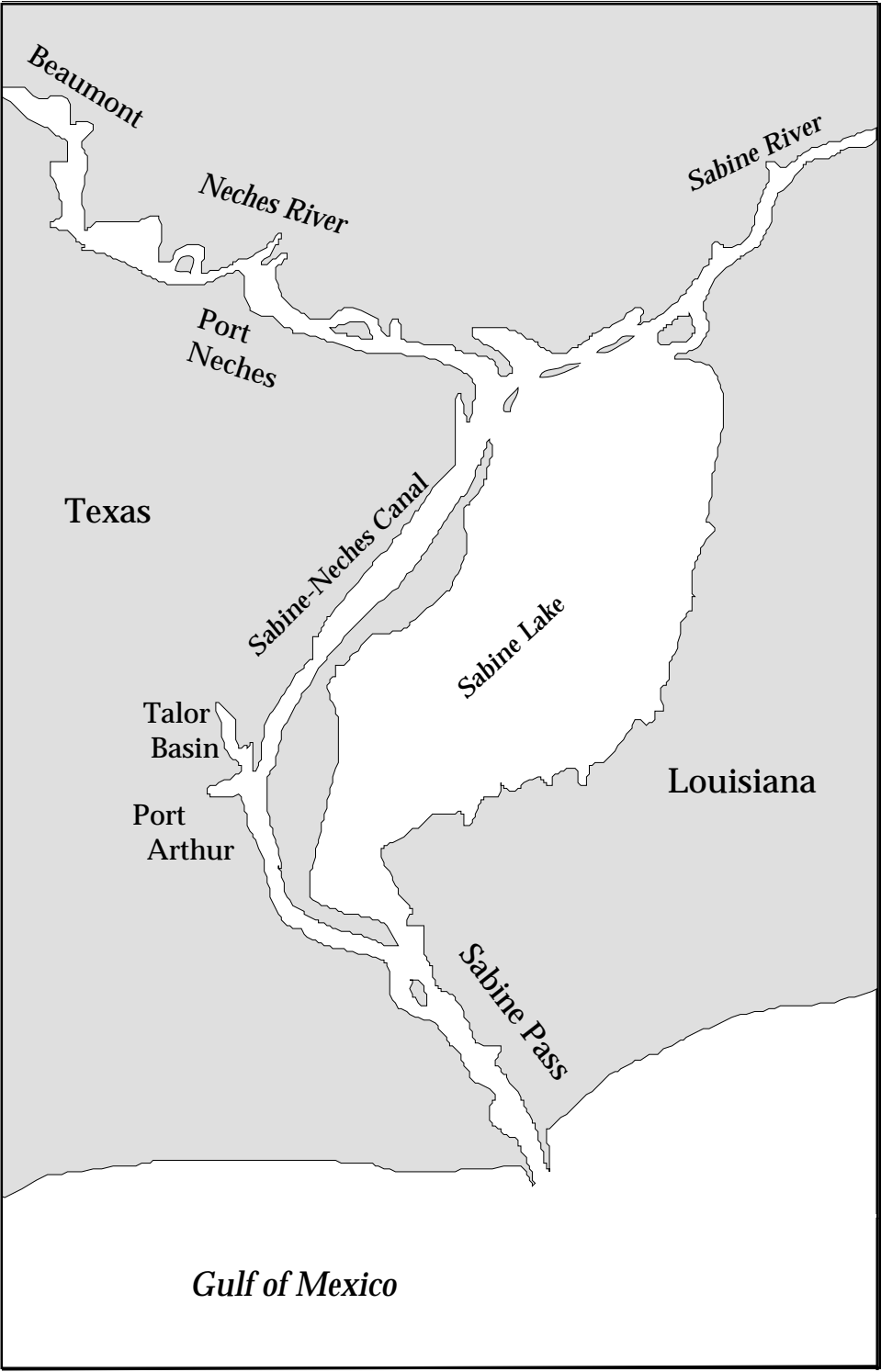


figure 1. Sabine Lake and surrounding study area.

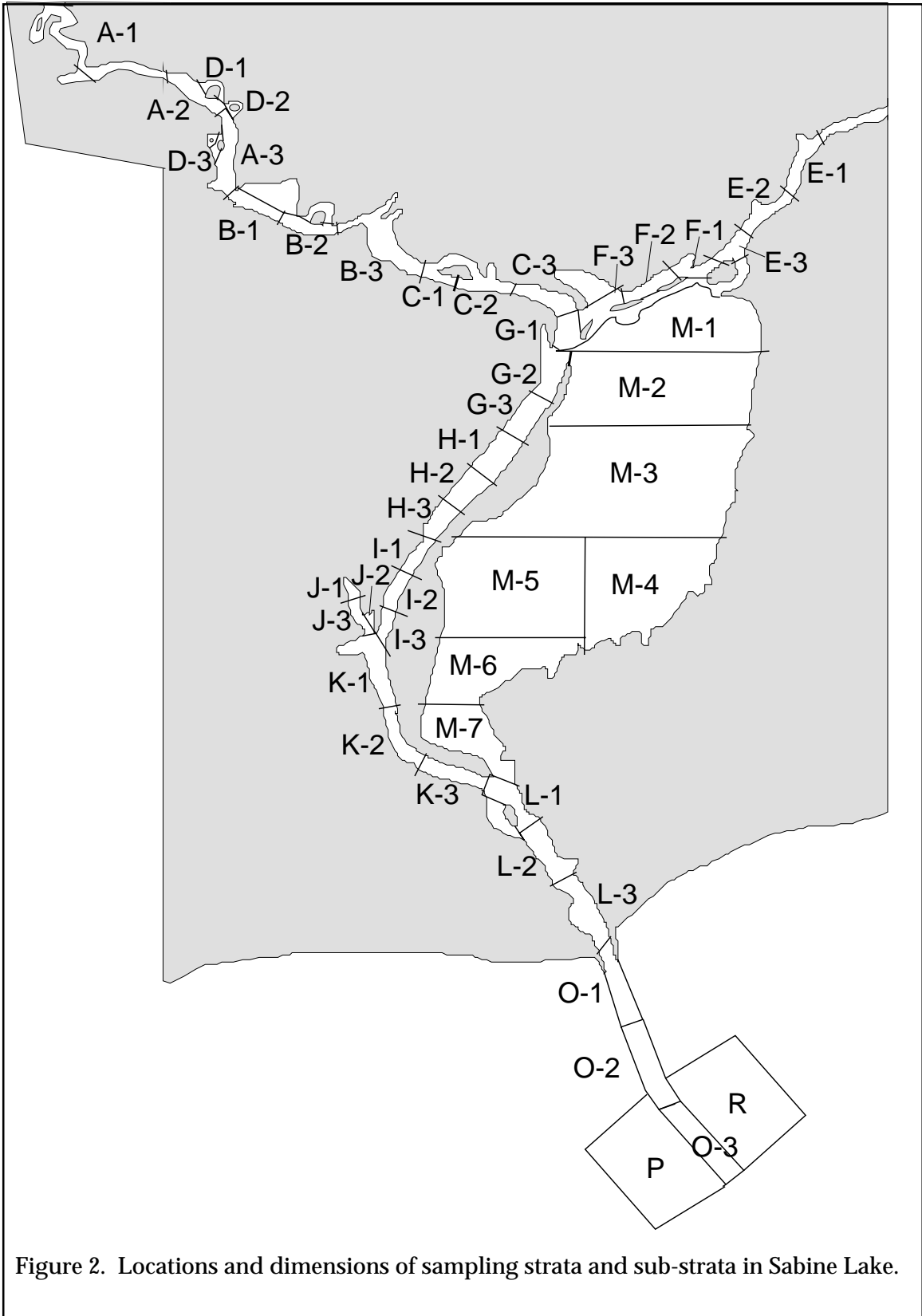
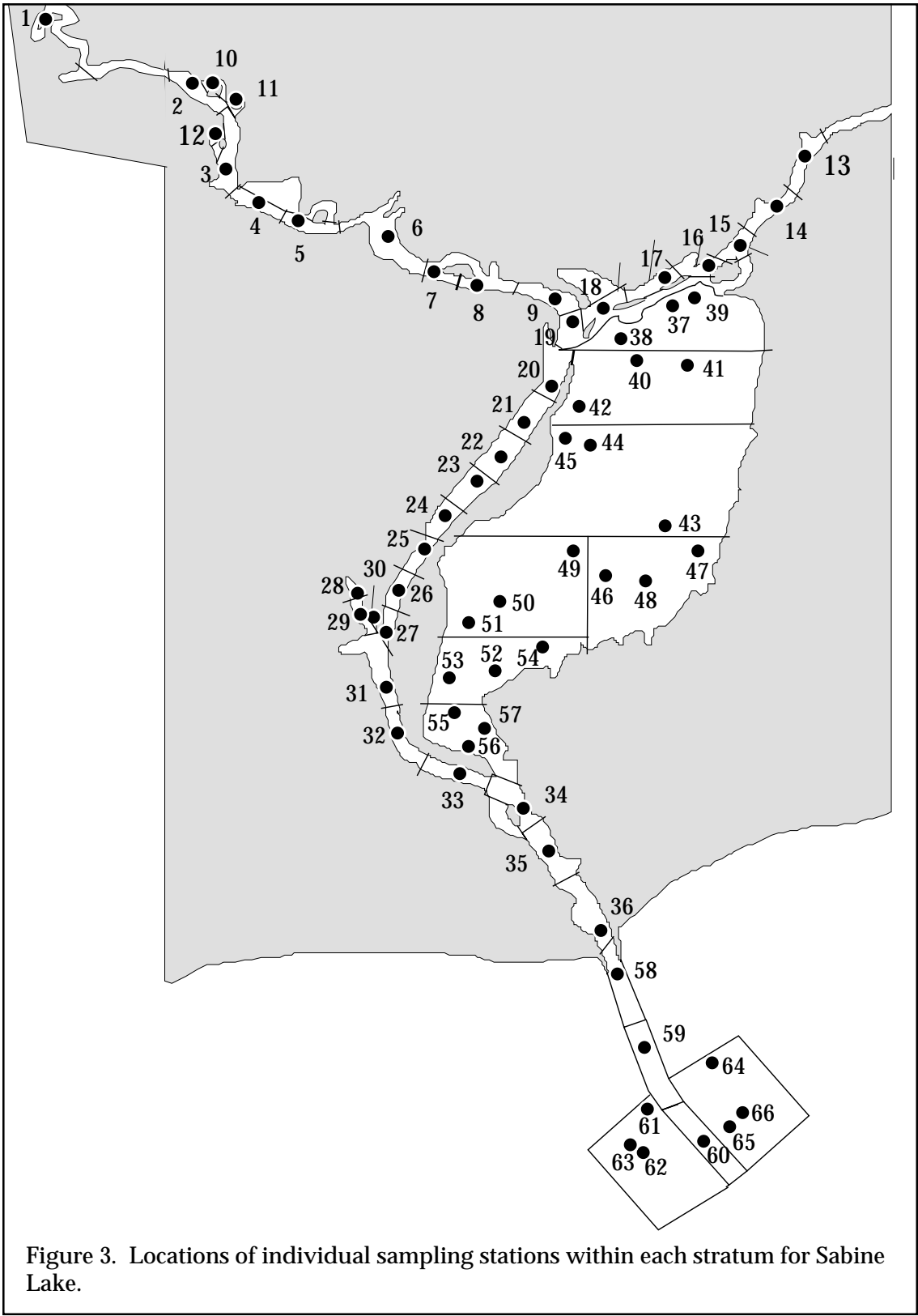


Figure 2. Locations and dimensions of sampling strata and sub-strata in Sabine Lake.



Sediments from a total of 66 stations were collected during August, 1995 with the NOAA vessel *Ferrel* and its launch. Each station was sampled only once.

Vessel positioning and navigation were aided with a differential-corrected, Global Positioning System (GPS) unit and a compensated LORAN C unit. The two systems generally agreed well when both were operational.

Samples for toxicity and chemical testing were collected with a Kynar-lined 0.1m² modified van Veen grab sampler (also, known as a Young grab) deployed with a hydraulic winch. The grab sampler and sampling utensils were acid washed with 10% HCl at the beginning of each day, and thoroughly cleaned with site water and acetone before each sample collection. Usually, 3 or 4 deployments of the sampler were required to provide a sufficient volume of material for the toxicity tests and chemical analyses. The upper 2-3 cm of the sediment were sampled with a plastic medical scoop and accumulated in a stainless steel pot. The pot was covered with a Teflon plate between deployments of the sampler to minimize sample oxidation and exposure to shipboard contamination. The material was carefully homogenized in the field with a stainless steel spoon before it was distributed to prepared containers for shipment to respective testing laboratories.

Samples for benthic community analyses were collected at one station randomly chosen within each stratum. Triplicate samples were collected at each station with a Young-modified, petite (0.413 cm²) van Veen grab. Samples for both toxicity/chemistry analyses and the benthic community analyses were collected at the same location. The entire contents of samples that were at least 5 cm deep were retained and sieved in the field with a 0.5 mm sieve. Material retained on the sieve was preserved in 10% buffered formalin with Rose Bengal. Samples were rejected if the jaws of the grab were open, if the sample was partly washed out or if the sample was less than 5 cm deep. A fourth sample was collected at each location and material retained for total organic carbon and grain size analyses.

Sample jars for each toxicity test and chemistry analysis were sealed to prevent leakage and outside contamination and shipped in ice chests packed with frozen water bottles or blue ice to the testing laboratories by overnight courier. Samples for toxicity tests were kept chilled until extractions or tests were initiated. Samples for chemical analyses and cytochrome P-450 tests were kept frozen until thawed for analyses. All samples were accompanied by chain of custody forms which included the date and time of the sample collection and station designation.

Locations, water depths, sampling dates, and visual sediment characteristics of the individual sampling stations for each sampling stratum are summarized in Appendix A. Multiple toxicity tests and complete chemical analyses were performed on all 66 sediment samples.

Laboratory toxicity tests.

Multiple toxicity tests were performed on aliquots of each sample to provide a weight of evidence. Tests were selected for which there were widely-accepted protocols, that would represent the toxicological conditions within different phases (partitions) of the sediments, and that would likely represent a range in response from physiological to acute lethal endpoints.

Amphipod survival test. The amphipod tests are the most widely and frequently used assays in sediment evaluations performed in North America. They are performed with subadult crustaceans exposed to relatively unaltered, bulk sediments. *Ampelisca abdita* has shown relatively little sensitivity to factors such as grain size, ammonia, and organic carbon in previous surveys. In previous surveys performed by the NS&T Program (Long et al., 1996), this test has provided wide ranges in responses among samples, strong statistical associations with elevated toxicant levels, and small within-sample variability.

Ampelisca abdita is a euryhaline benthic amphipod that ranges from Newfoundland to south-central Florida, and along the eastern Gulf of Mexico. Also, it is abundant in San Francisco Bay along the Pacific coast. The amphipod test with *A. abdita* has been routinely used for sediment toxicity tests in support of numerous EPA programs, including the Environmental Monitoring and Assessment Program (EMAP) in the Virginian, Louisianian, Californian, and Carolinian provinces (Schimmel et al., 1994).

Amphipod survival tests were conducted by Science Applications International Corporation, (SAIC) in Narragansett, R.I. All tests were initiated within 10 days of the date samples were collected. Samples were shipped by overnight courier in 4-liter high density polyethylene jugs which had been washed, acid-stripped, and rinsed with deionized water. Sample jugs were packed in shipping coolers with blue ice. Each was inspected to ensure it was within acceptable temperature limits upon arrival and then the jugs were stored at 4°C until testing was initiated. Prior to testing, sediments were mixed with a stainless steel paddle and press-sieved through a 1.0-mm mesh sieve to remove debris, stones, resident biota, etc.

Amphipods were collected by SAIC from tidal flats in the Pettaquamscutt (Narrow) River, a small estuary flowing into Narragansett Bay, RI. Animals were held in the laboratory in pre-sieved uncontaminated (“home”) sediments under static conditions. Fifty percent of the water in the holding containers was replaced every second day when the amphipods were fed. During holding, *A. abdita* were fed laboratory cultured diatoms (*Phaeodactylum tricorutum*). Control sediments were collected by SAIC from the Central Long Island Sound (CLIS) reference station of the U.S Army Corps of Engineers, New England Division. These sediments have been tested repeatedly with the amphipod survival test and other assays and found to be nontoxic (amphipod survival has exceeded 90% in 85% of the tests) and uncontaminated (Long et al., 1996). Sub-samples of the CLIS sediments were tested along with each series of samples from northern Puget Sound.

Amphipod testing followed the procedures detailed in the Standard Guide for conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods (ASTM, 1992). Briefly, amphipods were exposed to test and negative control sediments for 10 days with 5 replicates of 20 animals each under static conditions using filtered seawater. Aliquots of 200 mls of test or control sediments were placed in the bottom of one-liter test chambers, and covered with approximately 600 mL of filtered seawater (28-30 ppt). Air was provided by air pumps and delivered into the water column through a pipette to ensure acceptable oxygen concentrations. Pipettes were suspended in a manner to ensure that the sediments would not be disturbed.

Temperature was maintained at ~20°C by a temperature-controlled water bath. Lighting was continuous during the 10 day exposure period to inhibit emergence of the organisms

from the sediment, thereby maximizing the exposure of the amphipods to the test sediments. Information on temperature, salinity, dissolved oxygen, pH and ammonia in the test chambers was obtained during tests of each batch of samples to ensure compliance within acceptable ranges. Ammonia concentrations were determined in both pore waters (day 0 of the tests) and overlying waters (days 2 and 8 of the tests). Concentrations of the unionized form of ammonia were calculated, based upon measures of total ammonia and concurrent measures of pH, salinity and temperature.

Twenty healthy, active animals were placed in each test chamber, and monitored to ensure they burrowed into sediments. Non-burrowing animals were replaced. The jars were checked daily, and records kept of dead animals, and animals on the water surface, emerged on the sediment surface, or in the water column. Those on the water surface were gently freed from the surface film to enable them to burrow, and dead amphipods were removed.

Tests were terminated after ten days. Contents of each of the test chambers were sieved through a 0.5 mm mesh screen. The animals and any other material retained on the screen were examined under a stereomicroscope for the presence of amphipods. Total amphipod mortality was recorded for each test replicate.

A positive control (reference toxicant) test was used to document the sensitivity of each batch of test organisms. The positive control consisted of 96 hr water-only exposures to sodium dodecyl sulfate (SDS). LC50 values were calculated for each test run using results from tests of five SDS concentrations. Control charts provided by SAIC showed consistent results in tests of both the positive and negative controls.

Sea urchin fertilization test. Tests of sea urchin fertilization have been used in assessments of ambient water and effluents and in previous NS&T Program surveys of sediment toxicity (Long et al., 1996). Test results have shown wide ranges in responses among test samples, excellent within-sample homogeneity, and strong associations with the concentrations of toxicants in the sediments. This test combines the features of testing sediment pore waters (the phase of sediments in which dissolved toxicants are highly bioavailable) and exposures to early life stages of invertebrates (sperm cells) which often are more sensitive than adult forms. Tests of sediment pore water toxicity were conducted with the urchin *Arbacia punctulata* by the U.S. Geological Survey laboratory in Corpus Christi, Texas.

Sediments from each sampling location were shipped by overnight courier in one-gallon high density polyethylene jugs chilled in insulated coolers packed with blue ice. Upon arrival at the laboratory, samples were either refrigerated at 4°C or processed immediately. All samples were processed (i.e., pore waters extracted) within 10 days of the sampling date.

Pore water was extracted from sediments with a pressurized squeeze extraction device (Carr and Chapman, 1992). After extraction, porewater samples were centrifuged in polycarbonate bottles (@1200 g for 20 minutes) to remove any particulate matter. The supernatant was then frozen @-20°C. Two days before the start of a toxicity test, samples were transferred from a freezer to a refrigerator kept at 4° C, and one day prior to testing, thawed

in a tepid (20°C) water bath. Experiments performed by USGS have demonstrated no effects upon toxicity attributable to freezing and thawing of the pore water samples (Carr and Chapman, 1992).

Tests followed the methods of Carr and Chapman (1992) and USGS SOP F10.6, developed for *Arbacia punctulata*. Spawning was induced by a mild electrical shock. Test temperatures of the pore waters, the dilution waters and the tests themselves were maintained at 20±2° by incubation of in an environmental chamber. Adult *A. punctulata* were obtained from Gulf Specimen Co., Panacea, FL. Pore water from sediments collected in Redfish Bay, Texas, an area located near the testing facility, were used as negative (nontoxic) controls. Sediment pore waters from this location have been determined to be nontoxic in this test in repeated trials (Long et al., 1996). Porewater sample salinity was measured and adjusted to 30±1 ppt, if necessary, using either purified de-ionized water or concentrated brine. Following these adjustments of salinity, each of the pore water samples was tested in a dilution series of 100%, 50%, and 25% of the salinity – adjusted sample with 5 replicates per treatment. Dilutions were made with clean, filtered (0.45 μ m), Port Aransas laboratory seawater, which has been shown in previous trials to be nontoxic. A dilution series test with SDS was included as a positive control.

In addition to salinity, other water quality measurements were made for dissolved oxygen, pH, sulfide and total ammonia. Temperature and dissolved oxygen were measured with YSI meters; salinity was measured with Reichert or American Optical refractometers; pH, sulfide and total ammonia (expressed as total ammonia nitrogen, TAN) were measured with Orion meters and their respective probes. The concentrations of unionized ammonia (UAN) were calculated using TAN, salinity, temperature, and pH values.

For the sea urchin fertilization test, 50 μ L of appropriately diluted sperm were added to each vial, and incubated at 20±2°C for 30 minutes. One ml of a well mixed dilute egg suspension was added to each vial, and incubated an additional 30 minutes at 20± 2°C. Two mls of a 10% solution of buffered formalin solution was added to stop the test. Eggs with fertilization membranes were counted, and fertilization percentages calculated for each replicate test.

Microbial bioluminescence (Microtox™) tests. This is a test of the relative toxicity of sediment extracts prepared with an organic solvent. Therefore, it is immune to the effects of many environmental factors, such as grain size, ammonia and organic carbon. Organic toxicants, and to a lesser degree trace metals, that may or may not be readily bioavailable are extracted with the organic solvent. Therefore, this test can be considered as indicative of the potential toxicity of mixtures of substances bound to the sediment matrices. In previous NS&T Program surveys, the results of Microtox tests have shown extremely high correlations with the concentrations of mixtures of organic compounds. Microtox tests were run by the U.S. Geological Survey laboratory in Columbia, Mo, on dichloromethane (DCM) extracts prepared by ABC Laboratories. Final volume adjustments were made with the carrier solvent dimethylsulfoxide (DMSO). The organic extracts were tested at concentrations of 1.5 to 50 mg equivalent wet wt/mL.

The Microtox® assays were performed with dichloromethane (DCM) extracts of sediments following the basic procedures used in testing sediments from Pensacola Bay and elsewhere (Johnson and Long, 1998). All sediment samples were stored in the dark at 4°C for 5-10 days before processing was initiated. A 3-4 g sediment sample from each station was weighed, recorded, and placed into a DCM rinsed 50 mL centrifuge tube. A 15 g portion of sodium sulfate was added to each sample and mixed. Pesticide grade DCM (30 ml) was added and mixed in. The mixture was shaken for 10 seconds, vented and tumbled overnight.

Sediment samples were allowed to warm to room temperature and the overlying water discarded. They were then homogenized with a stainless steel spatula, and 15-25 grams of sediment from each were transferred to a centrifuge tube. The tubes were spun at 1000 g for 5 min. and the pore water was removed using a Pasteur pipette. Three replicate 3-4 g sediment sub-samples were placed in separate mortars each containing a 15 g portion of sodium sulfate and mixed. After 30 min sub-samples were ground with a pestle until dry and added to 50 mL centrifuge tubes. Then, 30 mL of DCM were added to each tube and shaken to dislodge sediments. Tubes were then shaken overnight on an orbital shaker at a moderate speed. The tubes were then centrifuged at 500 G for 5 min and the sediment extracts transferred to Turbovap™ tubes. Then, 20 mL of DCM was added to sediment, shaken by hand for 10 sec and spun at 500 g for 5 min. The previous step was repeated and all three extracts were combined in the Turbovap™ tube. Sample extracts were then placed in the Turbovap™ and reduced to a volume of 0.5 mL. The sides of the Turbovap™ tubes were then rinsed down with methylene chloride and again reduced to 0.5 mL. Then, 2.5 mL of dimethylsulfoxide (DMSO) were added to the tubes which were returned to the Turbovap™ for an additional 15 min. Sample extracts were then placed in clean vials and 2.5 mL of DMSO were added to obtain a final volume of 5 mL DMSO.

A suspension of luminescent bacteria, *Vibrio fischeri*, (Azur Environmental, Inc.) was thawed and hydrated with toxicant-free distilled water, covered and stored in a 4°C well on the Microtox analyzer. An aliquot of 10 μ L of the bacterial suspension was transferred to a test vial containing the standard diluent (2% NaCl) and equilibrated to 15°C using a temperature-controlled photometer. The amount of light lost per sample was proportional to the toxicity of that test sample. To determine toxicity, each sample was diluted into four test concentrations. Percent decrease in luminescence of each cuvette relative to the reagent blank was calculated. Light loss was expressed as a gamma value and defined as the ratio of light lost to light remaining.

Because organic sediment extracts were obtained with DCM, a strong nonpolar solvent, the final extract was evaporated and redissolved in DMSO. DMSO is compatible with the Microtox system because of its low test toxicity and good solubility with a broad spectrum of apolar chemicals (Johnson and Long, 1998). The logs of gamma values from these four dilutions were plotted and compared with the log of the samples' concentrations. The concentrations of the extract that inhibited luminescence by 50% after a 5-min exposure period, the EC50 value, was determined and expressed as mg equivalent sediment wet weight. Data were reduced using the Microtox Data Reduction software package. All EC50 values were average 5-min readings with 95% confidence intervals for three replicates.

A negative control (extraction blank) was prepared using DMSO, the test carrier solvent. A phenol standard (45 mg/L phenol) was run after reconstitution of each vial of freeze-dried *V. fischeri*. Tests of extracts of sediments from the Redfish Bay, Texas, site used in the urchin tests also were used as negative controls in the Microtox tests.

Cytochrome p-450 RGS assays.

All samples were analyzed by the P-450 reporter gene system (RGS) assay, which uses human liver cells to measure luciferase production in response to activation of CYP1A1 promoter sequences. This assay is responsive to the presence of mixed-function oxidase inducers such as dioxins, furans, high molecular weight PAHs, and coplanar PCBs in tissues and sediments (Anderson et al., 1995). Therefore, the RGS assay provides an estimate of the presence of contaminants bound to sediment that could produce chronic and/or carcinogenic effects in benthic biota and/or demersal fishes that feed in sediments. These tests were run by the Columbia Analytical Services, Inc. in Carlsbad, CA with solvent extracts prepared by their laboratory in Kelso, WA.

In these tests, standard protocols (Anderson et al., 1996; ASTM, 1997; APHA, 1996) were followed to ensure comparability with data derived for other areas. Approximately, 20 g of sediment from each station were extracted by EPA method 3550 to produce 2 mL of dichloromethane (DCM) extracts. This solvent was exchanged into DMSO, which is less volatile and less toxic to the test cells. Small portions (5 to 15 μ L) were applied to approximately one million human liver cells contained in three replicate wells with 2 mL of culture medium. After 16 hours of incubation (exposure), the cells were washed, then lysed, and the solution centrifuged to remove cell debris. Small portions (50 μ L) of the cell extracts were used in measures of luminescence in relative light units (RLU). Solvent blanks and the reference toxicants (2, 3, 7, 8 – dioxin and benzo[a]pyrene) were tested with each batch of samples.

Enzyme induction of the standards and samples was calculated (normalized) by dividing the mean RLU by the mean RLU produced by the solvent blank. The running average fold induction for dioxin at 3.1 nM (1ng/mL) is approximately 100 and that from 1 μ g/mL of benzo(a)pyrene (b[a]p) is 60 fold. A standard mixture of dioxins and furans, measured as Toxic Equivalency (TEQ), produces a RGS fold induction equal to the TEQ in pg/mL. Because the primary organic contaminants identified in previous marine studies have been PAHs, the RGS data were converted to benzo[a]pyrene equivalents (B[a]pEq) by multiplying the fold induction from the applied extract (e.g., 15 μ L) by a factor (133.3) to determine the total in the 2 mL extract, and then dividing by the dry weight of the samples, and 60(=1 μ g/gB[a]P). To convert the data to a TEQ(dioxins and furans) in pg/g, the calculations would be the same, except the factor of 60 would not be used. Tests were conducted with clean extracts spiked with TCDD and B[a]P to ensure compliance with the results of previous tests.

Chemical analyses – metals.

Chemical analyses of all samples were performed on all 66 samples by the analytical laboratory at Texas A&M University/Geochemical and Environmental Research Group (TAMU/GERG) in College Station, Texas on all 66 samples. All analytical methods conformed with performance-based analytical protocols and employed quality-assurance steps

of the NS&T Program (Lauenstein and Cantillo, 1993; 1998); including instrument calibration, use of internal standards, replication of some analyses, percent recoveries of spiked blanks, and analyses of standard reference materials.

Grain size was determined by the standard pipette method following sieving to remove the sand and gravel fractions. TOC was determined using a Leco Carbon Analyzer. Sediment samples were digested for final analysis by procedures specific to the instrument method used. Various concentrating and trapping techniques were used for selected analytes. The analysis for mercury was performed by cold vapor atomic absorption. Analyses for tin, arsenic, selenium, silver, and cadmium were performed by graphite furnace atomic absorption spectroscopy. All other metals concentrations were determined by flame atomic absorption spectroscopy and reported on a dry weight basis. Method detection limits (MDLs) attained in the analyses are listed in Table 1. Acid-volatile sulfide/simultaneously-extracted metals analyses were not performed.

Table 1. Trace metals measured in Sabine Lake sediments and method detection limits (MDLs).

<u>Parameter</u>	<u>Method Detection Limit</u> (ppm, based on dry weight)	<u>Analytical Method *</u>
Aluminum	440	FAA
Iron	40	FAA
Manganese	5.0	FAA
Arsenic	0.3	GFAAS
Cadmium	0.008	GFAAS
Chromium	0.1	GFAAS
Copper	0.44	GFAAS
Lead	0.35	GFAAS
Mercury	0.007	CVAA
Nickel	0.7	GFAAS
Selenium	0.2	GFAAS
Silver	0.03	GFAAS
Tin	0.1	GFAAS
Zinc	2.2	FAA

FAA = Flame atomic absorption spectroscopy;
 GFAAS = Graphite furnace atomic absorption spectroscopy
 CVAA = Cold vapor atomic absorption.

Chemical analyses – organic compounds.

The analytes determined in the organic analyses are listed in **Table 2**, along with their respective MDLs. Sediment samples for organic analysis were prepared by NaSO₄ drying, methylene chloride extraction, purified by silica gel/alumina chromatography and concentration. Quantification was performed using the internal standards method. Polycyclic aromatic hydrocarbons (PAHs) were analyzed by gas chromatography with a mass selective detector in the selective ion mode. Sediment samples to be analyzed for butyltins were dried with NaSO₄ and extracted with methylene chloride containing 2% tropolone, hexylated, purified by silica gel chromatography, and concentrated. Butyltins were detected by gas chromatography with

a tin selective flame photometric detector. Polychlorinated biphenyls and chlorinated pesticides were determined by gas chromatography/electron capture detection. Concentrations of sediment organic compounds are reported on a dry weight basis.

Table 2. Organic compounds measured in Sabine Lake sediments and method detection limits (MDLs).

<u>Parameter</u>	<u>MDL</u> (ng/g dry)	<u>Parameter</u>	<u>MDL</u> (ng/g dry)
2,4'Dichloro Diphenyl Ethylene (O,P'DDE)	0.28	Naphthalene	0.5
4,4'Dichloro Diphenyl Ethylene (P,P'DDE)	0.85	C1-Naphthalenes	
2,4'Dichloro Diphenyl Dichloroethylene (O,P'DDD)	0.13	C2-Naphthalenes	
4,4'Dichloro Diphenyl Dichloroethylene (P,P'DDD)	0.51	C3-Naphthalenes	
2,4'Dichloro Diphenyl Trichloroethylene (O,P'DDT)	0.25	C4-Naphthalenes	
4,4'Dichloro Diphenyl Trichloroethylene (P,P'DDT)	0.24	1- Methylnaphthalene	0.8
Aldrin	0.25	2- Methylnaphthalene	0.8
Cis-Chlordane	0.66	2,6-Dimethylnaphthalene	2.4
Oxychlordane		2,3,5- Trimethylnaphthalene	2.4
Alpha-Chlordane	0.23	Acenaphthalene	3.7
Trans-Nonachlor	0.1	Acenaphthylene	4.5
Cis-Nonachlor		Fluorene	2.5
Dieldrin	0.16	C1-Fluorenes	
Heptachlor	0.2	C2-Fluorenes	
Heptachloro-Epoxyde	0.16	C3-Fluorenes	
Hexachlorobenzene	0.37	Phenanthrenes	0.5
Alpha-Benzene Hexachloride (HCH)		C1-Phenanthrenes	
Beta-Benzene Hexachloride (HCH)		C2-Phenanthrenes	
Lindane (Gamma-Benzene Hexachloride-HCH)	0.22	C3-Phenanthrenes	
Delta-Benzene Hexachloride (HCH)	0.17	C4-Phenanthrenes	
Endrin		1- Methylphenanthrene	0.6
Mirex	0.08	Anthracene	4.1
Polychlorinated Biphenyls		Fluoranthene	0.4
PCB#8 (CL2)	0.08	Pyrene	3.1
PCB#18 (CL3)	0.25	Indeno-1,2,3-c,d-Pyrene	1.6
PCB#28 (CL3)	0.09	Dibenzothiophene	
PCB#44 (CL4)	0.09	C1-Dibenzothiophenes	
PCB#52 (CL4)	0.09	C2-Dibenzothiophenes	
PCB#66 (CL4)	0.14	C3-Dibenzothiophenes	
PCB#101 (CL5)	0.13	C1- Fluoranthene Pyrene	
PCB#105 (CL5)	0.1	Benzo-a-Anthracene	1.4
PCB#110/77 (CL5/4)	*	Chrysene	0.5
PCB#118/108/149 (CL5/5/6)	0.12	C1-Chrysenes	
PCB#128 (CL6)	0.13	C2-Chrysenes	
PCB#138 (CL6)	0.18	C3-Chrysenes	
PCB#126 (CL6)	*	C4-Chrysenes	
PCB#153 (CL6)	0.12	Benzo-b-Fluoranthene	1.8
PCB#170 (CL7)	0.81	Benzo-k-Fluoranthene	1.9
PCB#180 (CL7)	0.16	Benzo-a-Pyrene	1.2
PCB#187/182/159 (CL7/7/6)	0.14	Benzo-e-Pyrene	2.4
PCB#195 (CL8)	0.25	Perylene	3.3
PCB#206 (CL9)	0.09	Benzo-g,h,i-Perylene	0.3
PCB#209 (CL10)	0.78	Dibenzo-a,h-Anthracene	2.6
Biphenyl	2.4		

Chemistry QA/QC.

Quality assurance/quality control (QA/QC) procedures included analyses of duplicates, standard reference materials, and spiked internal standards. In the organic analyses, internal

standards were added at the start of the procedure and carried through the extraction, cleanup, and instrumental analysis steps and used to determine the concentrations of analytes. The following specific quality assurance steps were used to insure measurement accuracy and precision:

1. Trace and major metals: Two method blanks and three standard reference materials were run with each set of no more than 30 samples.
2. Physical/chemical measurements: Grain size duplicates were run every 20 samples. For TOC, one method blank, one duplicate, and one standard reference material were run every 20 samples.
3. Pesticides, PCBs and PAHs: One procedural blank, one matrix spike, one duplicate spike and one standard reference material were run with each batch of no more than 20 samples. Internal standard recoveries were tracked.

Benthic community.

Benthic community samples were collected at one-third of the station with a petite Ponar grab sampler. Results of these analyses will be reported in a separate technical report.

Statistical methods.

Several statistical methods were used to identify the significance of the toxicity tests, estimate spatial scales in toxicity and contamination, identify relationships between measures of toxicity and contamination, and identify chemicals of greatest concern.

Amphipod survival tests. Data from each station in which mean percent survival was less than that of the control were compared to the Central Long Island Sound control using a one-way, unpaired t-test ($\alpha = 0.05$) assuming unequal variance. Data were not transformed since examination of data from previous tests have shown that *A. abdita* percentage survival data meet the requirements for normality.

Significant toxicity for *A. abdita* is defined here as survival statistically less than that in the performance control ($\alpha = 0.05$). In addition, samples in which survival was significantly less than controls and less than 80% of CLIS control values were regarded as “highly toxic”. The 80% criterion is based upon statistical power curves created from SAIC’s extensive testing database with *A. abdita* (Thursby et al., 1997) that show that the power to detect a 20% difference from the control is approximately 90%. The minimum significant difference (i.e., “MSD” <80% of control response) was used as the critical value in calculations of the spatial extent of toxicity (Long et al., 1996).

Sea urchin fertilization tests. For the sea urchin fertilizations, statistical comparisons among treatments were made using ANOVA and Dunnett’s one-tailed *t*-test (which controls the experiment-wise error rate) on the arcsine square root transformed data with the aid of SAS (SAS, 1992). The trimmed Spearman-Kärber method (Hamilton et al., 1977) with Abbott’s correction (Morgan, 1992) was used to calculate EC₅₀ (50% effective concentra-

tion) values for dilution series tests. Prior to statistical analyses, the transformed data sets were screened for outliers (Moser and Stevens, 1992). Outliers were detected by comparing the studentized residuals to a critical value from a t-distribution chosen using a Bonferroni-type adjustment. The adjustment is based on the number of observations (n) so that the overall probability of a type 1 error is at most 5%. The critical value (CV) is given by the following equation: $cv = t(df_{Error}, .05/[2 \times n])$. After omitting outliers but prior to further analyses, the transformed data sets were tested for normality and for homogeneity of variance using SAS/LAB Software (SAS, 1992). Statistical comparisons were made with mean results from the Redfish Bay controls. Reference toxicant concentration results were compared to filtered seawater controls and each other using both Dunnett's t-test and Duncan's multiple range test to determine lowest observable effects concentrations (LOECs) and no observable effects concentrations (NOECs).

In addition to the Dunnett's one-tailed t-tests, data from field-collected samples were treated with an analysis similar to the MSD analysis used in the amphipod tests. Power analyses of the sea urchin fertilization data have shown MSDs of 15.5% for $\alpha = 0.05$ and 19% for $\alpha = 0.01$. However, to be consistent with the statistical methods used in previous surveys (Long et al., 1996), estimates of the spatial extent of toxicity were based upon the same critical value used in the amphipod tests (i.e., <80% of control response).

Microtox tests. Microtox data were analyzed using the computer software package developed by Microbics Corporation to determine concentrations of the extract that inhibit luminescence by 50% (EC50). This value was then converted to mg dry wt. using the calculated dry weight of sediment present in the original extract. To determine significant differences of samples from each station, pair-wise comparisons were made between survey samples and results from Redfish Bay control sediments using analysis of variance (ANOVA). Concentrations tested were expressed as mg dry wt based on the percentage extract in the 1 ml exposure volume and the calculated dry wt of the extracted sediment. Statistical comparisons among treatments were made using ANOVA and Dunnett's one-tailed t-tests on the log transformed data with the aid of SAS (SAS, 1992).

Two additional critical values were generated for the Microtox test results, both based upon statistical analyses of the existing data from NOAA surveys conducted thus far (n=1013). The two critical values are <0.06 mg/ml and <0.51 mg/mL. The first value (0.06 mg/mL) represented the 90% lower prediction limit (LPL) of the entire data set. The probability that a future observation from this data distribution would be less toxic (i.e., have a greater EC50 value) than 0.06 mg/ml would be 90%. Therefore, a sample with an EC50 less than 0.06 mg/mL would be considered to be extremely toxic in this test, but should occur rarely. The second value (0.51 mg/mL) represents the 80% LPL following removal of the lowest (most toxic) 10% of the data values from the database to eliminate the affects of these extremely toxic samples upon the distribution of the data.

Cytochrome P-450 RGS tests. Microsoft Excel 5.0 was used to determine the mean RGS response and the 99% confidence interval of the b[a]p equivalent values for all 66 samples. Two critical values were calculated and used to estimate spatial extent of toxicity in this test. The first value, 37.1 ug/g benzo(a)pyrene equivalents, represented the upper 90% prediction limit (UPL) of the entire data set gathered thus far in all NOAA studies (n=530).

This value agrees well with 32 $\mu\text{g/g}$, the RGS induction level equivalent to the ERL value (Long et al., 1995) for high molecular weight PAHs determined in regression analyses of the existing data for this test. Therefore, this value is viewed as a concentration above which toxicologically significant effects may begin in sediments. The second value, 11.1 $\mu\text{g/g}$, was the 80% UPL of the data distribution following elimination of the data above the 90th percentile of the entire data base. This value (11.1 $\mu\text{g/g}$) is viewed as the upper limit of background RGS responses.

Spatial patterns in toxicity Spatial patterns in toxicity were illustrated by plotting toxicity data on base maps of each major region. The incidence of toxicity was determined by dividing the numbers of samples identified as significantly different from controls or “highly toxic” by the total numbers of samples ($n=66$) tested.

Estimates of the spatial extent of toxicity were determined with cumulative distribution functions in which the toxicity results from each station were weighted to the dimensions (km^2) of the sampling stratum in which the samples were collected (Schimmel et al., 1994). The size of each stratum (km^2) was determined by use of an electronic planimeter applied to navigation charts, upon which the boundaries of each stratum were outlined. Stratum sizes were calculated as the averages of three trials, all of which were within 10% of each other. A critical value of less than 80% of control response was used in the calculations of the spatial extent of toxicity for all tests. That is, the sample-weighted sizes of each stratum in which toxicity test results were less than 80% of control responses were summed to estimate the spatial extent of toxicity. In addition, the critical values described above for the Microtox and RGS assays were used in these analyses.

Spatial patterns in chemical concentrations. Chemical data from the sample analyses were plotted on base maps to identify spatial patterns in concentrations. Maps were prepared for several chemicals representative of the different chemical classes. The spatial extent of contamination was determined with cumulative distribution functions in which the sizes of strata in which samples exceeded effects-based, numerical guidelines were summed, using the Effects Range-Median (ERM) values of Long et al. (1995).

Chemistry/toxicity relationships. Chemistry/toxicity relationships were determined in a multistep sequence. First, nonparametric, Spearman-rank correlations were determined (Statview software) for each toxicity test and each physical/chemical variable. The correlation coefficients (ρ) and their statistical significance (p values) were recorded and compared among chemicals to identify which chemicals co-varied with toxicity and which did not.

Second, for those chemicals in which a significant correlation was observed, the data were examined in scatterplots to determine if there was a reasonable pattern of increasing toxicity with increasing chemical concentration and if any chemical in the toxic samples equalled or exceeded published numerical guidelines. In this step, chemical concentrations in the scatterplots were compared with the ERM values to determine which samples, if any, were both toxic and had elevated chemical concentrations. The concentrations of unionized ammonia were compared to lowest observable effects concentrations (LOEC) determined for the sea urchin tests by the USGS (Carr et al., 1995) and no observable effects concentrations (NOEC) determined for amphipod survival tests (Kohn et al., 1994).

Third, the numbers (and percentages) of samples out of those that were analyzed that exceeded the respective guidelines were determined. The combined results of these steps were examined to determine which chemical(s), if any, may have contributed to the observed toxicity and which probably had a minor or no role in toxicity.

Correlations were determined for all the substances that were quantified, including total (bulk) trace metals, metalloids, unionized ammonia (UAN), percent fines, total organic carbon (TOC), chlorinated organic hydrocarbons (COHs), and polynuclear aromatic hydrocarbons (PAHs). In addition, a chemical index calculated as the sums of quotients formed by dividing the chemical concentrations in the samples by their respective ERM values were compared to the results of the toxicity tests. Those substances that showed significant correlations were indicated with asterisks (*for $p < 0.05$, ** for $p < 0.01$, ***for $p < 0.001$, and ****for $p < 0.0001$).

In correlation analyses involving a large number of variables such as in this survey, some correlations could appear to be significant by random chance alone. Statistical adjustments often are needed to account for this possibility. Accordingly, readers should note that in the results tables only those correlations shown with either three or four asterisks would remain significant if the number of variables (50) were taken into account in these analyses. However, the correlation coefficients are shown without these adjustments.

RESULTS

Severity and spatial patterns in toxicity.

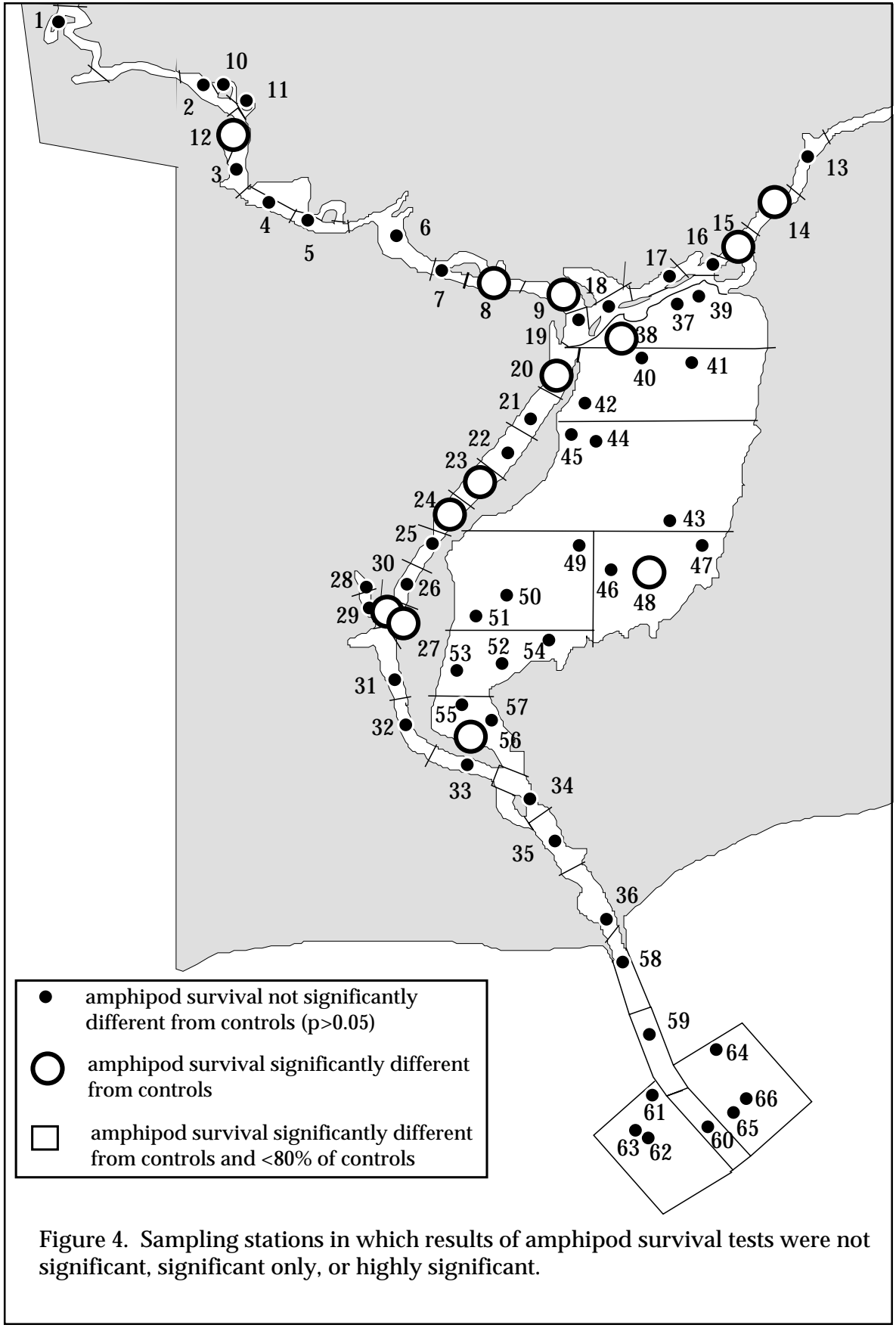
Results of the amphipod, sea urchin, Microtox, and cytochrome P-450 RGS tests are summarized for each sampling station in Table 3. Except in the case of the RGS assay, results are expressed as percentages of negative controls. RGS assay results are expressed as μg benzo(a)pyrene equivalents/g sediment.

Mean amphipod survival among the 66 samples ranged from 85% to 110% of negative controls. Amphipod survival was reduced significantly relative to controls in 13 samples; however, mean survival exceeded 80% of controls in all samples. Microtox test results were significantly different from controls in 52 samples and sample means ranged from 1.4% to 210% of controls. EC50 values were less than 0.51 mg/mL (indicating moderate loss in metabolic activity) in three samples. None of the mean EC50 values were less than 0.06 mg/mL, which would have indicated a severe response. In the sea urchin tests, mean percent fertilization was significantly reduced in 15 samples, 6 samples, and none of the samples in tests performed with 100%, 50%, and 25% pore water concentrations, respectively. In tests of 100% pore water, percent fertilization ranged from 4% to 108% of controls. Percent normal development was significantly reduced in 62 samples, 45 samples, and one of the samples in tests of 100%, 50%, and 25% pore water concentrations, respectively. Cytochrome P-450 RGS assay results ranged from 1.0 to 104 $\mu\text{g/g}$; responses exceeded 11.1 $\mu\text{g/g}$ in 21 samples (indicating moderate induction) and exceeded 37.1 $\mu\text{g/g}$ in 6 samples (indicating high induction).

The 13 samples in which mean amphipod survival was significantly less than controls are illustrated in Figure 4. Results were significant in only three stations within the lake

Table 3. Summary of toxicity test results for 66 sediment sampling stations in Sabine Lake.

Stratum Sta.	Ampelisca abdita %survival		Microtox (EC50)		Arbacia punctulata %fertilization (100%)		Arbacia punctulata %fertilization (50%)		Arbacia punctulata %fertilization (25%)		Arbacia punctulata %normal development (100%)		Arbacia punctulata % normal development (50%)		Arbacia punctulata % normal development (25%)		P450 BaP EQ(ug/g)
	Mean	% of ctrl	Mean	% of ctrl	Mean	% of ctrl	Mean	% of ctrl	Mean	% of ctrl	Mean	% of ctrl	Mean	% of ctrl	Mean	% of ctrl	
A 1	88	96	0.45	1.46 S	94.2	108	93.8	104	94.8	110	0	0 S	91.2	100	91.2	100	6.21
A 2	93	101	0.71	2.31 S	72.8	83 S	91.2	101	92.6	107	0	0 S	93.8	103	93.8	103	61.10
A 3	88	96	0.68	2.21 S	81.6	93	97.6	108	91	105	0	0 S	80.8	89	80.8	89	63.45
B 4	90	98	0.9	2.93 S	89.6	102	94.6	105	88	102	0	0 S	91.8	101	91.8	101	15.20
B 5	83	90	5.33	17.34 S	84.6	97	84.6	94	78	90	89	101	90.8	97	90.4	99	6.51
B 6	88	96	34.07	110.87 S	91.2	104	91.4	101	95.8	111	0	0 S	89.8	99	89.8	99	12.44
C 7	87	94	7.67	24.96 S	89.4	102	86	95	79.6	92	84.2	96	87.6	94	92.4	102	20.85
C 8	83	89 S	23.07	75.07 S	85.6	98	87.4	97	86.4	100	0	0 S	92.6	102	92.6	102	20.92
C 9	85	91 S	10.7	34.82 S	91	104	90.2	100	86.2	100	0	0 S	79.2	85	89.2	98	28.18
D 10	91	99	0.48	1.56 S	74.6	85 S	72.8	81 S	80.3	93	1.8	2 S	91.6	101	91.6	101	16.08
D 11	87	95	2.83	9.21 S	86.8	99	85.8	95	83.5	97	1.8	2 S	88.8	95	86.2	95	53.68
D 12	81	88 S	0.57	1.85 S	36.8	42 S	91	101	88	102	0.2	0 S	87.6	96	87.6	96	71.67
E 13	90	97	13.43	43.7 S	84.4	96	90.2	100	91.4	106	88.2	100	91.3	98	90.4	99	2.20
E 14	79	85 S	45.93	149.46 S	68.4	78 S	70.2	78 S	82.8	96	81.6	93	82	88	86	95	7.25
E 15	84	90 S	6.27	20.4 S	91.8	105	94.6	105	90.2	104	0.4	0 S	86.8	95	86.8	95	14.20
F 16	90	97	22.6	73.54 S	94.8	108	93.8	104	93.8	109	0	0 S	0.2	0 S	87.8	96	10.69
F 17	91	98	5.17	16.82 S	89.2	102	89.6	99	92.6	107	0	0 S	85.2	94	85.2	94	13.70
F 18	88	95	4.4	14.32 S	94.4	108	94.5	105	93.6	108	0.4	0 S	17.8	19 S	88	97	9.86
G 19	89	96	15.5	50.44 S	91.8	105	91.6	102	91.8	106	0	0 S	0	0 S	93	102	28.21
G 20	88	95 S	15.97	51.97 S	90.2	103	91.8	102	91.8	106	0.4	0 S	0.5	1 S	90.8	100	22.86
G 21	88	95	17.13	55.74 S	92.6	106	94.4	105	92	106	0.4	0 S	92.4	99	93.4	103	33.07
H 22	87	94	42.43	138.07 S	81	92	88.2	98	85.2	99	0	0 S	83	89	91.8	101	8.05
H 23	81	87 S	22.63	73.64 S	84.2	96	88.8	99	88.2	102	0.8	1 S	85.8	92	91.6	101	9.43
H 24	82	88 S	6.18	20.11 S	94	107	91.2	101	89.4	103	0	0 S	2.8	3 S	88.6	97	13.90
I 25	89	96	31.73	103.25 S	88	100	90.8	101	89.8	104	0	0 S	0	0 S	84	92	13.85
I 26	95	102	5.77	18.78 S	90.8	104	91	101	88.6	103	0	0 S	34	36 S	86	95	14.16
I 27	81	89 S	64.73	210.64 S	85.4	97	93	103	88.8	103	0	0 S	14	15 S	89.2	98	6.63
J 28	77	85	0.85	2.77 S	36.6	49 S	63.8	88	69.4	102	0	0 S	39.8	41 S	91.8	95	91.07
J 29	85	93	2.03	6.61 S	43	58 S	65.6	90	73.4	108	0	0 S	20.8	22 S	84	87	103.76
J 30	81	89 S	3.57	11.62 S	67.6	91	70.2	97	68.8	101	0	0 S	0	0 S	87.6	91	18.78
K 31	86	95	1.39	4.56 S	73.6	84	90.8	101	85.6	99	0	0 S	0	0 S	87	96	9.70
K 32	81	89	38.3	124.63 S	89	102	90.8	101	90.4	105	0	0 S	84.6	91	81.2	89	4.63



whereas survival was significantly reduced in 10 samples from the channels of the Intracoastal Waterway (ICW). Significantly reduced survival occurred in five of the samples from the Sabine-Neches Canal (stations 19 through 33). None of the stations in the lower portions of the ICW and entrance channel were significantly toxic in this test. Based upon the statistically determined criterion of <80% of control survival (Thursby et al., 1997), none of the samples was highly toxic in the amphipod tests.

As observed in the amphipod tests, toxicity in the urchin fertilization tests was scattered among the stations (Figure 5). None of the samples collected in Sabine Lake were toxic in the urchin fertilization tests. Several samples from the upper reaches of the Neches River (stations 1 – 9) were toxic in 100% pore water. One sample each from the Neches and Sabine rivers was toxic in both 100% and 50% pore water concentrations. Toxicity was much more apparent in samples from the Sabine Pass and entrance channel area (stations 34 - 35, and 58 – 66). However, none of the samples was toxic in 25% pore water concentrations.

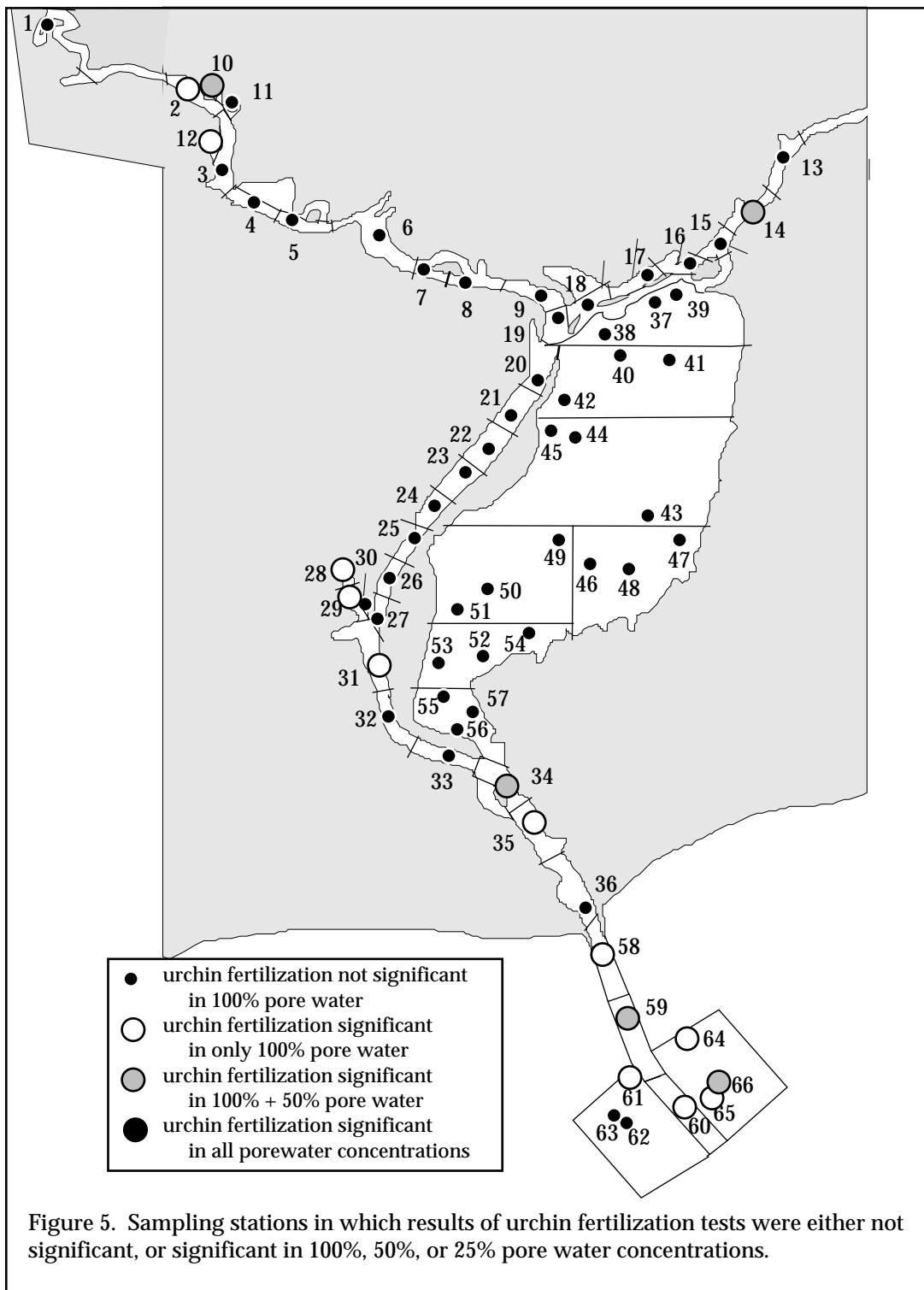
Toxicity was much more pervasive and severe as measured in the urchin embryo development tests (Figure 6) as compared to the other tests done with invertebrates. A large majority (45 of 66) of the samples were toxic in at least the 100% and 50% pore water concentrations. All samples seaward of the confluence of the Sabine-Neches canal and the Sabine Lake channel were toxic. The only sample in which a significant response was observed in 25% pore water was from an offshore station (number 63).

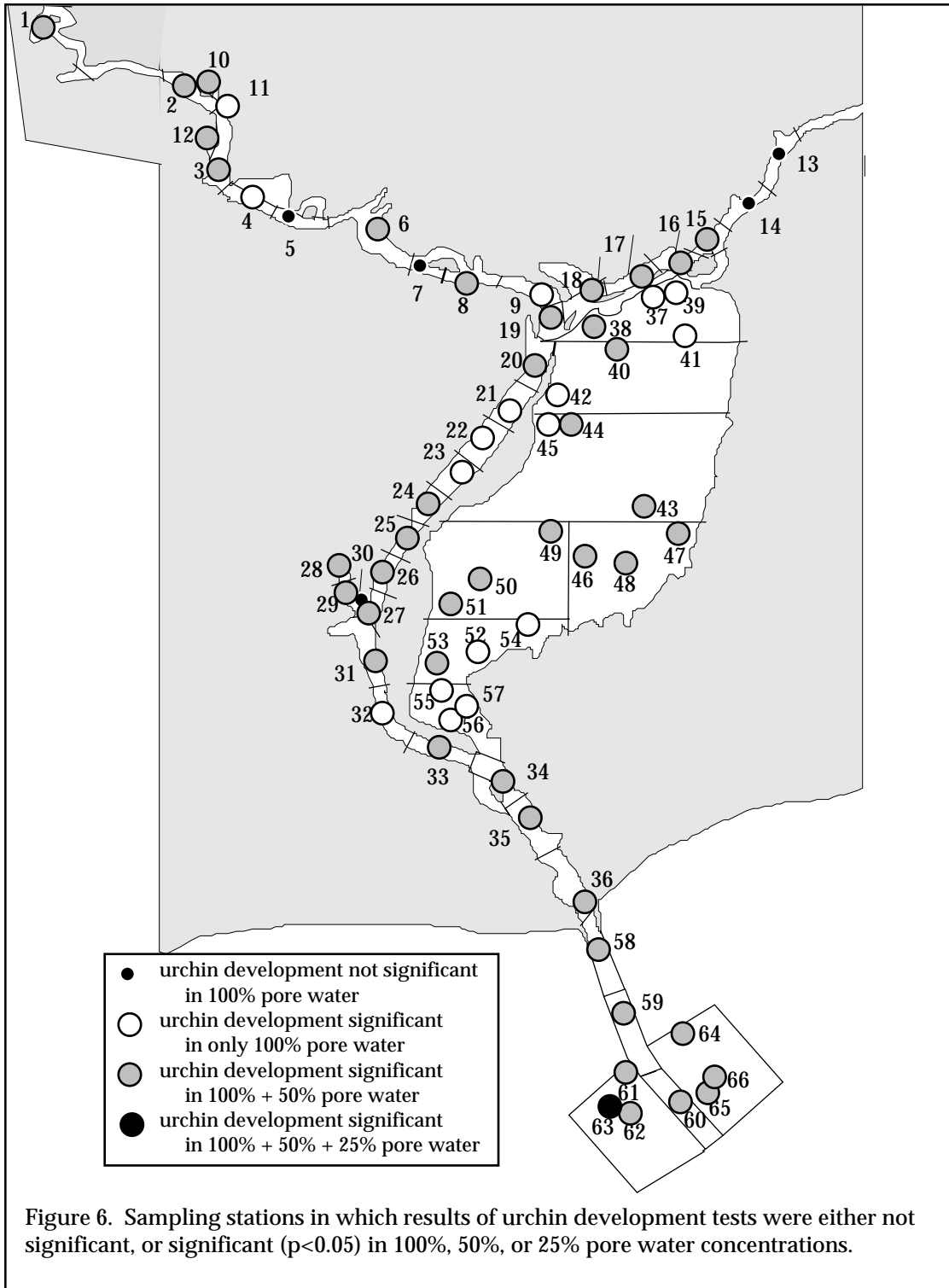
Microtox data are illustrated as histograms in Figure 7 to more clearly identify the spatial patterns in response. The data are shown as the ratio of the sample mean to the reference (Redfish Bay) mean; therefore, the toxic responses increased with the height of the bars. For example, the ratio between the sample mean for station 1 (EC50 = 0.45 mg/mL) and the reference mean (EC50 = 31 mg/mL) was 68, indicating a considerably smaller amount of extract caused a 50% reduction in bioluminescence. In contrast, the EC50 for station 6 was 34 mg/mL, resulting in a ratio to reference mean of 0.9. The data indicated there were three areas in which bioluminescence activity was reduced the most. These areas were the upper Neches River (stations 1- 4 and 10 – 12), the Taylor Basin and vicinity (stations 28 – 31), and the offshore area (stations 60 – 66). Sample means were less than 0.51 mg/ml at three stations (1, 10, and 65), indicating a moderate degree of response. Samples collected in Sabine Lake were among the least toxic.

Results of the RGS assays indicated that stations from the upper Neches River and Taylor Basin gave the highest induction responses (Figure 8); however, unlike the Microtox tests, the samples from the offshore stations did not cause relatively high responses. RGS assay results exceeded 37.1 ug/g in samples from four stations in the Neches River and two stations in Taylor Basin, indicating a relatively high level of response. As observed in the Microtox tests, samples collected in Sabine Lake were among the least toxic.

Spatial extent of toxicity.

The spatial extent of toxicity was estimated by weighting the data from the toxicity tests to the sizes of the sampling strata and calculating a sum of the strata sizes in which toxic responses were observed. Critical values (or criteria) for defining samples as “toxic” are listed for each test. The estimates are summarized in Table 4.





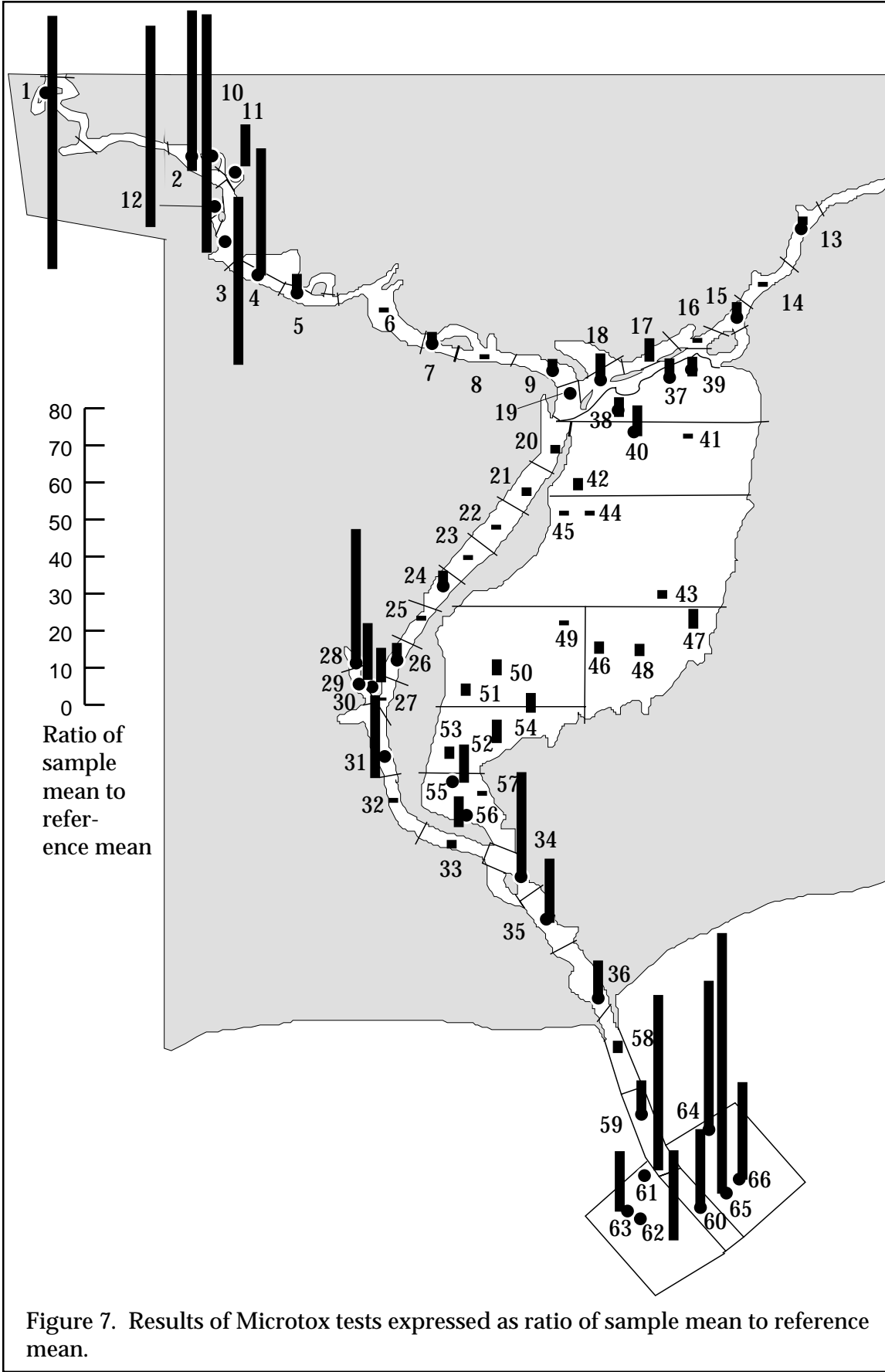


Figure 7. Results of Microtox tests expressed as ratio of sample mean to reference mean.

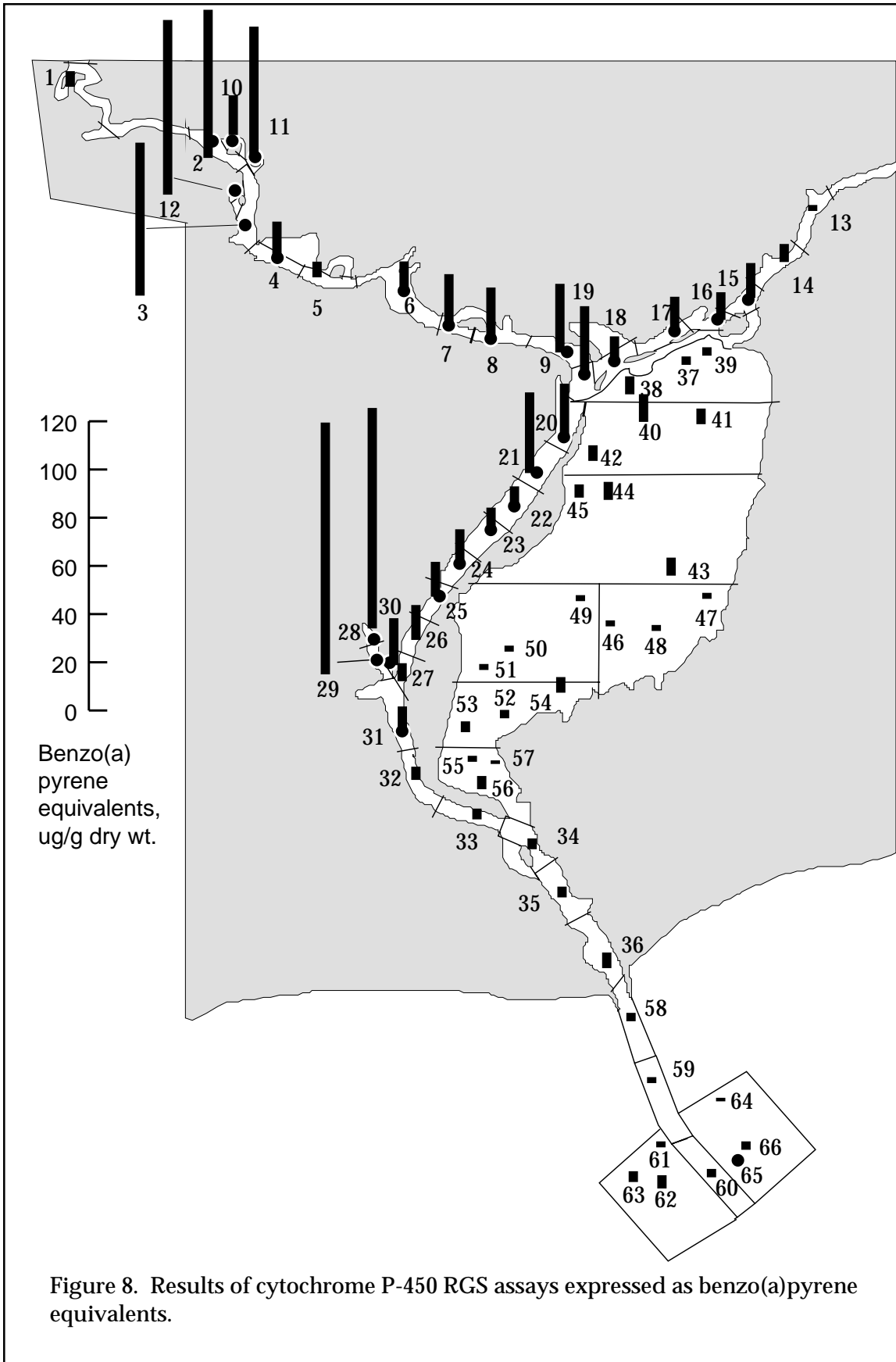


Table 4. Estimates of the spatial extent of toxicity in sediments of Sabine Lake based upon results from five independent tests.			
Toxicity test	Criterion	Toxic area (Km ²)	Percent of total*
Percent amphipod survival			
	• <80% of control	0.0	0.0
Percent urchin fertilization			
	• <80% of control in 100% pore water	14.5	5.9
	• <80% of control in 50% pore water	7.3	2.9
	• <80% of control in 25% pore water	0.0	0.0
Percent normal urchin development			
	• <80% of control in 100% pore water	244.6 ^A	99.4 ^A
	• <80% of control in 50% pore water	107.6 ^B	43.4 ^B
	• <80% of control in 25% pore water	0.0	0.0
Microtox bioluminescence EC50			
	• <80% of control	194.2	78.9
	• <0.51 mg/ml	3.6	1.4
	• <0.06 mg/ml	0.0	0.0
Cytochrome p-450 induction			
	• >11.1 ug/g	6.7 ^C	2.7 ^C
	• >37.1 ug/g	1.7 ^D	0.7 ^D
* total area: 246 km ²			
^A Toxic area: 243.4 km ² (99.4% of 244.8 km ²) accounting for second alternates at two stations			
^B Toxic area: 107.4 km ² (43.7% of 245.8 km ²) accounting for second alternate at one station			
^C Toxic area: 6.5 km ² (2.6% of 245.8 km ²) accounting for second alternate at one station			
^D Toxic area: 1.5 km ² (0.6% of 245.8 km ²) accounting for second alternate at one station			

Amphipod survival exceeded 80% of controls in all samples; therefore, the spatial extent of toxicity was estimated as 0% in that particular test. In the sea urchin tests of fertilization success, strata in which percent fertilization was less than 80% of controls represented 6%, 3%, and 0% of the area in tests of 100%, 50%, and 25% pore waters, respectively. In the urchin embryo development tests, results were less than 80% of controls in strata that represented 99%, 43%, and 0% of the area in the three pore water concentrations. Similarly, because of the large differences between the Redfish Bay control means and sample means in the Microtox tests, the spatial extent of toxicity as defined as <80% of controls was 79%. However, when defined as a EC50 response of <0.51 mg/mL and <0.06 mg/mL,

the estimates of the spatial extent of toxicity were 1.4% and 0%, respectively. Strata in which cytochrome p-450 RGS responses exceeded 11.1 $\mu\text{g/g}$ and 37.1 $\mu\text{g/g}$ represented 3% and 1% of the total area, respectively.

There were two sampling stations in which the first alternate set of coordinates were not sampled. At station 3, an active dredging operation precluded access to the target location. At station 57, oyster shells pinned the jaws of the sampler open and precluded collection of sediments. Therefore, samples were collected at second alternate locations for both stations. Estimates of the spatial extent of toxicity were adjusted for this situation by re-calibrating the areas of the strata in which these two stations were located (Table 4). The area of stratum A-3 was reduced by 0.23 km^2 (0.46 km^2 divided by 2) and the area of stratum M-7 was reduced by 1.0 km^2 (4.0 km^2 /4). The estimates based upon the adjusted areas are shown as footnotes on Table 4 for the tests in which the results were affected.

The estimates of spatial extent of toxicity in tests other than those with amphipods were highly influenced by the data from the Redfish Bay controls which proved to be unusually non-toxic. This situation was observed in analyses of data from another NOAA survey conducted in Puget Sound. Thus, the comparisons with the control responses probably exaggerated the degree of toxicity. Estimates based upon numerical critical values derived from analyses of the distribution of results in these tests in previous NOAA surveys are probably more reliable.

Summary of toxicity results. The results of the toxicity tests indicated that sediments in this survey area were not highly toxic (i.e., percent survival > 80% of controls) as measured with the acute amphipod survival tests. However, amphipod survival was significantly reduced in 13 samples and results of the sublethal tests performed with the urchin gametes and embryos were significant in additional samples. Urchin embryo development was significantly reduced in most samples. These data, collectively, suggest that sediments were slightly to moderately toxic, but not highly toxic, in parts of the study area. Results of the two tests performed with organic solvent extracts of the sediments showed that the potential for toxicity was highest in upper Neches River, Taylor Basin, and regions offshore beyond the entrance channel. Highly significant responses in these two tests, however, were neither pervasive nor widespread.

Spatial patterns in chemical concentrations. Distinct spatial patterns in chemical concentrations were difficult to identify because of the similarity in the data among the 66 stations. Most substances indicated very little variation in concentrations throughout the study area. For example, the concentrations of the trace metal arsenic ranged from 0.3 to 15 ppm, considerably below the ERM value of 70 ppm. Arsenic concentrations were higher in the Sabine-Neches canal (stations 6-27) than in the adjacent basin of Sabine Lake (Figure 9). Arsenic concentrations also were relatively high offshore (stations 58-66) as compared to the Sabine Lake stations. Although arsenic concentrations exceeded the ERL value of 8.2 ppm in many samples; none equalled or exceeded the ERM value of 70 ppm.

The pattern in the concentrations of total PAHs (sum of 13 compounds for which numerical guidelines have been derived) was clearer than that for arsenic. Total PAH concentrations were highest in samples from stations 2, 11, and 12 in the upper Neches River and from station 28 – 30 in the Taylor Basin (Figure 10). As observed with arsenic, total PAH con-

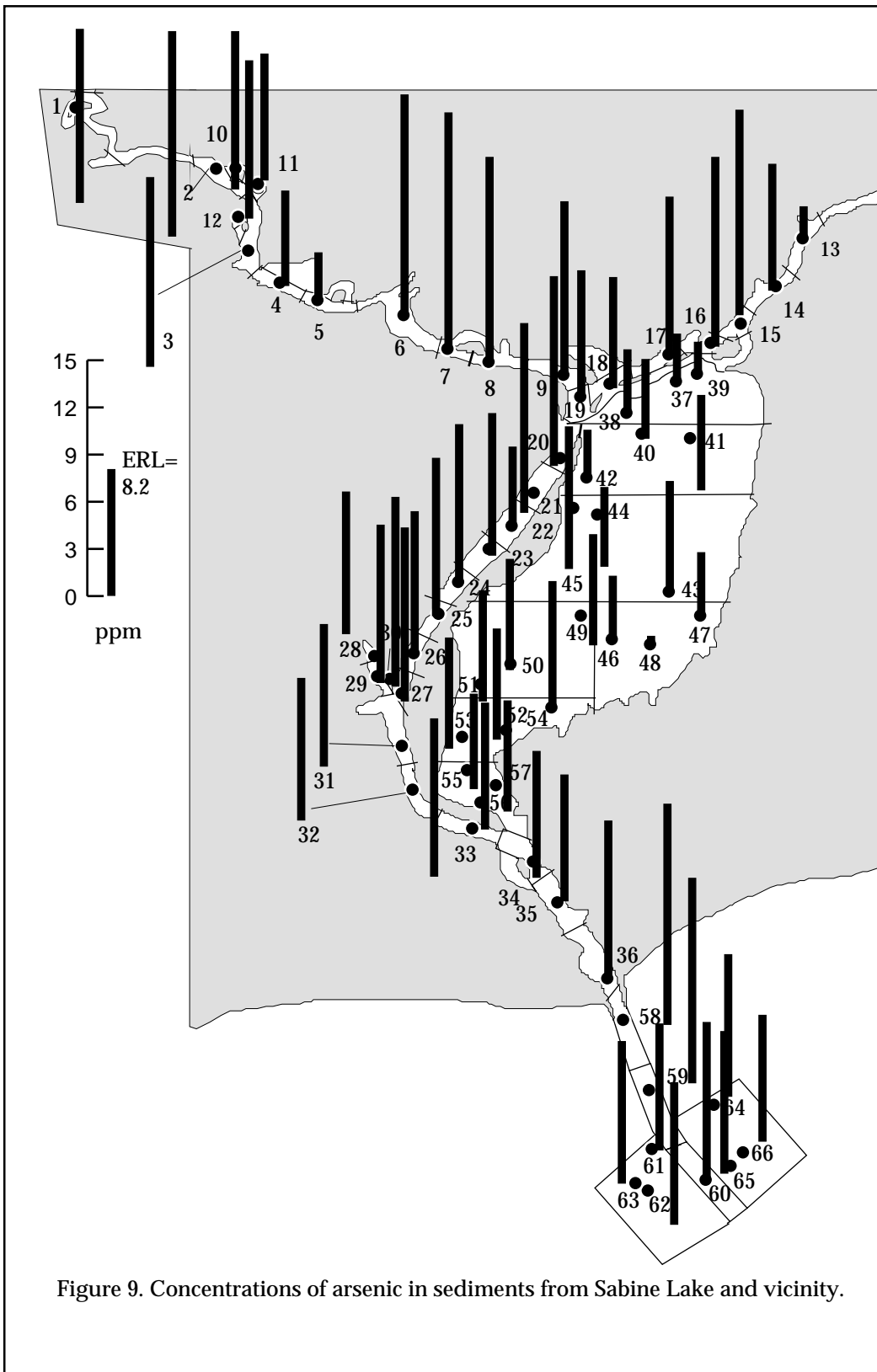
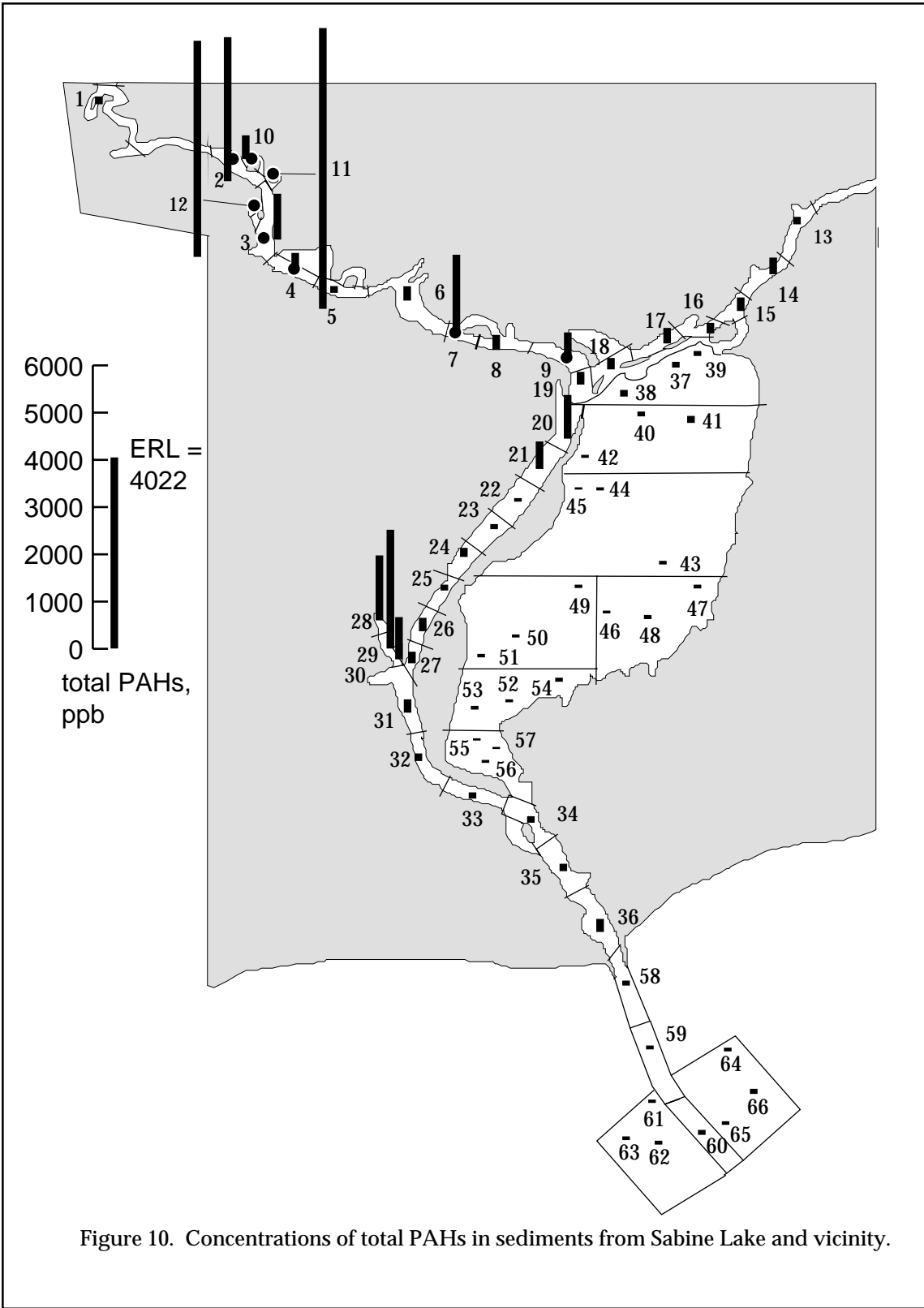


Figure 9. Concentrations of arsenic in sediments from Sabine Lake and vicinity.



centrations were somewhat higher in the canals than in the adjacent basin of Sabine Lake. However, unlike the pattern with arsenic, PAH concentrations decreased seaward and offshore.

Spatial extent of chemical contamination. The spatial extent of chemical contamination was estimated for 25 substances and/or classes of compounds by weighting the data to the sizes of the sampling strata, similar to the methods used to calculate the spatial extent of toxicity. ERL and ERM values from Long et al. (1995) were used as the critical values (Table 5). The data indicated that only one substance, acenaphthalene, equalled or exceeded an ERM value. The one sample in which the ERM value was exceeded represented 0.3 km², equivalent to 0.1% of the total study area. Concentrations of arsenic and nickel were elevated above the respective ERL values in the most stations, representing about 17% and 9% of the study area, respectively. The spatial extent of contamination by all other substances was less than 1.0% of the area. Similarly, the samples in which the mean ERM quotients exceeded 0.1 represented less than 1% of the area (Table 5).

Relationships between toxicity and chemical concentrations. The relationships between results of laboratory toxicity tests and concentrations of chemical substances in the sediments were determined with correlation analyses. Initially, correlations were calculated for classes of chemicals normalized to their respective ERM values and expressed as mean chemical:ERM quotients (Table 6) to identify which groups of chemicals, if any, co-varied with measures of toxicity. Chemical classes were designated as nine trace metals, thirteen PAHs, three chlorinated organic hydrocarbons, and all 25 substances combined. None of the chemical classes showed significant relationships with either the amphipod survival or urchin fertilization tests. Microtox results were significantly correlated, but only at a significant level of 0.05, with the mean ERM quotients for all 25 substances.

In contrast, the concentrations of all chemical groups were highly correlated with the urchin development and P-450 RGS test results. In the urchin development tests, the strongest correlations were with the trace metals. In the P-450 RGS assays, the strongest relationships were with the PAHs and to a lesser degree the chlorinated organics. These correlations do not establish a causative relationship, but, rather, a correlative association between a set of independent variables (chemical concentrations) and dependent variables (toxicity tests).

To further identify which organic compounds showed strong relationships with the results of the cytochrome P-450 RGS assays, correlation analyses were continued for a variety of different chemical classes (Table 7). Correlations were highly significant for all classes of chemicals: PAHs, DDTs, HCHs, and PCBs; therefore, indicating that results of this test co-varied with mixtures of several classes of organics in the sediments.

To further elucidate the associations between urchin development and trace metals, correlations were performed for individual trace metals (Table 8). The results also include the correlation coefficient for unionized ammonia, a substance commonly found in sediments that can be toxic. The correlations of percent normal development with concentrations of ammonia and all trace metals were significant. They were significant, except for silver, at a probability level of 0.0001 or greater. Zinc and tin showed the strongest relationships with percent normal development (i.e., Spearman-rank, $\rho > 0.7$).

Table 5. Estimates of the spatial extent of chemical contamination relative to effects - based numerical guidelines (ERL and ERM) for Sabine Lake. Data are shown as km² and percentages of total study area*.

Chemical or chemical class	>ERL		>ERM	
	km ²	Pct. of total	km ²	Pct. of total
arsenic	40.7	16.5	0	0
cadmium	0	0	0	0
chromium	0	0	0	0
copper	0	0	0	0
lead	0	0	0	0
mercury	0	0	0	0
nickel	22.2	9	0	0
silver	0	0	0	0
zinc	0	0	0	0
naphthalene	0	0	0	0
2-methyl naphthalene	0.2	0.1	0	0
acenaphthylene	0	0	0	0
acenaphthene	1.7	0.7	0.3	0.1
fluorene	1.7	0.7	0	0
phenanthrene	0.3	0.1	0	0
anthracene	0.9	0.4	0	0
fluoranthene	0.9	0.4	0	0
pyrene	0.5	0.2	0	0
benzo(a)anthracene	1.1	0.4	0	0
chrysene	0	0	0	0
benzo(a)pyrene	0	0	0	0
dibenz(a,h)anthracene	0.4	0.2	0	0
sum low PAHs	0.9	0.4	0	0
sum high PAHs	0.9	0.4	0	0
total PAHs	0.5	0.2	0	0
p,p' - DDE	0	0	0	0
total DDTs	1	0.4	0	0
total PCBs	0.8	0.3	0	0
mean ERM quotient >0.1	0.1	0.4		
total area: 246 km ²				

Table 6. Spearman-rank correlation coefficients (rho, corrected for ties) for toxicity test results and chemical concentrations normalized to ERM values in Sabine Lake sediments (n=66).

Chemical group	Percent amphipod survival	Percent urchin fertilization (100% pore water)	Percent urchin normal development (100% pore water)	Microtox EC50	Cytochrome P-450 RGS
mean ERM quotient: metals	0.012	-0.119	-0.698 ****	-0.19	-0.429 ***
mean ERM quotient: PAHs	-0.173	0.168	-0.408 ***	-0.13	0.827 ***
mean ERM quotient: COHs	0.080	0.126	-0.527 ****	-0.13	0.779 ***
mean ERM quotient: all	-0.040	-0.094	-0.677 ****	-0.28 *	0.662 ***
* p<0.05					
** p<0.01					
*** p<0.001					
**** p<0.0001					

Table 7. Spearman-rank correlation coefficients (rho, corrected for ties) for P-450 RGS test results and concentrations of classes of organic compounds in Sabine Lake sediments (n=66).

Class of compounds	P-450 RGS induction		
sum 7 LPAHs	0.802	****	
total LPAHs	0.794	****	
sum 6 HPAHs	0.828	****	
total HPAHs	0.812	****	
total 13 PAHs	0.827	****	
total PAHs	0.814	****	
total DDTs	0.840	****	
total HCHs	0.583	****	
total PCBs	0.779	****	
****p<0.0001			

Table 8. Spearman-rank correlation coefficients (rho, corrected for ties) for percent urchin normal development (100% porewater) and concentrations of trace metals and un-ionized ammonia in Sabine Lake sediments (n=66).

Chemical name	Percent sea urchin normal development		
un-ionized ammonia	-0.547	****	
arsenic	-0.665	****	
cadmium	-0.583	****	
chromium	-0.521	****	
copper	-0.739	****	
iron	-0.714	****	
lead	-0.632	****	
manganese	-0.646	****	
mercury	-0.600	****	
nickel	-0.655	****	
selenium	-0.592	****	
silver	-0.270	*	
tin	-0.731	****	
zinc	-0.699	****	
*p<0.05			
**p<0.01			
***p<0.001			
****p<0.0001			

The Lowest Observable Effects Concentrations (LOEC) for unionized ammonia in the fertilization test (800 ug/L) was not exceeded in the Sabine Lake samples. However, the LOEC of 90 ug/L for the embryological development test was exceeded in 35 of the 66 samples. Therefore, the data suggest that ammonia alone may have significantly contributed to the toxicity observed in many of the samples tested for embryological development.

Summary of chemical contamination. The chemical data for a wide variety of chemical substances indicated that the surficial sediments in Sabine Lake and vicinity were not highly contaminated. Chemical concentrations often were below respective ERL values. A few chemical concentrations equalled or exceeded the ERL values and only one chemical in one sample exceeded an ERM value. The spatial extent of chemical contamination was restricted to a small percentage of the total survey area. Sediments from Taylor Basin and a side-channel bayou of the Neches River had higher chemical concentrations than those from other areas. Samples from the basin of Sabine Lake often were among the least contaminated. Concentrations of organic compounds such as the PAHs decreased seaward and offshore into the Gulf of Mexico; however, the concentrations of trace metals such as arsenic did not follow this pattern. Concentrations of arsenic in offshore samples were equivalent to those from inland locations. Toxicity responses in the amphipod and urchin fertilization tests were not significantly correlated with chemical concentrations; however, results of urchin development and cytochrome P-450 RGS tests were highly correlated with all chemical classes. Urchin development was very highly correlated with the concentrations of trace metals and porewater unionized ammonia and P-450 induction was highly correlated with PAHs in the sediments.

Discussion and Conclusions

The survey of Sabine Lake and vicinity encompassed an area of approximately 246 km² and included analyses of 66 sediment samples for chemical contamination and toxicity. Samples from Sabine Lake, the Neches and Sabine rivers, the Intracoastal Waterway, and the entrance channel (Sabine Pass) to the Gulf of Mexico were collected for analyses. Toxicity was determined with a battery of acute and sublethal tests conducted on bulk (solid-phase) sediments, pore waters, and organic solvent extracts. Chemical analyses were conducted to determine the concentrations of many potentially toxic substances, including ammonia, trace metals, polynuclear aromatic hydrocarbons, and chlorinated organic compounds.

The results indicated that the sediments in this area were not significantly degraded. The spatial extent of chemical contamination when compared to effects-based ERM values was nil and the spatial extent when compared to ERL values was less than 1% for most substances. Chemical concentrations of organic substances were highest in Taylor Basin and an arm of the Neches River near Beaumont; whereas concentrations of trace metals failed to follow a clear spatial pattern. A very clear layer of oil was observed in the sediments from an arm of Neches River below the upper 3 cm depth that was sampled, possibly the result of a previous spill event. Overall, however, the sediments in the waterway channels were more contaminated than those from the basin of Sabine Lake.

The estimated spatial extent of toxicity as determined in the amphipod survival test is compared to comparable estimates for other estuarine regions studied by NOAA in Table 9. The surficial area represented by toxic samples in Sabine Lake (i.e., none or 0.0% of the area) was comparable with results from Galveston Bay, northern Puget Sound, Pensacola Bay, Charleston Harbor and a number of other areas. Sabine Lake ranked well below the

Table 9. A comparison of the spatial extent of toxicity (percentage of total survey area) in amphipod survival tests among 25 estuarine areas nationwide and "national estuarine averages" compiled from field studies conducted through 1995, 1996, and 1997.

Estuarine Study Area	Total area (Km ²)	<u>Amphipod survival</u> area (km ²)	(% of total area)
Newark Bay	13.0	10.8	85.0%
San Diego Bay	40.2	26.3	65.8%
California coastal lagoons	5.0	2.9	57.9%
Tijuana River	0.3	0.18	56.2%
Long Island Sound	71.9	36.3	50.5%
Hudson-Raritan Estuary	350.0	133.3	38.1%
San Pedro Bay	53.8	7.8	14.5%
Biscayne Bay	484.2	62.3	12.9%
National average: 1995	2 532.6	277.0	10.9%
National average: 1996	4 158.1	286.4	6.9%
Boston Harbor	56.1	5.7	10.0%
Delaware Bay	2346.8	145.4	6.20%
National average: 1997	7 278.8	431.8	5.93%
Savannah River	13.1	0.16	1.2%
St. Simons Sound	24.6	0.10	0.4%
Tampa Bay	550.0	0.5	0.1%
Galveston Bay	1351.1	0.0	0.0%
northern Puget Sound	773.9	0.00	0.0%
Pensacola Bay	273.0	0.04	0.0%
Choctawhatchee Bay	254.5	0	0.0%
Sabine Lake	245.9	0.00	0.0%
Apalachicola Bay	187.6	0	0.0%
St. Andrew Bay	127.2	0	0.0%
Charleston Harbor	41.1	0	0.0%
Winyah Bay	7.3	0	0.0%
Mission Bay	6.1	0.0	0.0%
Leadenwah Creek	1.7	0	0.0%
San Diego River	0.5	0.0	0.0%

national estuarine averages calculated with data gathered through the 1995, 1996, and 1997 field seasons.

In the urchin fertilization tests performed with 100% pore water concentrations, Sabine Lake also ranked among the least toxic areas (Table 10). The estimated spatial extent of toxicity in Sabine Lake (6%) was comparable to that for Pensacola Bay (5%) and Boston Harbor (7%) and less than the national estuarine averages for data compiled through the 1995, 1996, and 1997 field seasons.

Table 10. A comparison of the spatial extent of toxicity (percentage of total survey area) in urchin fertilization tests of 100% pore water among 22 estuarine survey areas and "national estuarine averages" compiled from field studies conducted through 1995, 1996, and 1997.

Estuarine study area	Total area (Km ²)	Urchin fertilization @ 100% area km ²	(% of total area)
San Pedro Bay	53.8	52.6	97.70%
Tampa Bay	550	463.6	84.30%
San Diego Bay	34	25.8	75.90%
Mission Bay	6.1	4.0	65.90%
Tijuana River	0.3	0.18	56.20%
San Diego River	0.5	0.26	52.00%
Biscayne Bay	484.2	229.5	47.40%
Choctawhatchee Bay	254.47	113.14	44.40%
California coastal lagoons	5	2.1	42.70%
National average: 1995	2 082.6	886.3	42.60%
Winyah Bay	7.3	3.1	42.20%
National average: 1996	3 717.06	1 439.9	38.67%
Apalachicola Bay	187.58	63.6	33.90%
Galveston Bay	1351.1	432.0	32.00%
Charleston Harbor	41.1	12.5	30.40%
National average: 1997	6 837.76	1 728.0	25.25%
Savannah River	13.12	2.42	18.40%
Delaware Bay	2346.8	247.5	10.50%
Boston Harbor	56.1	3.8	6.60%
Sabine Lake	245.9	14.0	5.70%
Pensacola Bay	273	14.4	5.30%
northern Puget Sound	773.9	40.6	5.20%
St. Simons Sound	24.6	0.65	2.60%
St. Andrew Bay	127.2	2.28	1.80%
Leadenwah Creek	1.69	0	0.00%

In sharp contrast to the other tests, results of the urchin tests of embryological development indicated toxic conditions throughout the area. The estimated spatial extent of toxicity in Sabine Lake (99%) in the embryological development tests was much higher than in most other areas studied by NOAA (Table 11). The degree of toxicity in Sabine Lake was only slightly lower than in Boston Harbor (100% of the area) and higher than in Biscayne Bay (84%) and in the national estuarine average (39%). The concentrations of the unionized form of ammonia in the pore waters were sufficiently high in 35 of the 66 samples to cause

or contribute significantly to decreased embryological development; thus, possibly leading - unlike the results of the other tests - to the observation of widespread toxicity in this test.

Table 11. A comparison of the spatial extent of toxicity (percentages of area) in urchin embryo development tests performed with 100% pore water of sediments from 8 U.S. bays and estuaries.

	Total area (Km ²)	Urchin development	
		Toxic area (km ²)	(percent of total area)
Boston Harbor	56.1	56.8	100.0%
Sabine Lake	246	245	99.4%
Biscayne Bay	484.2	408	84.3%
Apalachicola Bay	187.58	157.5	84.0%
Choctawhatchee Bay	254.47	116.1	45.4%
National average	2734	1066	39.0%
Galveston Bay	1351.1	314.8	23.3%
St. Andrew Bay	127.2	7.2	5.6%
Pensacola Bay	273	5.4	2.0%

Results of Microtox tests are compared among study areas in Table 12. Using 80% of controls as the critical value in these calculations, the spatial extent of toxicity in Sabine Lake (79%) was greater than comparable results from most other areas and the national estuarine averages. However, comparisons to the Redfish Bay negative controls may exaggerate the degree of toxicity in Sabine Lake. Using a critical value of <0.51 mg/ml (as done in the northern Puget Sound and Delaware Bay studies) provides an estimated spatial extent of toxicity of 1.4% in Sabine Lake. This estimate puts Sabine Lake toward the bottom of the list and is comparable with the estimates for the other two regions.

Comparable data from the cytochrome P450 assays are available from five other areas (Table 13). Estimates of the spatial extent of significant induction (i.e., critical value of 11.1 ug/g) and high induction (i.e., >37.1 ug/g) in Sabine Lake were somewhat lower than in other areas. Results were comparable with those from northern Puget Sound.

Based upon the compilation of results from chemical analyses and toxicity tests of surficial samples from 66 locations, sediments in Sabine Lake and vicinity did not appear to be severely degraded. Chemical concentrations rarely exceeded effects-based numerical guidelines, suggesting that toxicant-induced effects would not be expected in most areas. None of the samples was highly toxic in acute amphipod survival tests and a minority of samples was highly toxic in sublethal urchin fertilization tests. Although toxic responses occurred frequently in urchin embryo development tests performed with 100% pore water, toxicity diminished frequently in tests done with diluted pore waters. Microbial bioluminescent activity was not reduced to a great degree and cytochrome P-450 activity was not highly induced in tests done with organic solvent extracts. Urchin embryological development was highly correlated with concentrations of ammonia and many trace metals. Cytochrome P-450 induction was highly correlated with concentrations of a number of classes of organic compounds.

Table 12. Spatial extent of toxicity (km² and percentages of total area) in microbial bioluminescence tests performed with solvent extracts of sediments from 17 U. S. bays and estuaries.

Estuarine study area	Total area (Km ²)	Microtox bioluminescence	
		Area km ²	(% of total area)
Choctawhatchee Bay	254.47	254.47	100.00%
St. Andrew Bay	127.2	127	100.00%
Apalachicola Bay	187.58	186.84	99.60%
Pensacola Bay	273	262.8	96.40%
Galveston Bay	1351.1	1143.7	84.60%
Sabine Lake	245.9	194.2	79.00%
Winyah Bay	7.3	5.13	69.99%
Long Island Sound	71.86	48.8	67.90%
National average: 1996	4039.22	2670.69	66.12%
National average: 1995	2416.2	1482.3	61.30%
Savannah River	13.12	7.49	57.10%
Biscayne Bay	484.2	248.4	51.30%
St. Simons Sound	24.6	11.42	46.40%
Boston Harbor	56.1	25.8	44.90%
Charleston Harbor	41.1	17.6	42.90%
National average: 1997	7159.92	2802.39	39.10%
Hudson-Raritan Estuary	350	136.1	38.90%
Leadenwah Creek	1.69	0.34	20.10%
Delaware Bay (<0.51 mg/ml)	2346.8	114	4.90%
northern Puget Sound (<0.51 mg/ml)	773.9	17.7	2.20%
Tampa Bay	550	0.6	0.10%

Table 13. Spatial extent of toxicity (km² and percentages of total area) in P-450 RGS bioassays performed with solvent extracts of sediments from 6 areas.

	Total area (Km ²)	P-450 RGS (>11.1 ug/g)		P-450 RGS (>37.1 ug/g)	
		km ²	(% of total area)	km ²	(% of total area)
Biscayne Bay, 1996	271.4	8.8	3.3	0	0
northern Puget Sound	806.2	20.1	2.5	0.2	0.03
Delaware Bay	2346.8	145.2	6.2	80.5	3.4
Galveston Bay	1351.5	56.7	4.2	0	0
Sabine Lake	245.9	6.7	2.7	1.7	0.7
Southern Cal. Estuaries	5	2.30	46.8	0	0

Acknowledgments

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Appendix A. Field notes on sampling stations in Sabine Lake and vicinity.									
Location	Stratum	No Station	No. Sample	Id. Latitude	°N Longitude	°W Depth	Depth (ft.)	Depth(m)	Date
Neches River	A	1	1	30°05.5'	94°05.27'		34.3	10.46	8/8/95
		2	2	30°04.4833'	94°03.55'		39.5	12.04	8/17/95
		3	3	30°02.75'	94°01.7'		47.3	14.42	8/17/95
	B	1	1	30°01.18'	94°01.3'		47.2	14.39	8/17/95
		2	2	30°00.624'	93°59.282'		47.7	14.54	8/17/95
		3	3	29°59.910'	93°57.040'		47.7	14.54	8/17/95
	C	1	1	29°59.512'	93°56.084'		47.8	14.57	8/16/95
		2	2	29°59.097'	93°53.806'		41.5	12.65	8/16/95
		3	3	29°58.879'	93°52.421'		38.5	11.74	8/16/95
	D	1	1	30°04.72'	94°03.167'		49.1	14.97	8/18/95
		2	2	30°03.917'	94°02.00'		11.3	3.45	8/18/95
		3	3	30°02.617'	94°02.15'		28.5	8.69	8/18/95
Sabine River	E	1	13	30°02.833	93°43.75'		16.1	4.91	8/15/95
		2	14	30°01.2'	93°44.833'		22	6.71	8/15/95
		3	15	29°59.9'	93°46.42'		31.3	9.54	8/15/95
	F	1	16	29°59.4'	93°47.37'		32.8	10.00	8/15/95
		2	17	29°59.067'	93°48.5'		30.5	9.30	8/15/95
		3	18	29°58.38'	93°50.7'		22.8	6.95	8/15/95

Appendix A. Field notes on sampling stations in Sabine Lake and vicinity continued.									
Location	Stratum	No Station	No. Sample	Id.	Latitude °N	Longitude °W	Depth (ft.)	Depth(m)	Date
Sabine-Neches Canal									
	G		1	19	29°57.937	93°51.363	37.1	11.31	8/16/95
			2	20	29°56.801'	93°51.820	42.3	12.90	8/16/95
			3	21	29°55.35	93°52.917	32.5	9.91	8/14/95
	H		1	22	29°54.6'	93°53.53	35	10.67	8/14/95
			2	23	29°53.98'	93°54.017'	34.8	10.61	8/14/95
			3	24	29°53.017'	93°55.083'	38	11.59	8/14/95
	I		1	25	29°52.383'	93°55.73'	41.5	12.65	8/14/95
			2	26	29°51.083'	93°56.82'	32	9.76	8/14/95
			3	27	29°49.53'	93°57.467'	10.5 meters	10.50	8/8/95
	J		1	28	29°50.58 *	93°58.13 *	44	13.41	8/9/95
			2	29	29°50.22 *	93°58.067**	44	13.41	8/9/95
			3	30	29°50.05'	93°57.45'	41	12.50	8/9/95
	K		1	31	29°48.383'	93°57.267'	12 meters	12.00	8/8/95
			2	32	29°47.48	93°56.933'	12 meters	12.00	8/8/95
			3	33	29°45.933'	93°55.17'	12 meters	12.00	8/8/95
	L		1	34	29°44.872	93°52.936	51.8	15.79	8/10/95
			2	35	29°43.611	93°51.941	47.8	14.57	8/10/95
			3	36	29°42.148	93°50.881	49.3	15.03	8/10/95

Appendix A. Field notes on sampling stations in Sabine Lake and vicinity continued.									
Location	Stratum	No Station	No. Sample	Id.	Latitude °N	Longitude °W	Depth (ft.)	Depth(m)	Date
<u>Sabine Lake</u>	M-1		1	37	29°58.55'	93°48.23'	5.1	1.55	8/19/95
			2	38	29°57.2'	93°50.183'	5.2	1.59	8/19/95
			3	39	29°58.85'	93°47.9	3.9	1.19	8/19/95
	M-2		1	40	29°56.82	93°48.967	7.5	2.29	8/19/95
			2	41	29°56.7'	93°47.467'	7.6	2.32	8/19/95
			3	42	29°55.433	93°51.7	5.9	1.80	8/19/95
	M-3		1	43	29°52.533'	93°48.82'	8.4	2.56	8/21/95
			2	44	29°54.67'	93°50.733'	7.5	2.29	8/21/95
			3	45	29°54.883'	93°51.87'	6	1.83	8/21/95
	M-4		1	46	29°52.402	93°50.921	7.7	2.35	8/12/95
			2	47	29°52.446	93°47.368	8.2	2.50	8/12/95
			3	48	29°51.263	93°49.320	8.4	2.56	8/12/95
	M-5		1	49	29°52.405	93°51.710	7.3	2.23	8/13/95
			2	50	29°50.32'	93°54.92'	6.1	1.86	8/13/95
			3	51	29°50.7'	93°54.33'	6.4	1.95	8/13/95
	M-6		1	52	29°48.85'	93°53..333'	6.5	1.98	8/13/95
			2	53	29°50.67'	93°54.4'	6.1	1.86	8/13/95
			3	54	29°49.65'	93°52.96'	6.9	2.10	8/13/95
M-7 (a)		1	55	29°48.036	93°55.038	8.5	2.59	8/12/95	
		2	56	29°46.887	93°55.065	10.3	3.14	8/12/95	
		3	57	29°47.699	93°55.808	6.7	2.04	8/12/95	
<u>Sabine Pass</u>	O		1	58	29°41.098	93°50.144	55.5	16.92	8/10/95
			2	59	29°39.425	93°49.738	45	13.72	8/10/95
			3	60	29°37.476	93°48.833	29.1	8.87	8/11/95
	P		1	61	29°38.62'	93°49.87'	17.6	5.37	8/11/95
			2	62	29°37.005'	93°50.22'	20.9	6.37	8/11/95
			3	63	29°37.6'	93°50.233'	20.1	6.13	8/11/95
R		1	64	29°38.708	93°48.898	30	9.15	8/11/95	
		2	65	29°37.626	93°48.507	27.4	8.35	8/11/95	
		3	66	29°37.803	93°48.359	27.4	8.35	8/11/95	

* No coordinates obtained at the station, target coordinates are listed.

