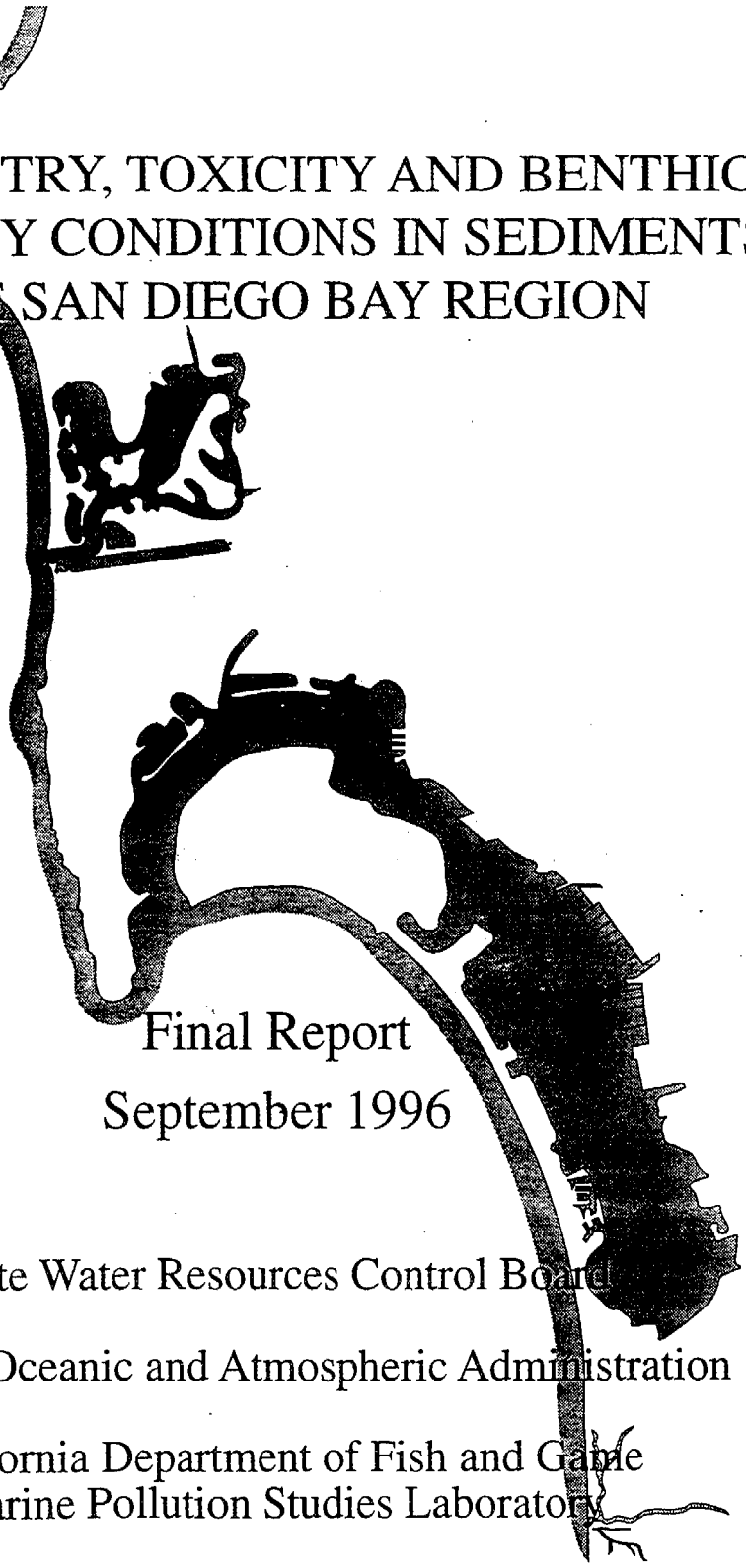
CHEMISTRY, TOXICITY AND BENTHIC COMMUNITY CONDITIONS IN SEDIMENTS OF THE SAN DIEGO BAY REGION



Final Report

September 1996

State Water Resources Control Board

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California Department of Fish and Game
Marine Pollution Studies Laboratory

Moss Landing Marine Laboratories

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EXECUTIVE SUMMARY

The following report describes and evaluates chemical and biological data collected from San Diego Bay and its historical tributaries between October, 1992 and May, 1994. The study was conducted as part of the ongoing Bay Protection and Toxic Cleanup Program, a legislatively mandated program designed to assess the degree of chemical pollution and associated biological effects in California's bays and harbors. The workplan for this study resulted from a cooperative agreement between the State Water Resources Control Board and the National Oceanic and Atmospheric Administration (NOAA). Monitoring and reporting aspects of the study were conducted by the Environmental Services Division, of the California Department of Fish and Game, and its subcontractors.

The study objectives were:

1. Determine presence or absence of adverse biological effects in representative areas of the San Diego Bay Region;

2. Determine relative degree or severity of adverse effects, and distinguish more severely impacted sediments from less severely impacted sediments;

3. Determine relative spatial extent of toxicant-associated effects in the San Diego Bay Region;

4. Determine relationships between toxicants and measures of effects in the San Diego Bay Region.

The research involved chemical analysis of sediments, benthic community analysis and toxicity testing of sediments and sediment pore water. Chemical analyses and bioassays were performed using aliquots of homogenized sediment samples collected synoptically at each station. Analysis of the benthic community structure was made on a subset of the total number of stations sampled.

Three hundred and fifty stations were sampled between October, 1992 and May, 1994. Areas sampled included San Diego Bay, Mission Bay, the San Diego River Estuary and the Tijuana River Estuary and are collectively termed "the San Diego Bay Region" in the following document. Two types of sampling designs were utilized: direct point sampling and stratified random sampling.

Chemical pollution was demonstrated by using comparisons to established sediment quality guidelines. Two sets of guidelines were used: the Effects Range-Low (ERL)/Effects Range-Median (ERM) guidelines developed by NOAA (Long and Morgan, 1990; Long *et al.*, 1995) and the Threshold Effects Level (TEL)/Probable Effects Level (PEL) guidelines used in Florida (McDonald, 1993; McDonald, 1994). Copper, mercury, zinc, total chlordane, total PCBs and the PAHs were most often found to exceed critical ERM or PEL values

and were considered the major chemicals or chemical groups of concern in the San Diego Bay Region. ERM and PEL summary quotients were used to develop chemical indices for addressing the pollution of sediments with multiple chemicals. An ERM summary quotient >0.85 or a PEL summary quotient >1.29 was indicative of stations where multiple chemicals were significantly elevated. Stations with any chemical concentration >4 times its respective ERM or >5.9 times its respective PEL were considered to exhibit elevated chemistry. Summary quotients and magnitude of sediment quality guideline exceedances were used as additional information to help prioritize stations of concern for Regional Water Quality Control Board staff.

Identification of degraded and undegraded habitat (as determined by macrobenthic community structure) was conducted using a cumulative, weight-of-evidence approach. Analyses were performed to identify relationships between community structure within and between each station or site (*e.g.*, diversity/evenness indices, analyses of habitat and species composition, construction of dissimilarity matrices for pattern testing, assessment of indicator species, and development of a benthic index, cluster analyses, and ordination analyses).

Analyses of the 75 stations sampled for benthic community structure identified 23 undegraded stations, 43 degraded and 9 transitional stations. All sampled stations with an ERM summary quotient >0.85 were found to have degraded communities. All sampled stations with P450 Reporter Gene System responses above 60 μg/g BaPEq. were similarly found to have degraded benthic communities.

The statistical significance of toxicity test results was determined using two approaches: the reference envelope approach and laboratory control comparison approach used by the United States Environmental Protection Agency- Environmental Monitoring and Assessment Program and NOAA- National Status and Trends programs. The reference envelope approach indicated that toxicity for the *Rhepoxynius* (amphipod) sediment test was significant when survival was less than 48% in samples tested. No reference envelope was calculated for the urchin fertilization or development tests due to high variability in pore water data from reference stations.

The laboratory control comparison approach was used to compare test sediment samples against laboratory controls for determination of statistically significant differences in test organism response. Criteria for toxicity in this approach were 1) survival less than 80% of the control value and 2) significant difference between test samples and controls, as determined using a t-test. Using this approach, there was no absolute value below which all samples could be considered toxic, although survival below a range of 72-80% was generally considered toxic.

Using the EMAP definition of toxicity, 56% of the total area sampled was toxic to *Rhepoxynius*. For the *Strongylocentrotus*

larval development test, percent of total area toxic was 29%, 54%, and 72% respectively for 25%, 50%, and undiluted pore water concentrations. Samples representing 14%, 27%, or 36% of the study area were toxic to both *Strongylocentrotus* in pore water (25%, 50%, or undiluted, respectively) and *Rhepoxynius* in solid phase sediment.

Linear regression analyses failed to reveal strong correlations between amphipod survival and chemical concentration. It is suspected instead of a linear response to chemical pollutants, most organisms are tolerant of pollutants until a threshold is exceeded. Comparisons to established sediment quality guideline thresholds demonstrate an increased incidence of toxicity for San Diego Bay Region samples with chemical concentrations exceeding the ERM or PEL values. It is further suspected toxicity in urban bays is caused by exposure to complex mixtures of chemicals. Comparisons to ERM summary quotients (multiple chemical indicators) demonstrate that the highest incidence of toxicity (>78%) is found in samples with elevated ERM summary quotients (>0.85).

Statistical analyses of the P450 Reporter Gene System responses versus the PAHs in sediment extracts demonstrated that this biological response indicator was significantly correlated ($r^2 = 0.86$) with sediment PAH (total and high molecular weight) concentration.

Stations requiring further investigation were prioritized based on existing evidence. Each station receiving a high, moderate or low priority ranking meets one or more of the criteria under evaluation for determining hot spot status in the Bay Protection and Toxic Cleanup Program. Those meeting all criteria were given the highest priority for further action. A ranking scheme was developed to evaluate stations of lower priority.

Seven stations (representing four sites) were given a high priority ranking, 43 stations were given a moderate priority ranking, and 57 stations were given a low priority ranking. The seven stations receiving the high priority ranking were in the Seventh Street channel area, two naval shipyard areas near the Coronado Bridge, and the Downtown Anchorage area west of the airport. The majority of stations given moderate rankings were associated with commercial areas and naval shipyard areas in the vicinity of the Coronado Bridge. Low priority stations were interspersed throughout the San Diego Bay Region.

A review of historical data supports the conclusions of the current research. Recommendations are made for complementary investigations which could provide additional evidence for further characterizing stations of concern.

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LIST OF ABBREVIATIONS

AA	Atomic Absorption
ASTM	American Society for Testing Materials
AVS	Acid Volatile Sulfide
BPTCP	Bay Protection and Toxic Cleanup Program
CDF	Cumulative Distribution Frequencies
CDFG	California Department of Fish and Game
CH	Chlorinated Hydrocarbon
COC	Chain of Custody
COR	Chain of Records
EDTA	Ethylenediaminetetraacetic Acid
EMAP	Environmental Monitoring and Assessment Program
ERL	Effects Range Low
ERM	Effects Range Median
ERMQ	Effects Range Median Summary Quotient
EqP	Equilibrium Partitioning Coefficient
FAAS	Flame Atomic Absorption Spectroscopy
GC/ECD	Gas Chromatograph Electron Capture Detection
GFAAS	Graphite Furance Atomic Absorption Spectroscopy
HCl	Hydrochloric Acid
HDPE	High-density Polyethylene
HMW PAH	High Molecular Weight Polynuclear Aromatic Hydrocarbons
HNO_3	Nitric Acid
HPLC/SEC	High Performance Liquid Chromatography Size Exclusion
H_2S	Hydrogen Sulfide
IDORG	Identification and Organizational Number
KCL	Potassium Chloride
LC_{50}	Lethal Concentration (to 50 percent of test organisms)
LMW PAH	Low Molecular Weight Polynuclear Aromatic Hydrocarbons
MDL	Method Detection Limit
MDS	Multi-Dimensional Scaling
MLML	Moss Landing Marine Laboratories
MPSL	Marine Pollution Studies Laboratory
NH_3	Ammonia
NOAA	National Oceanic and Atmospheric Administration
NOEC	No Observed Effect Concentration
NS&T	National Status and Trends Program
P450	Cytochrome P450 Enzyme System
PAH	Polynuclear Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyl
PEL	Probable Effects Level
PELQ	Probable Effects Level Summary Quotient
PPE	Porous Polyethylene
PVC	Polyvinyl Chloride
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
REF	Reference
RGS	P450 Reporter Gene System
RWQCB	Regional Water Quality Control Board
SCCWRP	Southern Calif. Coastal Waters Research Project

SPARC	Scientific Planning and Review Committee
SQC	Sediment Quality Criteria
SWRCB	State Water Resources Control Board
T	Temperature
TBT	Tributyltin
TFE	Tefzel Teflon®
TEL	Threshold Effects Level
TIE	Toxicity Identification Evaluation
TOC	Total Organic Carbon
TOF	Trace Organics Facility
UCSC	University of California Santa Cruz
USEPA	U.S. Environmental Protection Agency
WCS	Whole Core Squeezing

Units

liter = 1 l
milliliter = 1 ml
microliter = 1 µl
gram = 1 g
milligram = 1 mg
microgram = 1 µg
nanogram = 1 ng
kilogram = 1 kg
1 part per thousand (ppt) = 1 mg/g
1 part per million (ppm) = 1 mg/kg, 1 µg/g
1 part per billion (ppb) = 1 µg/kg, 1 ng/g

INTRODUCTION

Purpose

In 1992, the State Water Resources Control Board (SWRCB) and the National Oceanic and Atmospheric Administration (NOAA) entered into a three-year cooperative agreement to assess potential adverse biological effects from sediments in coastal bays and harbors of Southern California (SWRCB and NOAA, 1991, 1992, 1993). The study area for the three-year cooperative agreement extended south of the Palos Verdes Peninsula to the USA/Mexico border. The majority of work focused on selected coastal bays, harbors and lagoons where depth ranged from approximately 60 meters to the upper limit of the tidal range. In the first phase of the study, data were collected, analyzed, and reported from the Los Angeles/Long Beach areas (SWRCB and NOAA, 1994).

This report presents results from data collected in the San Diego Bay area during the second and third years of the cooperative agreement. The study was performed in San Diego Bay, Mission Bay, San Diego River Estuary, and Tijuana River Estuary in southern California (Figure 1).

The purposes of the present study were:

1. Determine presence or absence of statistically significant toxicity effects in representative areas of the San Diego Bay Region;

2. Determine relative degree or severity of observed effects, and distinguish more severely impacted sediments from less severely impacted sediments;

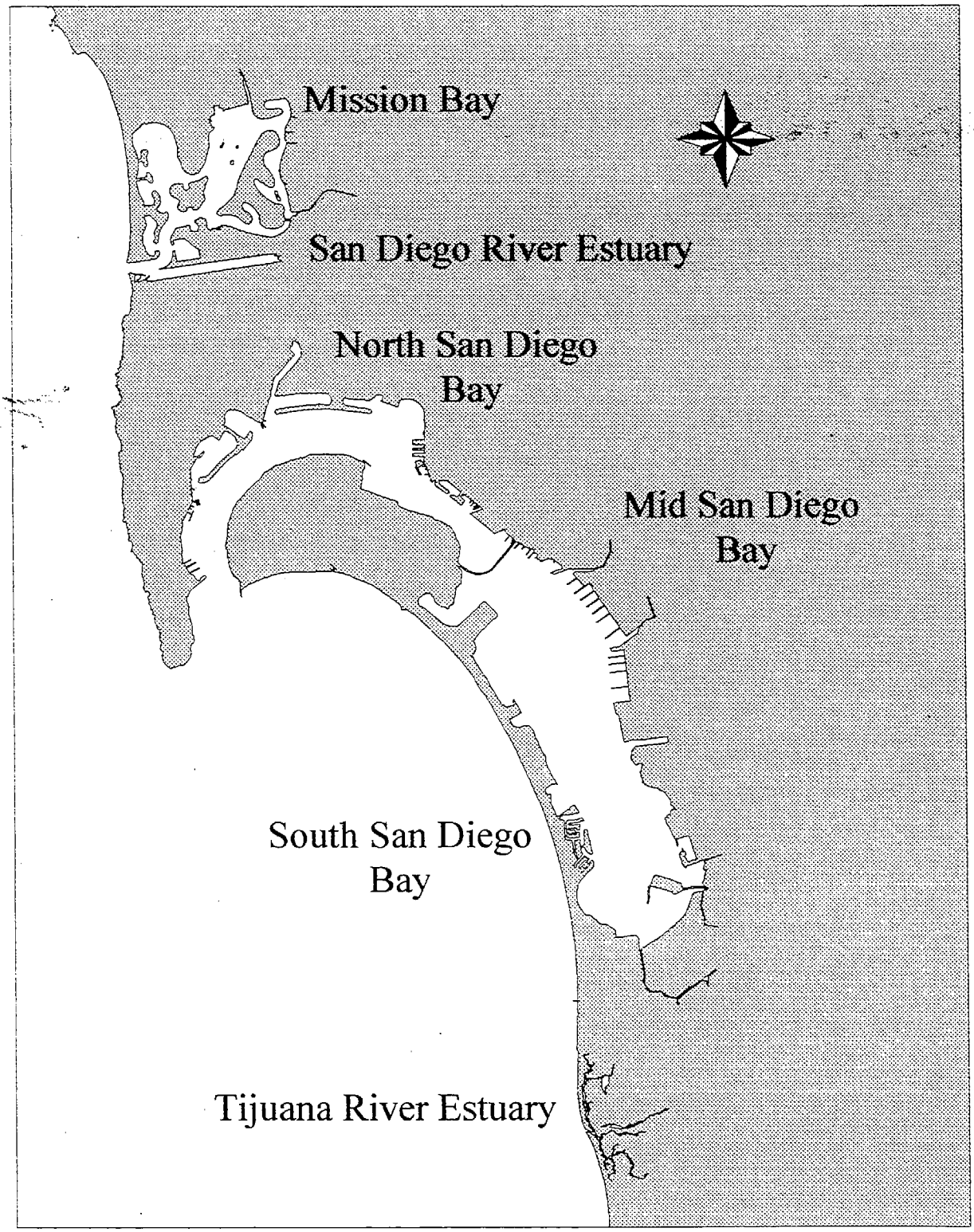
3. Determine relative areal extent of significant toxicity in the San Diego Bay Region;

4. Determine relationships between pollutants and measures of effects in these bays.

Programmatic Background and Needs

Due to the long history of human activity in San Diego Bay and its surrounding waters, there is a need to assess any environmentally detrimental effects which have been associated with those activities. The cooperative agreement between NOAA and SWRCB was designed to investigate these environmental effects by evaluating the biological and chemical state of San Diego Bay sediments. The methods used to assess environmental impacts include sediment and interstitial water bioassays, sediment chemistry analysis, and benthic community analysis. The study areas included San Diego Bay, Mission Bay, Tijuana River Estuary, and the San Diego River. Although these water bodies are separated physically, and are quite different in character, for simplicity they will often be referred to collectively as the "San Diego Bay Region" in this report (Figure 1). The SWRCB and NOAA have common programmatic needs for this research, however, some differences exist. NOAA is mandated by Congress to conduct a

Figure 1
San Diego Bay Region Study Area



program of research and monitoring on marine pollution. Much of this research is conducted through the National Status and Trends (NS&T) Program and the Coastal Ocean Program. The NS&T Program performs intensive regional studies on the magnitude and extent of toxicant-associated bioeffects in selected coastal embayments and estuaries. Areas chosen for these regional studies were those in which pollutant concentrations indicate the greatest potential for biological effect. These biological studies augment regular chemical monitoring activities of the NS&T Program, and provide a means for estimating the extent of toxicity associated with measured concentrations of sediment pollutants.

The California Water Code, Division 7, Chapter 5.6, Section 13390 mandates the State Water Resources Control Board and the Regional Water Quality Control Boards to provide the maximum protection of existing and future beneficial uses of bays and estuarine waters and to plan for remedial actions at those identified toxic hot spots where the beneficial uses are being threatened by toxic pollutants.

A cooperative agreement between NOAA and SWRCB has been implemented through the Bay Protection and Toxic Cleanup Program (BPTCP). Sediment characterization approaches currently used by the BPTCP range from chemical or toxicity monitoring only, to monitoring designs which attempt to generally correlate the presence of pollutants with toxicity or benthic community degradation. Studies were designed, managed, and coordinated by the SWRCB's Bays and Estuaries Unit as a cooperative effort with NOAA's Bioeffects Assessment Branch, and the California Department of Fish and Game's (CDFG) Marine Pollution Studies Laboratory. Funding was provided by the SWRCB and NOAA's Coastal Ocean Program.

Research for the San Diego Bay Region involved toxicity testing and chemical analysis of sediments and sediment pore water. Toxicity tests and chemical analysis were performed using aliquots of homogenized sediment samples collected synoptically from each station, resulting in paired data. Analyses of benthic community structure and P450 enzyme induction were also made on a subset of the total number of stations sampled.

Field and laboratory work was accomplished under interagency agreement with, and under the direction of, the CDFG. Sample collections were performed by staff of the San Jose State University Foundation at the Moss Landing Marine Laboratories, Moss Landing, CA (MLML). Trace metals analyses were performed by CDFG personnel at the trace metal facility at Moss Landing Marine Laboratories. Synthetic organic pesticides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) were analyzed at the UCSC trace organics analytical facility at Long Marine Laboratory in Santa Cruz, California. MLML staff also performed total organic carbon (TOC) and grain size analyses, as well as benthic community analyses. Toxicity testing was conducted by the University of California at Santa Cruz (UCSC) staff at the CDFG toxicity testing laboratory at Granite Canyon,

California. P450 Reporter Gene System analyses were conducted by Columbia Analytical Services in Carlsbad, CA.

Study Area

San Diego Bay

San Diego Bay is the southern-most embayment on the west coast of the United States. It is located within the Southern California Bight and is the largest embayment along the 1450 kilometer stretch of coastline between San Francisco and Central Baja California. Located 16 kilometers northwest of the Mexico border, it is considered one of the finest natural harbors in the world. This reputation is due mainly to its deep entrance and protection from weather it provides ships. San Diego Bay lies entirely in the county of San Diego, extending from the entrance at Point Loma southward to the mouth of the Otay River.

San Diego Bay is a natural, nearly-enclosed, crescent-shaped estuary that encompasses approximately 52 square kilometers. It is approximately 24 kilometers (km) in length and varies from 0.4 km to 5.8 km in width. Depths in the Bay vary from 18 meters near the mouth to less than 1 meter in the southern part of the bay, with the average depth for the entire bay being slightly more than 12 meters. The Bay is much deeper and narrower than it was historically, due mainly to dredging of channels and filling of nearshore areas.

San Diego Bay opens to the Pacific Ocean and is classified as an estuarine system due to its fresh water dilution. The diversion of the San Diego River to Mission Bay by the U.S. Army Corps of Engineers in 1857 was the first major reduction of freshwater input into the bay (Smith, 1977). Sweetwater River and the Otay River were also main sources of freshwater for San Diego Bay, although these sources have been greatly reduced over the years as a result of dam construction, extensive ground water use, and limited rainfall in recent years. Freshwater input is now limited to periodic surface drainage from the metropolitan area and intermittent flow from several rivers and creeks during periods of rainfall. Because of the dry Mediterranean-like climate that characterizes San Diego Bay, average annual rainfall in the Bay is usually between 10 and 13 inches, the majority of which falls between November and February.

Tides in San Diego Bay demonstrate marked variation between the heights of two high tides and two low tides that occur daily, classifying them as diurnal. The range between mean higher high water (MHHW) and mean lower low water (MLLW) is 1.6 meters and the extreme range of tides within the Bay is approximately 2.9 meters (Browning and Speth, 1973). Tidal currents are strongest in the northern part of the Bay where surface velocities reach 2.9 knots on ebb tide and 2.2 knots on flood tide (U.S. Army Corps of Engineers, 1973). Tidal currents are reduced considerably in the shallower central and south bay areas. Average tidal flushing for San Diego Bay is about 30% of the

entire Bay water volume exchanged per tidal cycle (12.5 hours). This volume of water is referred to as the tidal prism and in San Diego Bay represents approximately 74,000,000 cubic meters. Tidal flushing rates differ drastically between the Bay entrance and South Bay. Complete tidal flushing for the South Bay requires seven to fourteen days, whereas, the entrance of the Bay may only require one to two days. It has been estimated over the last century, tidal flushing in San Diego Bay has been reduced by 30% due to channel dredging and landfill projects (Browning and Speth, 1973).

San Diego Bay is a sedimentary environment with the bay floor and bay margins characterized by sand, silt and clay deposits (Peeling, 1974). Sand deposits are found near the Bay's mouth and along western margins, while finer silt and clay deposits are located on the eastern margins and at the southern end of the Bay.

An early navigation chart issued by the U.S. Coastal Survey in 1859 shows an undredged Bay fifteen miles long with a channel varying in depth from 22.2 meters decreasing to 3.6 meters. This natural channel stretched for 13 kilometers from the tip of Point Loma to the South Bay. Salt marshes existed at the mouths of seven creeks and river tributaries.

The early residents of the San Diego Bay area were Native Americans, who hunted and fished in the Bay; Spanish, Mexican, and American ranchers, who traded hides and tallow; and the early Yankee whalers who established camps in North Bay. These groups appeared to have little impact on the water quality in the Bay. By 1830 there were 16 American whaling vessels operating out of San Diego Bay. The whaling industry reached its peak in 1871-72 when 55,000 gallons of oil and 200 tons of whalebone were shipped from Point Loma. Americans participating in the New Town land boom of the 1880's settled in the central San Diego Bay area, site of the present downtown San Diego. This settlement soon represented a considerable increase in the population of the area as well as a dramatic threat to water quality in the Bay.

The Cuyamaca Dam and a flume were completed in 1888, diverting freshwater from eastern mountains into what is now Chollas Reservoir. Forty miles of sewers coupled with a sewage reservoir and outfall located in San Diego Bay off Market street were also completed in 1888. This sewage system marked the beginning of the decline in water quality for the Bay. Conditions within the Bay continued to decline because of the increase in population (30,000 in 1901) and acceptance of the Bay as a major harbor for the U.S. Navy and civilian commerce.

During the next four decades communications and aviation stations were added and docking facilities expanded. Naval facilities expanded greatly during World War II as business and industry boomed. In 1940, the population had increased to 200,000 causing a failure of the overloaded sewage collection and treatment facilities. In 1943, raw or minimally treated sewage was being

discharged into the Bay from 15 outfalls. After World War II and the Korean War, San Diego Bay was subject to the dumping of more than 50 million gallons of sewage and industrial waste per day (San Diego Interagency Water Quality Panel, 1989).

In 1950, the population of the San Diego metropolitan area had increased to over 400,000. In an attempt to curtail the flow of raw sewage into the Bay, San Diego and several neighboring communities combined their sewage outfalls into one system. Unfortunately, this new system was constantly operating on overload and discharging directly into the Bay. Simultaneously, the Bay received untreated industrial discharge from five fish canneries, a large rendering operation, a kelp processing plant, four aircraft manufacturing plants, several shipyards, and the Pacific coast's largest naval base, naval air station, and submarine base (San Diego Interagency Water Quality Panel, 1989).

The California Regional Water Quality Control Board was established in 1950 (following the passage of the Dickey Act in 1949). Through extensive water sampling it was concluded that the entire Bay had become contaminated, due to heavy loading of domestic and industrial wastes. Dissolved oxygen concentrations in the Bay had declined to about half normal levels and turbidity in the water resulted in a visibility of less than 1 meter. Bait and game fish had virtually disappeared from the Bay. Coliform bacteria were routinely isolated from the Bay at significant levels. In 1955, the State Board of Public Health and the San Diego Department of Public Health declared much of the Bay contaminated, and posted quarantine and warning signs along 10 miles of shoreline. By 1963, sludge deposits from the treatment plant outfall were two meters deep, extended 200 meters seaward, and along 9000 meters of the shoreline.

A report in the early 1950's from the Regional Board and the San Diego Sewerage Survey report indicated sewage discharge into the Bay was becoming a major problem which had to be corrected. In 1960, San Diego voters approved a bond ($42.5 million) which allowed construction to begin on the Metropolitan Sewerage System. In August of 1963, a massive collection, treatment, and ocean disposal system began operation and by February, 1964, domestic sewage disposal had been eliminated from San Diego Bay. Following the completion of the new sewage treatment plant, dissolved oxygen concentrations rose to an average of more than 5 parts per million, visibility increased to 2 meters, and coliform bacteria counts dropped within the federal safety standards. Plankton blooms were scarce and sludge deposits of more than 30 cm were seldom reported. The sewage system currently processes 170 million gallons of waste per day (City of San Diego, 1995)

Routine sampling, beginning in the 1970's, revealed new information regarding the presence of industrial wastes in the Bay. Regulatory standards were developed for the protection of humans and wildlife based on new sampling systems and more refined analytical techniques. The conventional engineering and

bacteriological data gathered earlier did not adequately address the issue of toxic waste in the Bay. During the late 1980's, the press regarded San Diego Bay as being heavily contaminated, particularly for PCBs. Although conditions in the Bay are similar to other urban influenced embayments in the United States, San Diego Bay has serious problems with chemical pollution. A number of toxic hotspots in the Bay have been identified on lists of water quality impairment such as Clean Water Act Section 303(d), Section 319, Section 304(l) and Section 131.11.

Mission Bay

Mission Bay is located 9 kilometers north of Point Loma and encompasses an area of 1860 hectares. It has two main tributaries, Tecolote creek and Rose creek (Dexter, 1983). Originally named False Bay because its entrance was near San Diego Bay and occasionally fooled ship captains, it is now considered a recreational small-craft harbor (United States Coast Pilot, 1994). Prior to the development of Mission Bay park in 1946, Mission Bay was a natural estuary of over 2020 hectares of salt marshes, tidal channels, and a shallow central bay. Between 1946 and 1962 major dredging within the Bay and modifications to the San Diego River flood control channel gave way to its present-day configuration. Today it is a highly modified lagoon which receives freshwater input only during infrequent, heavy rains. The major additions of freshwater into Mission Bay occur at Rose Inlet, in the northeastern portion of the Bay, and Tecolote Creek, in the southeast. Because of this limited amount of freshwater, the salinities throughout the Bay do not change markedly. Mean tidal range is 1.2 meters and the mean diurnal range is 1.7 meters at the Bay entrance (Levin, 1983).

As a result of circulation patterns within Mission Bay, a variety of sediments are found. In the mouth of the Bay and near the main channel, water movement is sufficient to maintain a sandy bottom. In other parts of the Bay, such as Sail Bay and sites located further east, sediments are muddy with a high silt and clay content (Dexter, 1983).

Tecolote and Rose creeks carry urban pollutants such as oil, grease, fertilizers, and high sediment loads into the back bay. Furthermore, sewer lines back up occasionally into the back bay. The lack of water circulation in the back bay allows these pollutants to accumulate and has resulted in quarantines for several months at a time (Marcus, 1989).

Tijuana River Estuary

The Tijuana River Estuary is located 16 kilometers southeast of Point Loma. Although the estuary is situated entirely within the boundaries of San Diego County, three-fourths of its watershed is in Mexico. It is a wetland dominated estuary with no major embayment, however, a series of channels allows for a relatively narrow ocean connection (Herron, 1972). In the classification scheme developed by Prichard (1967), Tijuana Estuary is

considered an intermittent coastal plain estuary due to the large freshwater input during the winter wet season. During most years, the river mouth has been open and tidal flushing has prevailed. The intertidal area supports salt marsh vegetation (*Salicornia virginica, Spartina foliosa*), whereas mudflats and sandflats occupy only a small fraction of the estuary (Zedler *et al.*, 1992).

The Tijuana River Estuary has been altered substantially by natural and human disturbances. In the early 1900's, sewage disposal practices led to dredging of the east-west channel in order to connect an adjacent waste collecting lagoon with the estuary. Dikes were then created to subdivide the lagoon into three wastewater receiving ponds, however, these dikes were later removed to increase tidal flow. Gravel extraction for street and dike construction created isolated ponds within the estuary. Long-term dumping and filling altered most of the peripheral topography, while extensive damage to the southern half of the estuary from military, agricultural, and horse-raising activities is evident (Marcus, 1989).

Wastewater flow from Tijuana has been a serious threat to water quality in the estuary. In 1988, approximately 30 million gallons of sewage per day were produced while only 17 million gallons were collected. The remaining 13 million gallons emptied directly into the Tijuana River and estuary (Seamans, 1988). Breaks in the Tijuana sewer line, which carried collected sewage to an ocean outfall, were also common.

Recent U.S. projects have reduced the threat of sewage pollution. An interceptor on the Tijuana River, completed in early October 1991, diverts approximately 15 million gallons of sewage a day to the San Diego wastewater facility (Zedler, 1992). A sewage treatment plant is planned for the U.S. side of the border, and a new ocean outfall is under evaluation.

METHODS

Sampling Design

Two basic sampling designs were used to meet both SWRCB's and NOAA's goals. A directed point sampling design was required to address SWRCB's need to identify specific toxic hot spots. A stratified random sampling design was required to address NOAA's need to evaluate spatial extent of pollution. This has resulted in a data set of 350 samples collected between October, 1992 and May, 1994. Of the 350 total samples, 229 were collected from directed point sampled stations and 121 were collected from randomly sampled stations.

When directed point sampling design was required, a two step process was used. Areas of interest were identified, by regional and state water board staff, for sampling during an initial "screening phase". Station locations (latitude & longitude) were predetermined by agreement with the SWRCB, NOAA, Regional Water Quality Control Boards, and DFG personnel. Changing of the site location during sediment collection was allowed only under the following conditions:

1. Lack of access to predetermined site,
2. Inadequate or unusable sediment (i.e. rocks or gravel)
3. Unsafe conditions
4. Agreement of appropriate staff

This phase of work was intended to give a broad assessment of toxicity throughout the San Diego Bay area using multiple test species and toxicity endpoints. Fifty-six stations were sampled during the period between October, 1992 and January, 1993. Chemical analysis was performed on selected samples in which toxicity results prompted further analysis. Stations which met certain criteria during the screening phase, or during the random sampling phase, were then selected for a second round of sampling, termed the "confirmation phase". During this phase sampling was replicated and chemical analysis of samples was more extensive. In addition, benthic community analysis was performed on all confirmation stations sampled during the summer of 1993. Evidence from this two step process is used to establish a higher level of certainty for stations which may later be identified as "toxic hot spots".

Stratified random sampling began in March, 1993 and continued through August, 1993, with a total of 121 stations sampled. The San Diego Bay Region was stratified into areas of similar physical characteristics or uses, such as transit channels, anchorages, marinas, commercial shipping or military uses, and designated as 95 blocks of known size (Figures 2a & 2b). Station coordinates were chosen randomly within the boundaries of each sampling block by USEPA Environmental Monitoring and Assessment Program (USEPA-EMAP) personnel using a computer program developed for that purpose. Eight alternate locations were chosen for each block, a maximum of two of which were actually sampled (Weisberg *et al.*, 1993). This stratified random design "forces"

Figure 2a
Sampling Blocks for Random Stations
San Diego Bay

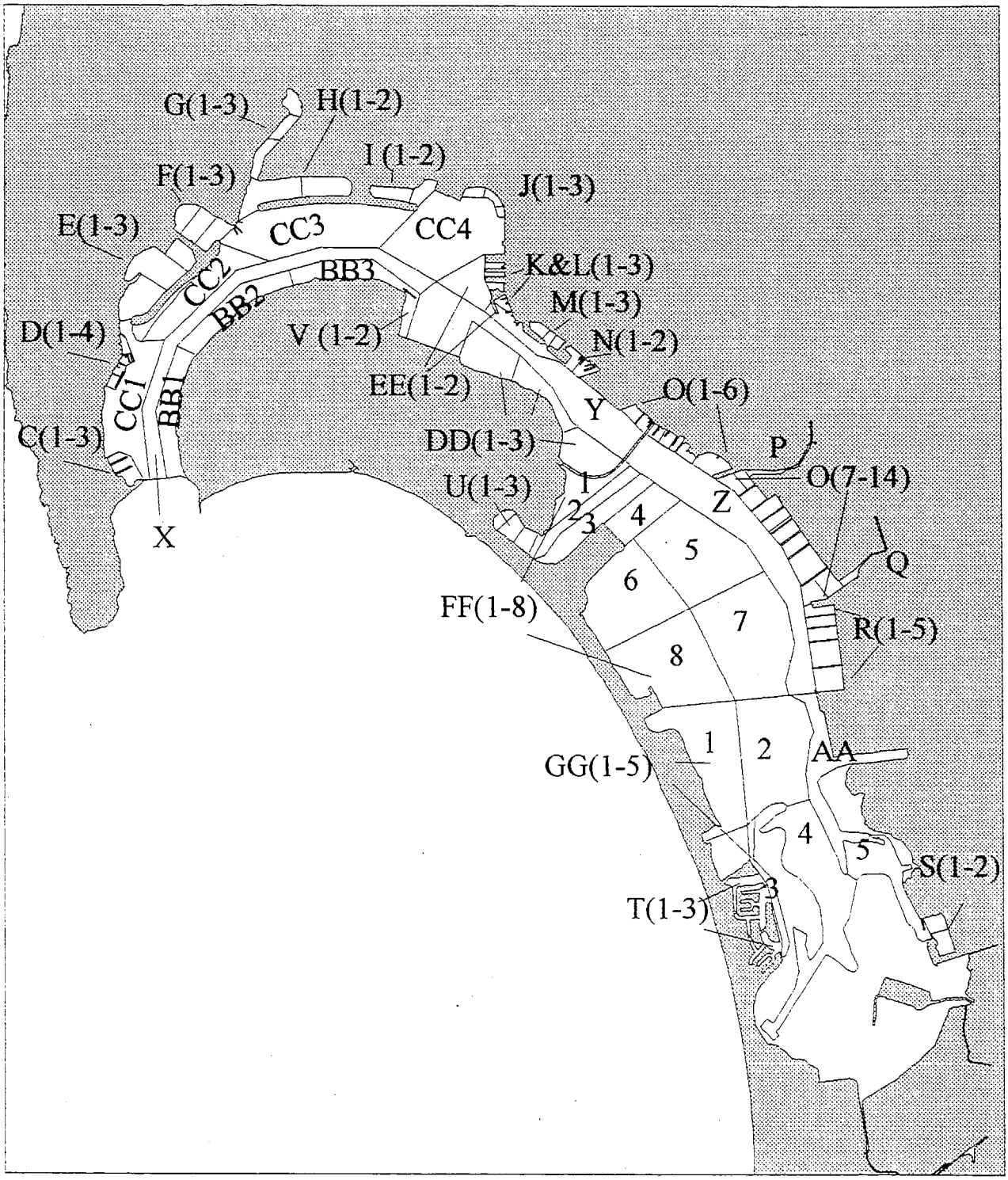
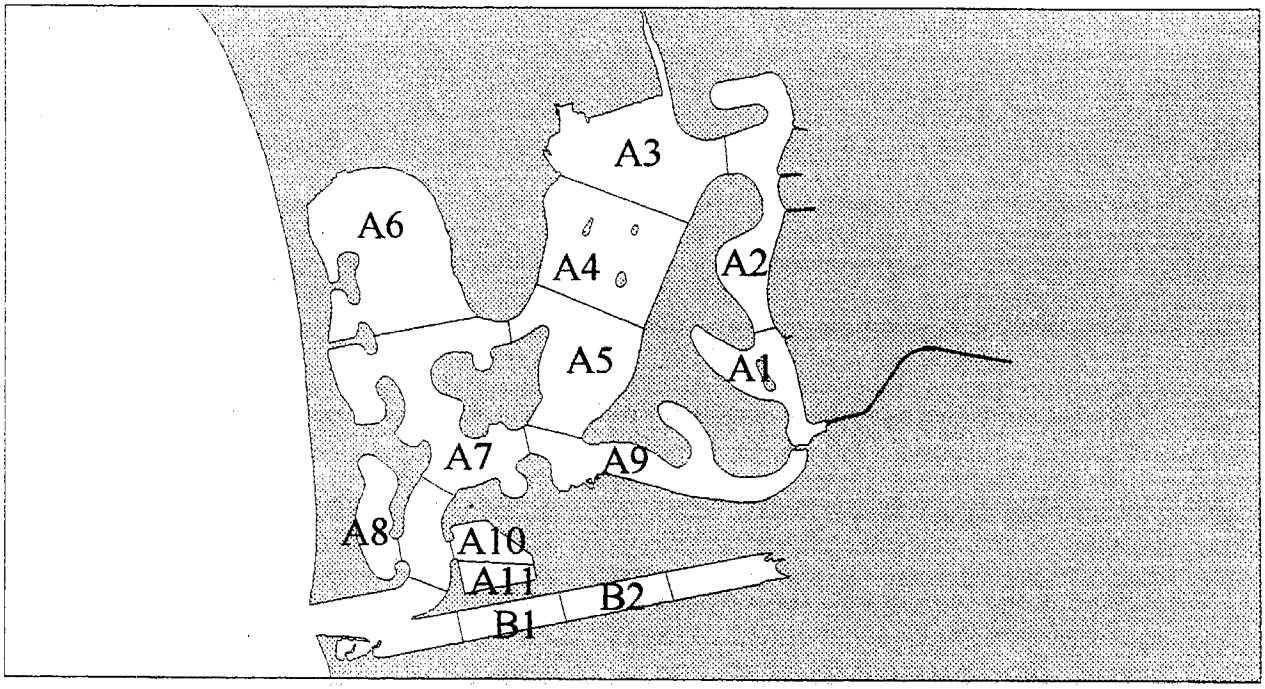
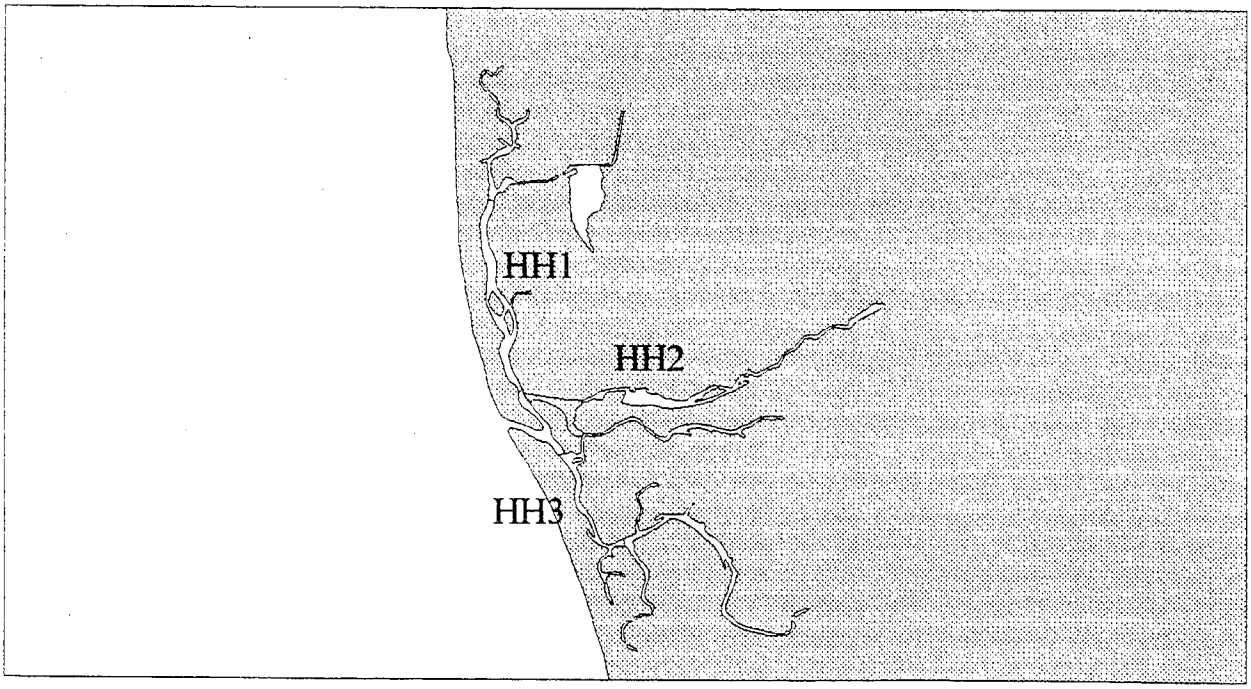


Figure 2b
Sampling Blocks for Random Stations
Mission Bay and San Diego River Estuary



Tijuana River Estuary



random samples to cover all areas of the Bay, whereas a pure random design most likely would miss some areas and oversample others. In the field, sampling was attempted at each designated location (x1-x8), beginning with x1, until a sample was retrieved which met sample acceptability criteria. For example, in block FF2, Station number 93124 was sampled at the random location x1 while in block FF3, Station #93172 was sampled at random location x4 because the grain size was too coarse at locations x1, x2 and x3. Of the 121 stations sampled, ≈15% could not be sampled at the random x1 location, due to the location being inaccessible by boat because of obstructions, vessel moorings, piers or shallow depths. Similarly, ≈3% were not sampled because the grain size was too coarse at the x1 location. Samples were collected successfully at alternate locations (x2, x3, x4, ...) for all stations where x1 was not sampled. This sampling design allows data from random stations to be used for calculation of areal extent of toxicity in the San Diego Bay Region. Chemical analyses were only performed on a limited number of random station samples.

From the combined sampling designs, a total of 350 samples were collected from 183 station locations in the San Diego Bay Region (Figure 3(a-d)). Station locations which were sampled more than once were always resampled at the original location using navigational equipment and lineups. Bioassay tests, grain size and total organic carbon analyses were performed on all 350 samples. Trace metal analysis was performed on 217 samples. Trace synthetic organic analysis was performed on 229 samples. Benthic community analysis was performed on 75 samples.

Sample Collection and Processing

Summary of Methods

Specific techniques used for collecting and processing samples are described in this section. Because collection of sediments influences the results of all subsequent laboratory and data analyses, it was important that samples be collected in a consistent and conventionally acceptable manner. Field and laboratory technicians were trained to conduct a wide variety of activities using standardized protocols to ensure comparability in sample collection among crews and across geographic areas. Sampling protocols in the field followed the accepted procedures of EMAP, NS&T, and ASTM and included methods to avoid cross-contamination; methods to avoid contamination by the sampling activities, crew, and vessel; collection of representative samples of the target surficial sediments; careful temperature control, homogenization and subsampling; and chain of custody procedures.

Cleaning Procedures

All sampling equipment (*i.e.*, containers, container liners, scoops, water collection bottles) was made from non-contaminating materials and was precleaned and packaged protectively prior to entering the field. Sample collection gear and samples were handled only by personnel wearing non-contaminating

Figure 3a
Sampling Locations
North San Diego Bay

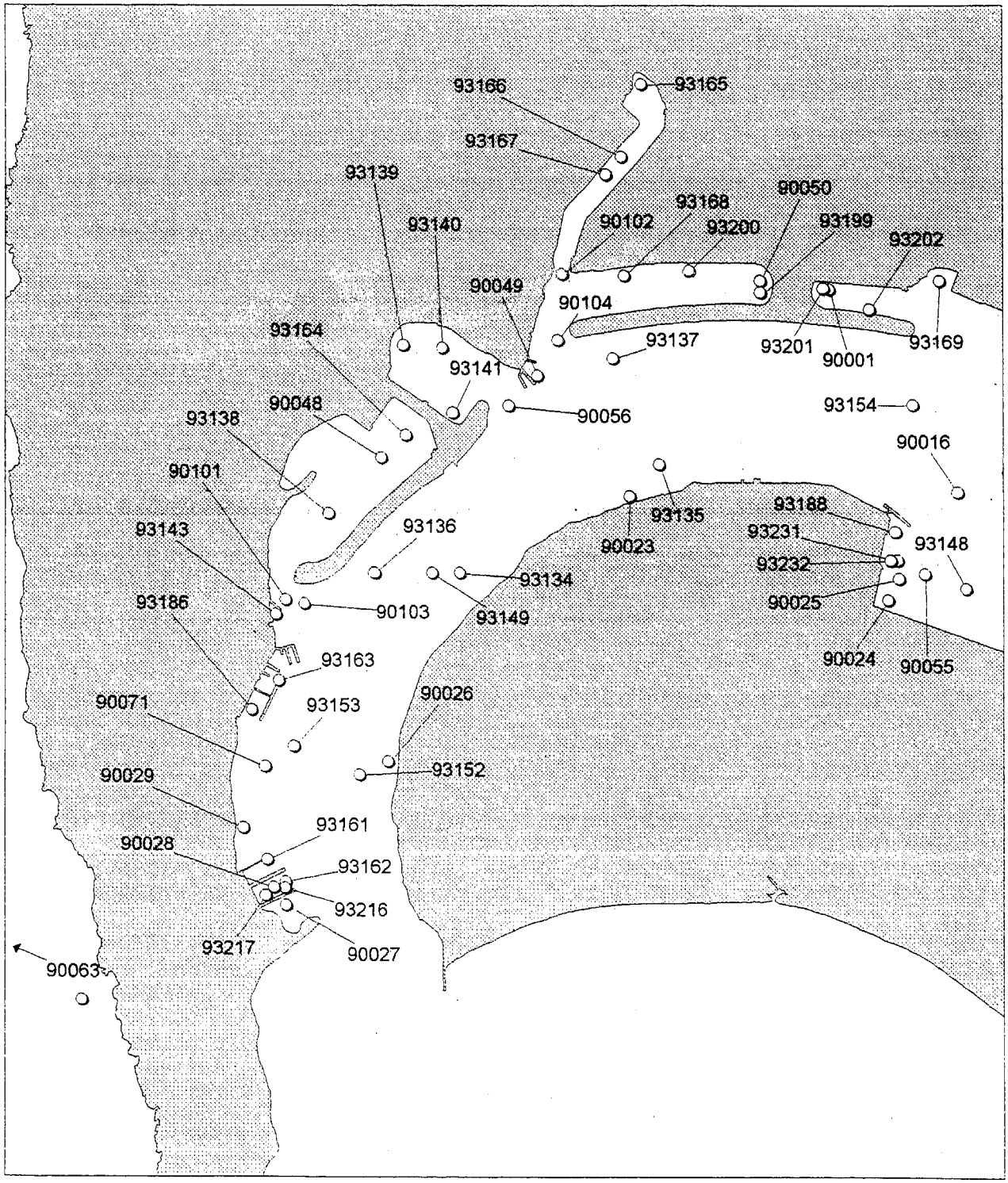


Figure 3b
Sampling Locations
Mid San Diego Bay

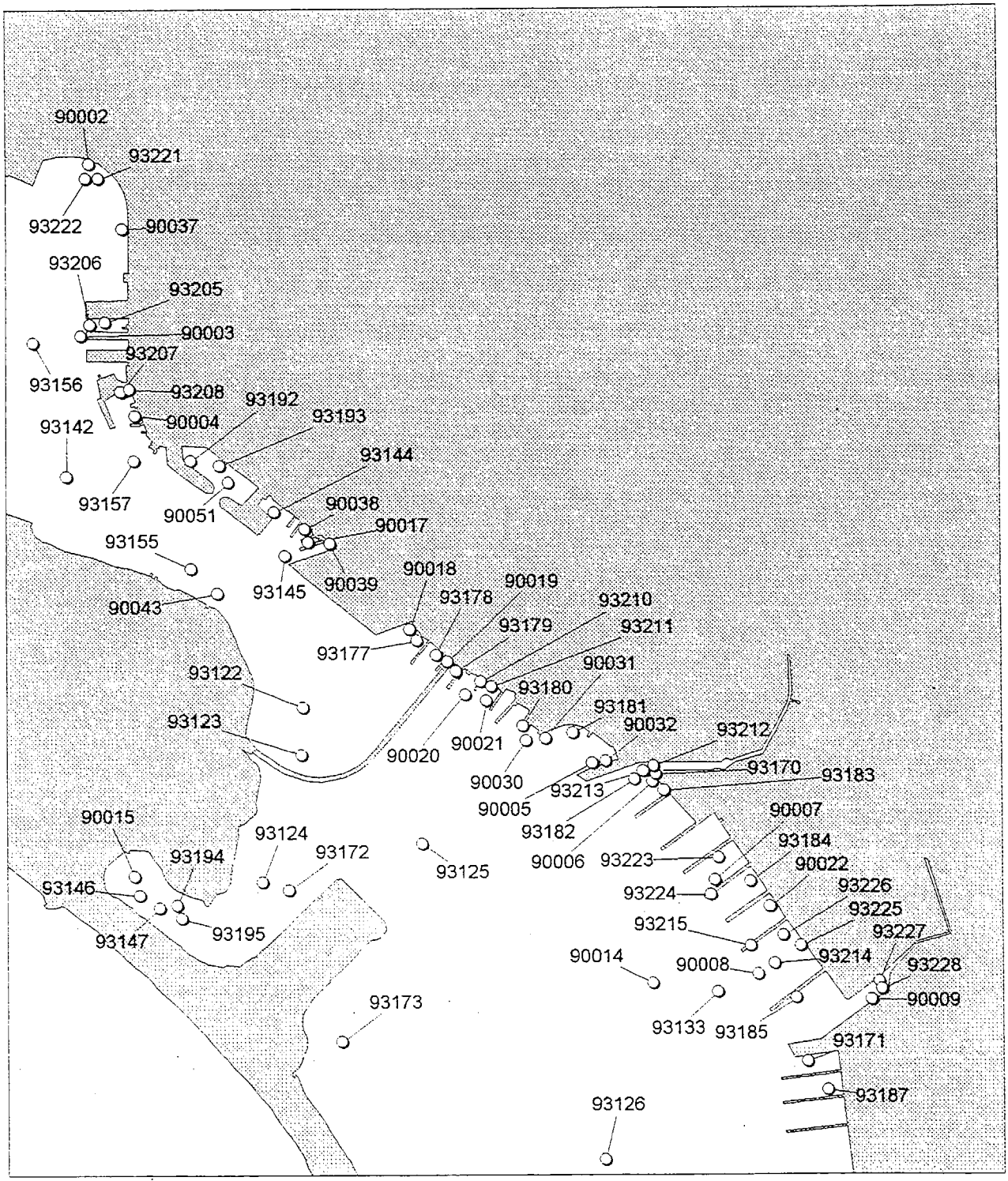


Figure 3c
Sampling Locations
South San Diego Bay

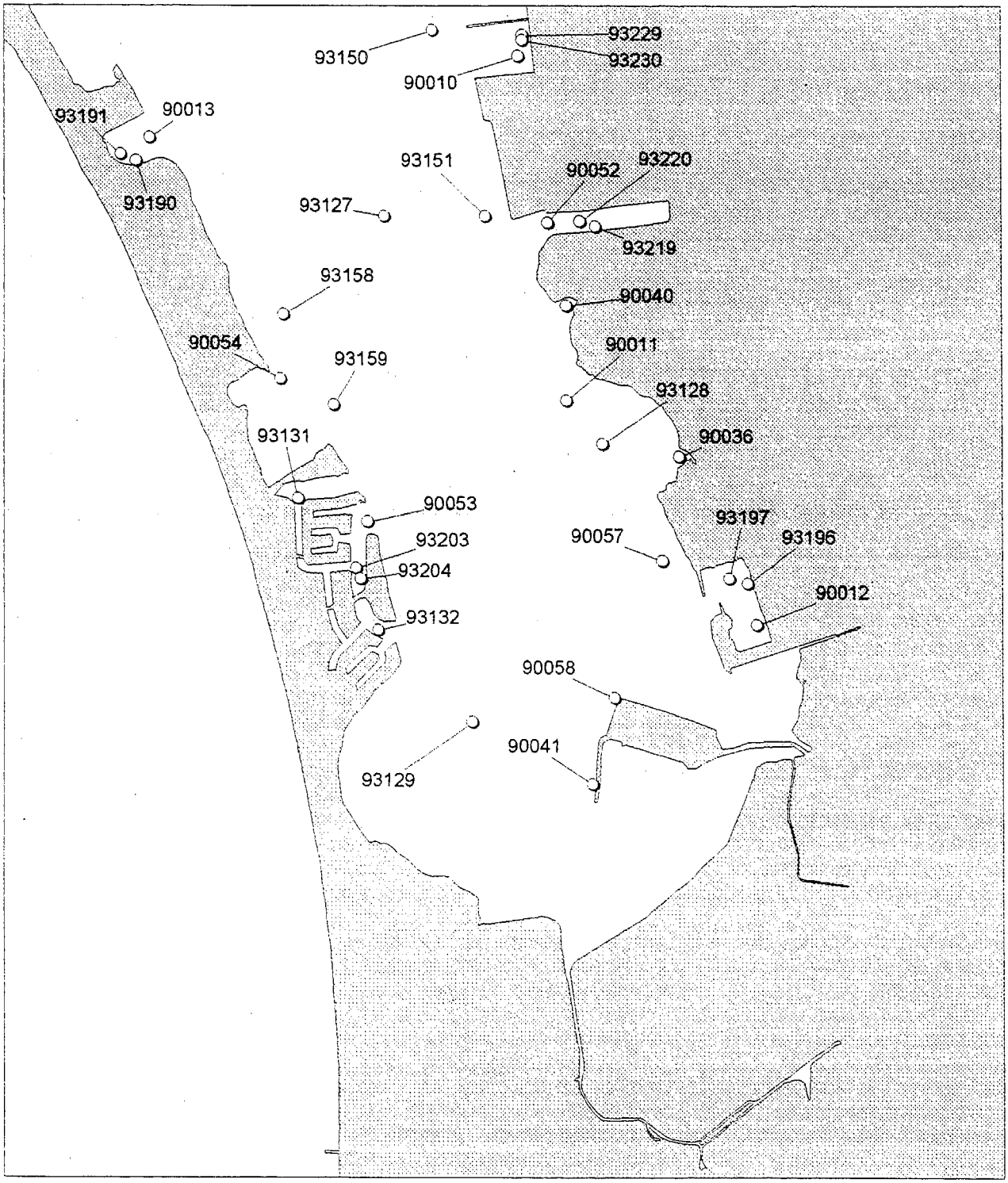
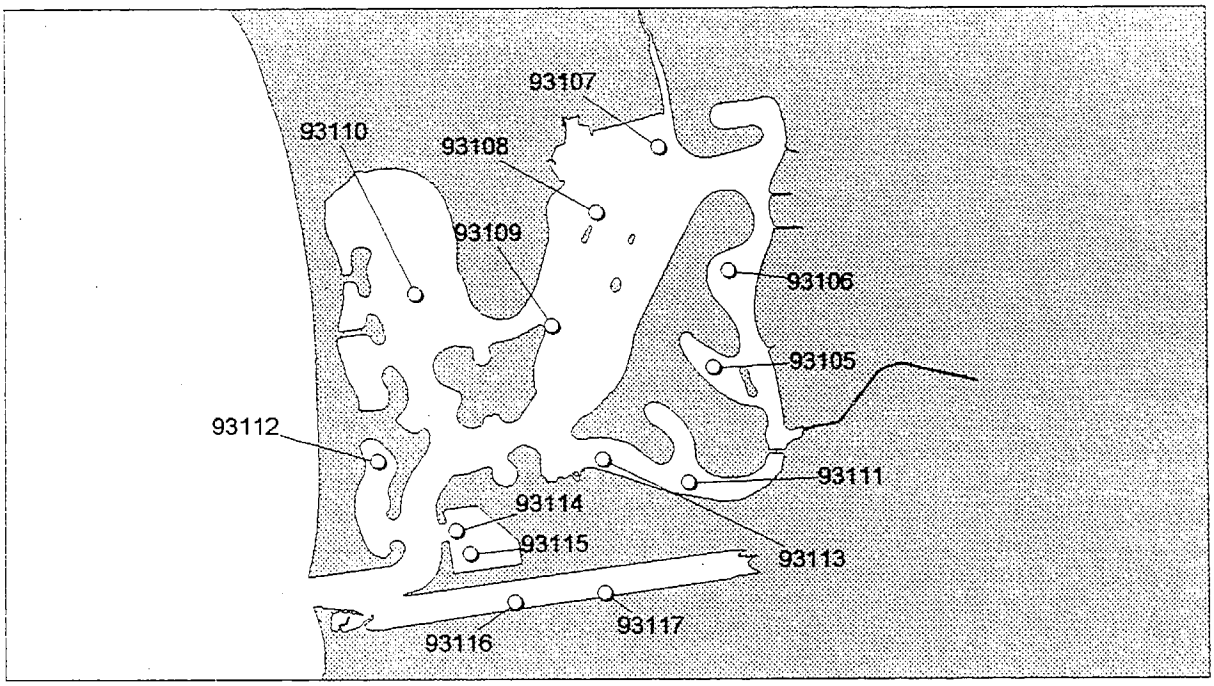
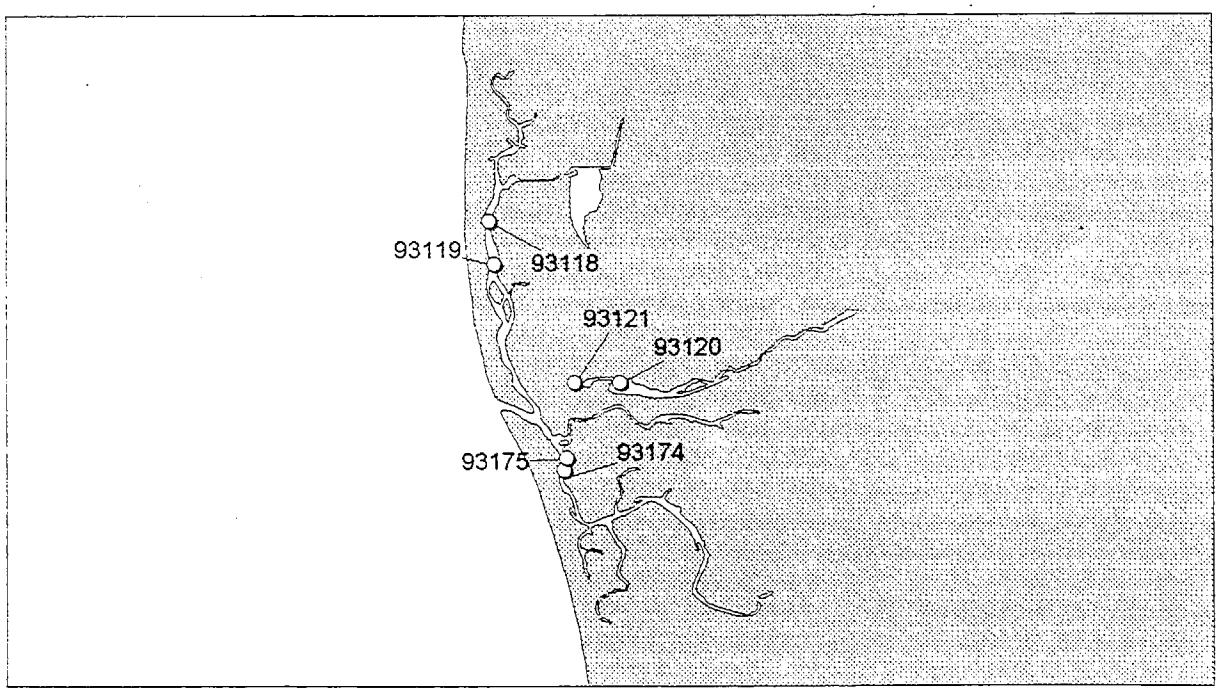


Figure 3d
Sampling Locations
Mission Bay and San Diego River Estuary



Tijuana River Estuary



polyethylene gloves. All sample collection equipment (excluding the sediment grab) was cleaned by using the following sequential process:

> Two-day soak and wash in Micro® detergent, three tap-water rinses, three deionized water rinses, a three-day soak in 10% HCl, three ASTM Type II Milli-Q® water rinses, air dry, three petroleum ether rinses, and air dry.

All cleaning after the Micro® detergent step was performed in a positive pressure "clean" room to prevent airborne contaminants from contacting sample collection equipment. Air supplied to the clean room was filtered.

The sediment grab was cleaned prior to entering the field, and between sampling stations, by utilizing the following sequential steps: a vigorous Micro® detergent wash and scrub, a sea-water rinse, a 10% HCl rinse, and a methanol rinse. The sediment grab was scrubbed with seawater between successive deployments at the same station to remove adhering sediments from contact surfaces possibly originating below the sampled layer.

Sample storage containers were cleaned in accordance with the type of analysis to be performed upon its contents. All containers were cleaned in a positive pressure "clean" room with filtered air to prevent airborne contaminants from contacting sample storage containers.

Plastic containers (HDPE or TFE) for trace metal analysis media (sediment, archive sediment, pore water, and subsurface water) were cleaned by: a two-day Micro® detergent soak, three tap-water rinses, three deionized water rinses, a three-day soak in 10% HCl or HNO_3, three Type II Milli-Q® water rinses, and air dry.

Glass containers for total organic carbon, grain size or synthetic organic analysis media (sediment, archive sediment, pore water, and subsurface water) and additional teflon sheeting cap-liners were cleaned by: a two-day Micro® detergent soak, three tap-water rinses, three deionized water rinses, a three-day soak in 10% HCl or HNO_3, three Type II Milli-Q® water rinses, air dry, three petroleum ether rinses, and air dry.

Sediment Sample Collection

All sampling locations (latitude & longitude), whether altered in the field or predetermined, were verified using a Magellan NAV 5000 Global Positioning System, and recorded in the field logbook. The primary method of sediment collection was by use of a $0.1m^2$ Young-modified Van Veen grab aboard a sampling vessel. Modifications include a non-contaminating Kynar coating which covered the grab's sample box and jaws. After the filled grab sampler was secured on the boat gunnel, the sediment sample was inspected carefully. The following acceptability criteria were met prior to taking sediment samples. If a sample did not meet all the criteria, it was rejected and another sample was collected.

1. Grab sampler was not over-filled (*i.e.*, the sediment surface was not pressed against the top of the grab).
2. Overlying water was present, indicating minimal leakage.
3. Overlying water was not excessively turbid, indicating minimal sample disturbance.
4. Sediment surface was relatively flat, indicating minimal sample disturbance.
5. Sediment sample was not washed out due to an obstruction in the sampler jaws.
6. Desired penetration depth was achieved (*i.e.*, 10 cm).
7. Sample was muddy (>30% fines), not sandy or gravelly.
8. Sample did not include excessive shell, organic or man-made debris.

It was critical that sample contamination be avoided during sample collection. All sampling equipment (*i.e.*, siphon hoses, scoops, containers) was made of non-contaminating material and was cleaned appropriately before use. Samples were not touched with un-gloved fingers. In addition, potential airborne contamination (*e.g.*, from engine exhaust, cigarette smoke) was avoided. Before sub-samples from the grab sampler were taken, the overlying water was removed by slightly opening the sampler, being careful to minimize disturbance or loss of fine-grained surficial sediment. Once overlying water was removed, the top 2 cm of surficial sediment was sub-sampled from the grab. Subsamples were taken using a precleaned flat bottom scoop. This device allowed a relatively large sub-sample to be taken from a consistent depth. When subsampling surficial sediments, unrepresentative material (e.g., large stones or vegetative material) was removed from the sample in the field. Small rocks and other small foreign material remained in the sample. Determination of overall sample quality was determined by the chief scientist in the field. Such removals were noted on the field data sheet. For the sediment sample, the top 2 cm was removed from the grab and placed in a pre-labeled polycarbonate container. Between grabs or cores, the sediment sample in the container was covered with a teflon sheet, and the container covered with a lid and kept cool. When a sufficient amount of sediment was collected, the sample was covered with a teflon sheet assuring no air bubbles. A second, larger teflon sheet was placed over the top of the container to ensure an air tight seal, and nitrogen was vented into the container to purge it of oxygen.

If water depth did not permit boat entrance to a site (*e.g.*, <1 meter), divers sampled that site using sediment cores (diver cores). Cores consisted of a 10 cm diameter polycarbonate tube, 30 cm in length, including plastic end caps to aid in transport. Divers entered a study site from one end and sampled in one direction, so as to not disturb the sediment with feet or fins. Cores were taken to a depth of at least 15 cm. Sediment was extruded out of the top end of the core to the prescribed depth of 2-cm, removed with a polycarbonate spatula and deposited into a cleaned polycarbonate tub. Additional samples were taken with the same seawater rinsed core tube until the required total sample volume was attained. Diver core samples were treated the

same as grab samples, with teflon sheets covering the sample and nitrogen purging. All sample acceptability criteria were met as with the grab sampler.

Replicate benthic samples (n=5) were obtained at predetermined sites from separate deployments of the sampler. Three of the replicates were positioned according to the BPTCP sampling protocol (*e.g.*, located by previously assigned lat/long coordinates), while the other two replicates were chosen within the location range of the previous three samples. The coring device was 10 cm in diameter and 14 cm in height, enclosing a 0.0075 m^2 area. Corers were placed into sediment with minimum disruption of the surface sediments, capturing essentially all surface-active fauna as well as species living deeper in the sediment. Corers were pushed about 12 cm into the sediment and retrieved by digging along one side, removing the corer and placing the intact sediment core into a pvc screening device. Sediment cores were sieved through a 0.5 mm screen and residues (*e.g.*, organisms and remaining sediments) were rinsed into pre-labeled storage bags and preserved with a 10% formalin solution. After 3 to 4 days, samples were rinsed and transferred into 70% isopropyl alcohol and stored for future taxonomy and enumeration.

Transport of Samples
Six-liter sample containers were packed (three to an ice chest) with enough ice to keep them cool for 48 hours. Each container was sealed in precleaned, large plastic bags closed with a cable tie to prevent contact with other samples or ice or water. Ice chests were driven back to the laboratory by the sampling crew or flown by air freight within 24 hours of collection.

Homogenization and Aliquoting of Samples
Samples remained in ice chests (on ice, in double-wrapped plastic bags) until the containers were brought back to the laboratory for homogenization. All sample identification information (station numbers, etc.) was recorded on Chain of Custody (COC) and Chain of Record (COR) forms prior to homogenizing and aliquoting. A single container was placed on plastic sheeting while also remaining in original plastic bags. The sample was stirred with a polycarbonate stirring rod until mud appeared homogeneous.

All prelabeled jars were filled using a clean teflon or polycarbonate scoop and stored in freezer/refrigerator (according to media/analysis) until analysis. The sediment sample was aliquoted into appropriate containers for trace metal analysis, organic analysis, pore water extraction, and bioassay testing. Samples were placed in boxes sorted by analysis type and leg number. Sample containers for sediment bioassays were placed in a refrigerator (4°C) while sample containers for sediment chemistry (metals, organics, TOC and grain size) were stored in a freezer (-20°C).

Procedures for the Extraction of Pore Water
The BPTCP primarily used whole core squeezing to extract pore

water. The whole core squeezing method, developed by Bender *et al.* (1987), utilizes low pressure mechanical force to squeeze pore water from interstitial spaces. The following squeezing technique was a modification of the original Bender design with some adaptations based on the work of Fairey (1992), Carr *et al.* (1989), and Long and Buchman (1989). The squeezer's major features consist of an aluminum support framework, 10 cm i.d. acrylic core tubes with sampling ports and a pressure regulated pneumatic ram with air supply valves. Acrylic subcore tubes were filled with approximately 1 liter of homogenized sediment and pressure was applied to the top piston by adjusting the air supply to the pneumatic ram. At no time during squeezing did air pressure exceed 200 psi. A porous prefilter (PPE or TFE) was inserted in the top piston and used to screen large (> 70 microns) sediment particles. Further filtration was accomplished with disposable TFE filters of 5 microns and 0.45 microns in-line with sample effluent. Sample effluent of the required volume was collected in TFE containers under refrigeration. Pore water was subsampled in the volumes and specific containers required for archiving, chemical or toxicological analysis. To avoid contamination, all sample containers, filters and squeezer surfaces in contact with the sample were plastics (acrylic, PVC, and TFE) and cleaned with previously discussed clean techniques.

Chain of Records & Custody
Chain-of-records documents were maintained for each station. Each form was a record of all sub-samples taken from each sample. IDORG (a unique identification number for only that sample), station numbers and station names, leg number (sample collection trip batch number), and date collected were included on each sheet. A Chain-of-Custody form accompanied every sample so that each person releasing or receiving a subsample signed and dated the form.

Authorization/Instructions to Process Samples
Standardized forms entitled "Authorization/Instructions to Process Samples" accompanied the receipt of any samples by any participating laboratory. These forms were completed by DFG personnel, or its authorized designee, and were signed and accepted by both the DFG authorized staff and the staff accepting samples on behalf of the particular laboratory. The forms contain all pertinent information necessary for the laboratory to process the samples, such as the exact type and number of tests to run, number of laboratory replicates, dilutions, exact eligible cost, deliverable products (including hard and soft copy specifications and formats), filenames for soft copy files, expected date of submission of deliverable products to DFG, and other information specific to the lab/analyses being performed.

Trace Metals Analysis of Sediments

Summary of Methods
Trace Metals analyses were conducted at the California Department of Fish and Game's (CDFG) Trace Metals Facility at Moss Landing, CA. Table 1 indicates the trace metals analyzed and lists method

detection limits for sediments. These methods were modifications of those described by Evans and Hanson (1993) as well as those developed by the CDFG (California Department of Fish and Game, 1990). Samples were selected for chemical analyses by SWRCB staff based on results from toxicity tests.

Analytes and Detection Limits
Table 1 - Trace Metal Detection Limits in Sediments (µg/g, dry weight).

Aluminum	1	Antimony	0.1
Arsenic	0.1	Cadmium	0.01
Chromium	0.1	Copper	0.1
Iron	0.1	Lead	0.1
Manganese	0.05	Mercury	0.03
Nickel	0.1	Selenium	0.2
Silver	0.01	Tin	0.02
Tributyltin	0.013	Zinc	0.05

Sediment Digestion Procedures
One gram aliquot of sediment was placed in a pre-weighed Teflon vessel, and one ml concentrated 4:1 nitric:perchloric acid mixture was added. The vessel was capped and heated in a vented oven at 130° C for four hours. Three ml Hydrofluoric acid were added to vessel, recapped and returned to oven overnight. Twenty ml of 2.5% boric acid were added to vessel and placed in oven for an additional 8 hours. Weights of vessel and solution were recorded, and solution transfered to 30 ml polyethylene bottles.

Atomic Absorption Methods
Samples were analyzed by furnace AA on a Perkin-Elmer Zeeman 3030 Atomic Absorption Spectrophotometer, with an AS60 auto sampler, or a flame AA Perkin Elmer Model 2280. Samples, blanks, matrix modifiers, and standards were prepared using clean techniques inside a clean laboratory. ASTM Type II water and ultra clean chemicals were used for all standard preparations. All elements were analyzed with platforms for stabilization of temperatures. Matrix modifiers were used when components of the matrix interferes with adsorption. The matrix modifier was used for Sn, Sb and Pb. Continuing calibration check standards (CLC) were analyzed with each furnace sheet, and calibration curves were run with three concentrations after every 10 samples. Blanks and standard reference materials, MESS1, PACS, BCSS1 or 1646 were analyzed with each set of samples for sediments.

Trace Organic Analysis of Sediments (PCBs, Pesticides, and PAHs)

Summary of Methods
Analytical sets of 12 samples were scheduled such that extraction and analysis will occur within a 40 day window. The methods employed by the UCSC-TOF were modifications of those described by Sloan *et al.* (1993). Tables 2 and 3 indicate the pesticides, PCBs, and PAHs currently analyzed and list method detection limits for sediments on a dry weight basis.

Analytes and Detection Limits

Table 2. Organochlorine Pesticides Analyzed and Their Detection Limits in Sediment, ng/g dry weight.

Aldrin	0.5
cis-Chlordane	0.5
trans-Chlordane	0.5
alpha-Chlordene	0.5
gamma-Chlordene	0.5
Chlorpyrifos	1.0
Dacthal	0.2
o,p'-DDD	1.0
p,p'-DDD	0.4
o,p'-DDE	1.0
p,p'-DDE	1.0
p,p'-DDMS	3.0
p,p'-DDMU	2.0
o,p'-DDT	1.0
p,p'-DDT	1.0
p,p'-Dichlorobenzophenone	3.0
Dieldrin	0.5
Endosulfan I	0.5
Endosulfan II	1.0
Endosulfan sulfate	2.0
Endrin	2.0
Ethion	2.0
alpha-HCH	0.2
beta-HCH	1.0
gamma-HCH	0.2
delta-HCH	0.5
Heptachlor	0.5
Heptachlor Epoxide	0.5
Hexachlorobenzene	0.2
Methoxychlor	1.5
Mirex	0.5
cis-Nonachlor	0.5
trans-Nonachlor	0.5
Oxadiazon	2.0
Oxychlordane	0.5
Toxaphene	10

Table 3. PCB Congeners and PAHs Analyzed and Their Detection Limits in Sediment, ng/g dry weight.

NIST Congeners:

PCB Congener 8	PCB Congener 128
PCB Congener 18	PCB Congener 138
PCB Congener 28	PCB Congener 153
PCB Congener 44	PCB Congener 170
PCB Congener 52	PCB Congener 180
PCB Congener 66	PCB Congener 187
PCB Congener 87	PCB Congener 195
PCB Congener 101	PCB Congener 206

Table 3 (cont.). PCB Congeners and PAHs Analyzed and Their Detection Limits in Sediment, ng/g dry weight.

PCB Congener 105	PCB Congener 209
PCB Congener 118	

Additional Congeners:

PCB Congener 5	PCB Congener 137
PCB Congener 15	PCB Congener 149
PCB Congener 27	PCB Congener 151
PCB Congener 29	PCB Congener 156
PCB Congener 31	PCB Congener 157
PCB Congener 49	PCB Congener 158
PCB Congener 70	PCB Congener 174
PCB Congener 74	PCB Congener 177
PCB Congener 95	PCB Congener 183
PCB Congener 97	PCB Congener 189
PCB Congener 99	PCB Congener 194
PCB Congener 110	PCB Congener 201
PCB Congener 132	PCB Congener 203

All individual PCB Congener detection limits were 1 ng/g dry weight.

Aroclors:

Aroclor 5460	50

Polycyclic Aromatic Hydrocarbons

Naphthalene	5
2-Methylnaphthalene	5
1-Methylnaphthalene	5
Biphenyl	5
2,6-Dimethylnaphthalene	5
Acenaphthylene	5
Acenaphthene	5
2,3,5-Trimethylnaphthalene	5
Fluorene	5
Phenanthrene	5
Anthracene	5
1-Methylphenanthrene	5
Fluoranthrene	5
Pyrene	5
Benz[a]anthracene	5
Chrysene	5
Benzo[b]fluoranthrene	5
Benzo[k]fluoranthrene	5
Benzo[e]pyrene	5
Benzo[a]pyrene	5
Perylene	5
Indo[1,2,3-cd]pyrene	5
Dibenz[a,h]anthracene	5
Benzo[ghi]perylene	5

Extraction and Analysis
Samples were removed from the freezer and allowed to thaw. A 10 gram sample of sediment was removed for chemical analysis and an independent 10 gram aliquot was removed for dry weight determinations. The dry weight sample was placed into a pre-weighed aluminum pan and dried at 110°C for 24 hours. The dried sample was reweighed to determine the sample's percent moisture. The analytical sample was extracted 3 times with methylene chloride in a 250-mL amber Boston round bottle on a modified rock tumbler. Prior to rolling, sodium sulfate, copper, and extraction surrogates were added to the bottle. Sodium sulfate dehydrates the sample allowing for efficient sediment extraction. Copper, which was activated with hydrochloric acid, complexes free sulfur in the sediment.

After combining the three extraction aliquots, the extract was divided into two portions, one for chlorinated hydrocarbon (CH) analysis and the other for polycyclic aromatic hydrocarbon (PAH) analysis.

The CH portion was eluted through a silica/alumina column, separating the analytes into two fractions. Fraction 1 (F1) was eluted with 1% methylene chloride in pentane and contains > 90% of p,p'-DDE and < 10% of p,p'-DDT. Fraction 2 (F2) analytes were eluted with 100% methylene chloride. The two fractions were exchanged into hexane and concentrated to 500 µL using a combination of rotary evaporation, controlled boiling on tube heaters, and dry nitrogen blow downs.

F1 and F2 fractions were analyzed on Hewlett-Packard 5890 Series gas chromatographs utilizing capillary columns and electron capture detection (GC/ECD). A single 2 µl splitless injection was directed onto two 60m x 0.25mm i.d. columns of different polarity (DB-17 & DB-5; J&W Scientific) using a glass Y-splitter to provide a two dimensional confirmation of each analyte. Analytes were quantified using internal standard methodologies. The extract's PAH portion was eluted through a silica/alumina column with methylene chloride. It then underwent additional cleanup using size-exclusion high performance liquid chromatography (HPLC/SEC). The collected PAH fraction was exchanged into hexane and concentrated to 250 µL in the same manner as the CH fractions.

Total Organic Carbon Analysis of Sediments

Summary of Methods
Samples were received in the frozen state and allowed to thaw at room temperature. Source samples were gently stirred and sub-samples were removed with a stainless steel spatula and placed in labeled 20 ml polyethylene scintillation vials. Approximately 5 grams equivalent dry weight of the wet sample was sub-sampled.

Sub-samples were treated with two, 5 ml additions of 0.5 N, reagent grade HCl to remove inorganic carbon (CO^{-3}), agitated, and centrifuged to a clear supernate. Some samples were retreated

with HCl to remove residual inorganic carbon. The evolution of gas during HCl treatment indicates the direct presence of inorganic carbon (CO^{-3}). After HCl treatment and decanting, samples were washed with approximately 15 ml of deionized-distilled water, agitated, centrifuged to a clear supernate, and decanted. Two sample washings were required to remove weight determination and analysis interferences.

Prepared samples were placed in a 60° C convection oven and allowed to come to complete dryness (approx. 48 hrs.). Visual inspection of the dried sample before homogenization was used to ensure complete removal of carbonate containing materials, (shell fragments). Two 61 mm (1/4") stainless steel solid balls were added to the dried sample, capped and agitated in a commercially available ball mill for three minutes to homogenize the dried sample.

A modification of the high temperature combustion method, utilizing a Weatstone bridge current differential was used in a commercially available instrument, (Control Equipment Co., 440 Elemental Analyzer) to determine carbon and nitrogen concentrations. The manufactures suggested procedures were followed. The methods are comparable to the validation study of USEPA method MARPCPN I. Two to three aliquotes of 5-10 mg of dried prepared sub-sample were used to determine carbon and nitrogen weight percent values. Calibration of the instrument was with known standards using Acetanilide or L-Cystine. Detection limits are 0.2 ug/mg, carbon and 0.01 ug/mg nitrogen dry weight.

The above methods and protocols are modifications of several published papers, reference procedures and analytical experimentation experience (Franson, 1981; Froelich, 1980; Hedges and Stern, 1983; MARPCPN I, 1992).

Quality Control/Quality Assurance

Quality control was tested by the analysis of National Research Council of Canada Marine Sediment Reference Material, BCSS-1 at the beginning and end of each sample analysis set (20-30 individual machine analyses). All analyzed values were within suggested criteria of $\pm$ 0.09% carbon (2.19% Average). Nitrogen was not reported on the standard data report, but was accepted at $\pm$ 0.008% nitrogen (0.195% Average) from the EPA study. Quality assurance was monitored by re-calibration of the instrument every twenty samples and by the analysis of a standard as a unknown and comparing known theoretical percentages with resultant analyzed percentages. Acceptable limits of standard unknowns were less than $\pm$ 2%. Duplicate or triplicate sample analysis variance (standard deviation/mean) greater than 7% is not accepted. Samples were re-homogenized and re-analyzed until the variance between individual runs fell below the acceptable limit of 7.0%.

Grain Size Analysis of Sediments

Summary of Methods
The procedure used combined wet and dry sieve techniques to determine particle size of sediment samples. Methods follow those of Folk (1974).

Sample Splitting and Preparation
Samples were thawed and thoroughly homogenized by stirring with a spatula. Spatulas were rinsed of all adhering sediment between samples. Size of the subsample for analysis was determined by the sand/silt ratio of the sample. During splitting, the sand/silt ratio was estimated and an appropriate sample weight was calculated. Subsamples were placed in clean, pre-weighed beakers. Debris was removed and any adhering sediment was washed into the beaker.

Wet Sieve Analysis (separation of coarse and fine fraction)
Beakers were placed in a drying oven and sediments were dried at less than 55°C until completely dry (approximately three days). Beakers were removed from drying oven and allowed to equilibrate to room temperature for a least a half-hour. Each beaker and its contents were weighed to the nearest .01 g. This weight minus the empty beaker weight was the total sample weight. Sediments in beakers were disaggregated using 100 ml of a dispersant solution in water (such as 50g Calgon/L water) and the sample was stirred until completely mixed and all lumps disappear. The amount and concentration of dispersant used was recorded on the data sheet for each sample. Sample beakers were placed in an ultrasonic cleaner for 15 minutes for disaggregation. Sediment dispersant slurry was poured into a 63 μm (ASTM #230, 4 phi) stainless steel or brass sieve in a large glass funnel suspended over a 1L hydrometer cylinder by a ring stand. All fine sediments were washed through the sieve with water. Fine sediments were captured in a 1L hydrometer cylinder. Coarse sediments remaining in sieve were collected and returned to the original sample beaker for quantification.

Dry Sieve Analysis (coarse fraction)
The coarse fraction was placed into a preweighed beaker, dried at 55-65°C, allowed to acclimate, and then weighed to 0.01 g. This weight, minus the empty beaker weight, was the coarse fraction weight. The coarse fraction was poured into the top sieve of a stack of ASTM sieves having the following sizes: No. 10 (2.0 mm), 18 (1.0 mm), 45 (0.354 mm), 60 (0.25 mm), 80 (0.177 mm), 120 (0.125 mm), and 170 (0.088 mm). The stack was placed on a mechanical shaker and shaken at medium intensity for 15 minutes. After shaking, each sieve was inverted onto a large piece of paper and tapped 5 times to free stuck particles. The sieve fractions were added cumulatively to a weighing dish, and the cumulative weight after each addition determined to 0.01g. The sample was returned to its original beaker, and saved until sample computations were completed and checked for errors.

Analytical Procedures
Fractional weights and percentages for various particle size fractions were calculated. If only wet sieve analysis was used, weight of fine fraction was computed by subtracting coarse fraction from total sample weight, and percent fine composition was calculated using fine fraction and total sample weights. If dry sieve was employed as well, fractional weights and percentages for the sieve were calculated using custom software on a Macintosh computer. Calibration factors were stored in the computer.

Benthic Community Analysis

Summary of Methods
Each catalogued sample was processed individually in the laboratory to obtain an accurate assessment of species diversity and abundance. All macroinvertebrates were sorted from residues under a dissecting microscope, identified to lowest possible taxon, and counted. Laboratory processing of benthic cores consists of both rough and fine sorting. Initial sorting separates animals into large taxonomic groups such as polychaetes, crustaceans, mollusks and other (*e.g.*, phoronids). Bound laboratory logbooks were maintained and used to record number of samples processed by each technician, as well as results of any sample resorts, if necessary. Sorters were required to sign and date a Milestone Progress Checksheet for each replicate sample processed. Specimens of similar taxonomic groups were placed in vials and labelled internally and externally with project, date collected, site/station information, and IDORG. Samples were selected for benthic community analysis by SWRCB staff based on results from toxicity tests.

In-house senior taxonomists and outside specialists processed and verified the accuracy of species identification and enumeration. An archived voucher specimen collection was established at this time.

Toxicity Testing

Summary of Methods
All toxicity tests were conducted at the California Department of Fish and Game's Marine Pollution Studies Laboratory (MPSL) at Granite Canyon. Toxicity tests were conducted by personnel from the Institute of Marine Sciences, University of California, Santa Cruz.

Pore Water Samples
Once at MPSL, frozen pore water samples were stored in the dark, at -12°C, until required for testing. Experiments performed by the U.S. National Biological Survey have shown no effects of freezing porewater upon the results of toxicity tests (Carr *et al.*, 1995). Samples were thawed on the day of a test, and pH, temperature, salinity, and dissolved oxygen were measured in all samples to verify water quality criteria were within the limits

defined for test protocol. Pore water samples with salinities outside specified ranges for each protocol were adjusted to within the acceptable range. Salinities were increased by the addition of hypersaline brine, 60 to 80 parts per thousand (ppt), drawn from partially frozen seawater. Dilution water consisted of Granite Canyon seawater (32 to 34 ppt). Water quality parameters were measured at the beginning and end of each test. Dissolved oxygen concentrations and pH were measured using an Orion EA940 expandable ion analyzer. Salinity was measured with a refractometer. Temperature of each sample was measured with a mercury thermometer.

Measurement of Ammonia and Hydrogen Sulfide

Total ammonia concentrations were measured using an Orion Model 95-12 Ammonia Electrode. The concentration of unionized ammonia was derived from the concentration of total ammonia using the following equation (from Whitfield 1974, 1978):

$$[NH_3] = [\text{total ammonia}] \times ((1 + \text{antilog}(pK_a^\circ - pH))^{-1}),$$

where pK_a° is the stoichiometric acidic hydrolysis constant for the test temperature and salinity. Values for pK_a° were experimentally derived by Khoo *et al.* (1977). The method detection limit for total ammonia was 0.1 mg/L.

Total sulfide concentrations were measured using an Orion Model 94-16 Silver/Sulfide Electrode, except that samples tested after February, 1994, were measured on a spectrophotometer using a colorimetric method (Phillips et al. in press). The concentration of hydrogen sulfide was derived from the concentration of total sulfide by using the following equation (ASCE 1989):

$$[H_2S] = [S^{2-}] \times (1 - ((1 + \text{antilog}(pK_a^\circ - pH))^{-1})),$$

where temperature and salinity dependent pK_a° values were taken from Savenko (1977). The method detection limit for total sulfide was 0.1 mg/L for the electrode method, and 0.01 mg/L for the colorimetric method. Values and corresponding detection limits for unionized ammonia and hydrogen sulfide were an order of magnitude lower than those for total ammonia and total sulfide, respectively.

Subsurface Water Samples

The subsurface water toxicity tests are water column toxicity tests (abalone development, mussel development, etc..) performed on water collected with the modified Van Veen grab. A water sample bottle on the frame of the grab and a stopper is pulled as the jaws of the grab close for a sediment sample. The water sample is consequently collected approximately 0.5 meters above the bottom. Subsurface water samples were held in the dark at 4°C until testing. Toxicity tests were initiated within 14 days of the sample collection date. Water quality parameters, including ammonia and sulfide concentrations, were measured in one replicate test container from each sample in the overlying water

as described above. Measurements were taken at the beginning and end of all tests.

Sediment Samples
Bedded sediment samples were held at 4°C until required for testing. All *Rhepoxynius abronius* and *Neanthes arenaceodentata* solid phase sediment tests were initiated within 14 days of the sample collection date. All sediment samples were processed according to procedures described in ASTM (1992). Water quality parameters, including ammonia and sulfide concentrations, were measured in one replicate test container from each sample in the overlying water as described above. Measurements were taken at the beginning and end of all *Rhepoxynius* and *Neanthes* tests, and during overlying water renewals in the *Neanthes* tests.

Sea Urchin Larval Development Test
The sea urchin (*Strongylocentrotus purpuratus*) larval development test was conducted on all pore water samples. Details of the test protocol were given in Dinnel (1992). A brief description of the method follows.

Sea urchins were collected from the Monterey County coast near Granite Canyon, and held at MPSL at ambient seawater temperature and salinity (approx. 32±2 ppt) until testing. Adult sea urchins were held in complete darkness to preserve gonadal condition. On the day of a test, urchins were induced to spawn in air by injection with 0.5M KCl. Eggs and sperm collected from the urchins were mixed in seawater at a 500 to 1 sperm to egg ratio, and embryos were distributed to test containers within 1 hour of fertilization. Test containers were polyethylene-capped, sea-water leached, 20ml glass scintillation vials containing 5 mls of pore water. Each test container was inoculated with approximately 150 embryos (30/ml). All pore water samples were tested at three concentrations: 100, 50 and 25% pore water, each having three replicates. Pore water samples were diluted when necessary with one micron-filtered Granite Canyon seawater. Laboratory controls were included with each set of samples tested. Controls include a dilution water control consisting of Granite Canyon seawater, a brine control with all samples that require brine adjustment, and in some tests a frozen seawater control consisting of Granite Canyon seawater that has been frozen along with the pore water samples. Tests were conducted at ambient seawater salinity (usually 33±2 ppt). A positive control reference test was conducted concurrently with each pore water test using a dilution series of copper chloride as a reference toxicant.

After an exposure of 72 or 96 hours (no difference in results was detectable between these periods), larvae were fixed in 5% buffered formalin. Approximately 100 larvae in each container were examined under an inverted light microscope at 100x to determine the proportion of normally developed larvae as described by Dinnel (1992). Visual clues used to identify embryos as normal included development of skeletal rods (spicules) that extend beyond half the length of the larvae and normal

development of a three part gut. Slow growing embryos were considered abnormal.

Percent normal development was calculated as:

$$\frac{\text{(Number of normally developed larvae) X 100}}{\text{(Total number of observed larvae + number of abnormal larvae)}}$$

Sea Urchin Fertilization Test
The sea urchin (*Strongylocentrotus purpuratus*) fertilization test was conducted on pore water samples. Details of the test protocol were described in Dinnel *et al.* (1987).

Sea urchins were from the same stock described for the sea urchin larval development test. On the day of a test, urchins were induced to spawn in air by injection with 0.5M KCl. Sperm were exposed in test containers for sixty minutes before approximately 1000 eggs were added. After twenty minutes of fertilization, the test was fixed in a 5% buffered formalin solution. A constant sperm to egg ratio of 500 to 1 was used in all tests. This ratio maintained fertilization in the 70-90% range required by the test protocol. Fertilization was determined by the presence or absence of a fertilization membrane (raised chorion completely surrounding the egg). Test containers were polyethylene-capped, sea-water leached, 20ml glass scintillation vials containing 5 mls of pore water. All pore water samples were tested at three concentrations: 100, 50 and 25% pore water, each having three replicates. Pore water samples were diluted with one micron-filtered Granite Canyon seawater. Laboratory controls were included with each set of samples tested. Controls included a dilution water control consisting of Granite Canyon seawater, a brine control with all samples that require brine adjustment, and in some tests a frozen seawater control consisting of Granite Canyon seawater that has been frozen along with the pore water samples. Tests were conducted at ambient seawater salinity (usually 33±2 ppt). A positive control reference test was conducted concurrently with each pore water test using a dilution series of copper chloride as a reference toxicant. All eggs in each container were examined under an inverted light microscope at 100x, and counted as either fertilized or unfertilized.

Percent fertilization was calculated as:

$$\frac{\text{(Number of fertilized eggs) x 100}}{\text{(Number of fertilized eggs + number of unfertilized eggs)}}$$

Sea Urchin Cytogenetics Test
Analysis of cytogenetic abnormalities using sea urchin embryos followed methods described in Hose (1985). Sea urchin embryos were exposed to pore water for 48 hours then preserved in 5% buffered formalin. Embryos were placed on a clean glass microscope slide and excess formalin removed with tissue paper. Embryos were then treated with a few drops of aceto-orcein stain (19 parts aceto-orcein:one part propionic acid) for approximately 1 to 3 minutes, and a cover slip was then applied to the darkly

stained embryos. Excess stain was removed by blotting, and embryos were compressed into a monolayer by application of direct pressure. Embryo monolayer preparations were observed under oil immersion using either an Olympus BH2 or Tiyoda light microscope at 100x magnification. Cytogenetic abnormalities were observed in mitotic cells in anaphase and telophase. Possible aberrations observed followed those described in Hose (1985), including: stray or lagging chromosomes, accentric or attached chromosome fragments, and translocated or side-arm bridges . Because a majority of the embryos exposed to the 100 and 50% pore water concentrations displayed gross developmental abnormalities, mitotic aberrations were generally assessed using embryos exposed to 25% pore water.

Red Abalone Larval Development Test

The red abalone (*Haliotis rufescens*) larval development test was conducted on all subsurface water samples. Details of the test protocol were described in Anderson *et al.* (1990). The following was a brief description of the method. Adult male and female abalone were induced to spawn separately using a dilute solution of hydrogen peroxide in sea water. Fertilized eggs were distributed to the test containers within 1 hour of fertilization. Test containers were polyethylene-capped, seawater leached scintillation vials containing 10 mls of sample water. Each of five replicate test containers were inoculated with 100 embryos (10/ml).

Positive control reference tests using zinc sulfate as a reference toxicant were conducted concurrently with each batch of samples. A negative sea water control consisting of one micron-filtered Granite Canyon seawater was tested along with sub-surface water samples and zinc concentrations. After 48 hours of exposure, developing larvae were fixed in 5% buffered formalin. Approximately 100 larvae in each container were examined under an inverted light microscope at 100x to determine the proportion of veliger larvae with normal shells as described in Anderson et al. (1990).

Percent normal development was calculated as:

$$\frac{\text{(Number of normally developed larvae) x 100}}{\text{Total number of observed larvae}}$$

Amphipod Tests

Solid-phase sediment sample toxicity was assessed using the 10-day amphipod survival toxicity test protocol for *Rhepoxynius abronius* (ASTM 1993).

All test organisms were obtained from Northwest Aquatic Sciences in Yaquina Bay, Oregon. Amphipods were separated into groups of approximately 100 each, placed in polyethylene boxes containing Yaquina Bay collection site sediment, and then shipped on ice via overnight courier. Upon arrival at Granite Canyon, the amphipods were acclimated slowly (<2 ppt per day) to 28 ppt sea water (T =15°C). Once acclimated to 28 ppt, the animals were held for

an additional 48 hours prior to inoculation into the test containers.

Test containers were one liter glass beakers or jars containing two cm of sediment and filled to the 700 ml line with seawater adjusted to 28 ppt using spring water or distilled well water. Test sediments were not sieved for indigenous organisms prior to testing although at the conclusion of the test, the presence of predators was noted and recorded on the data sheet. Test sediment and overlying water were allowed to equilibrate for 24 hours, after which 20 amphipods were placed in each beaker along with 28 ppt seawater to fill test containers to the one liter line. Test chambers were aerated gently and illuminated continuously at ambient laboratory light levels.

Five laboratory replicates of each sample were tested for ten days. A negative sediment control consisting of five lab replicates of Yaquina Bay home sediment was included with each sediment test. After ten days, the sediments were sieved through a 0.5 mm Nytex screen to recover the test animals, and the number of survivors was recorded for each replicate.

Positive control reference tests were conducted concurrently with each sediment test using cadmium chloride as a reference toxicant. For these tests, amphipod survival was recorded in three replicates of four cadmium concentrations after a 96 hour water-only exposure. A negative seawater control consisting of one micron-filtered Granite Canyon sea water, diluted to 28 ppt was compared to all cadmium concentrations.

Amphipod survival for each replicate was calculated as:

$$\frac{\text{(Number of surviving amphipods) X 100}}{\text{(Initial number of amphipods)}}$$

Polychaete Tests

A subset of sediment samples was tested using *Neanthes arenaceodentata*. The protocol follows procedures described by Johns *et al.* (1990). Newly emergent juvenile *Neanthes* (2 to 3 weeks old) were obtained from Dr. Donald Reish in Long Beach, California. Worms were shipped in seawater in plastic bags at ambient temperature via overnight mail. Upon arrival at MPSL, worms were allowed to acclimate gradually to 28 ppt with <2 ppt daily incremental salinity adjustments. Once acclimated, the worms were maintained for at least 48 hours, and no longer than 10 days, before the start of a test.

The test setup was similar to the amphipod test. Test containers were one liter glass beakers or jars, each containing 2 cm of sediment and filled to the 700 ml line with 28 ppt seawater. Seawater was adjusted to the appropriate salinity using spring water or distilled well water. After test sediment and overlying water were allowed to equilibrate for 24 hours, 5 worms were placed in each of 5 replicate beakers per sample, and 28 ppt seawater was added up to the one liter line. Test chambers were

aerated and illuminated continuously during the 20-day test period. Worms were fed TetraMin® every 2 days, and water was renewed every 3 days. At the end of 20 days, samples were sieved through 0.5mm Nitex® screens, and the number of surviving worms recorded. Surviving worms were placed in pre-weighed foil in a drying oven until they reached a constant weight. Worms were weighed to the nearest 0.1mg.

Worm survival for each replicate was calculated as:

$$\frac{\text{(Number of surviving worms)} \times 100}{\text{Initial number of worms}}$$

Mean weight/worm for each replicate was calculated as:

$$\frac{\text{(Total weight)} - \text{(foil weight)}}{\text{Number of surviving worms}}$$

Positive control reference tests were conducted using cadmium chloride as a reference toxicant. Worm survival for 10 worms was recorded in three replicates of four cadmium concentrations in seawater after 96 hours of exposure. A negative seawater control consisting of one micron-filtered Granite Canyon seawater was compared to all cadmium concentrations. A negative sediment control consisting of Yaquina Bay amphipod home sediment was also included in each test.

Mussel Development Test

The bay mussel (*Mytilus edulis*) larval development test was conducted on pore water and sub-surface water samples for which salinity was in the range of 0-26 parts per thousand (ppt). Details of the test protocol are given in ASTM (1992). A brief description of the method follows.

Mussels were shipped via overnight courier and held at MPSL at ambient temperature (11-13°C) and salinity (32-34 ppt) until testing. On the day of a test, adult mussels were transferred to 25°C water to induce spawning through heat stress. Sperm and eggs were mixed in 25 ppt water to give a final sperm-to-egg ratio of 15 to 1. After approximately 20 minutes, fertilized eggs were rinsed on a 25 µm screen to remove excess sperm. Embryos were distributed to the test containers after approximately 90% of the embryos exhibited first cell cleavage (approximately 1 hour).

Test containers were polyethylene-capped, sea water-leached, 20 ml glass scintillation vials containing 10 mls of test solution. Each test container was inoculated with approximately 250 embryos (25/ml). Pore water samples were tested at 25 ± 2 ppt. Low salinity samples were adjusted to 25 ppt using frozen seawater brine. Controls consisted of one micron-filtered Granite Canyon sea water adjusted to 25 ppt, and a separate brine control consisting of sea water brine adjusted to 25 ppt with distilled water. A positive control reference test was conducted concurrently with each test using a dilution series of cadmium chloride as a reference toxicant.

After a 48-hour exposure period, larvae were fixed in 5% buffered formalin. All larvae in each container were examined under an inverted light microscope at 100x to determine the proportion of normally developed larvae as described in ASTM (1992). The percentage normally developed larvae was calculated as:

$$\frac{\text{Observed number of live normal larvae} \times 100}{\text{Mean number of live embryos inoculated at start of test}}$$

Statistical Analysis of Toxicity Test Data

A total of three hundred fifty solid-phase sediment samples were tested for toxicity to amphipods (*Rhepoxynius abronius)* as part of this study. A subset of 154 samples of solid-phase sediment samples were tested with the polychaete *Neanthes arenaceodentata*. Two hundred twenty-five pore water samples were tested using the purple sea urchin *(Strongylocentrotus purpuratus*) fertilization test; 196 samples were tested using the sea urchin larval development test; and 65 subsurface water (water column) samples were tested with the red abalone *(Haliotis rufescens)* larval development test. The bivalve mollusc *(Mytilus edulis)* larval development test was used to test eight sub-surface water and three pore water samples that had salinities below the threshold (26 ppt) selected for use of the sea urchin test.

There were three primary objectives for the toxicity testing portion of this study:
(1) Investigate the areal extent of toxicity in the San Diego Bay region by estimating the percent area considered toxic, based on toxicity test data for each individual protocol; (2) Identify those sites which were most toxic to assist in prioritization and designation of "toxic hot spots"; and (3) Evaluate the performance of each toxicity test protocol.

The first objective (investigating the spatial extent of toxicity) was primarily for use of the National Oceanic and Atmospheric Administration (NOAA)- National Status and Trends Program. The second objective (identifying and prioritizing individual sites as "toxic hot spots") was primarily for the California State Water Resources Control Board.

The different objectives required different sampling designs and different statistical approaches. The first objective, determination of the areal extent of toxicity, was accomplished through a process this report will refer to as the "EMAP approach": statistical procedures that compared samples from randomly selected stations against the test controls. In this approach, classification of a particular test sample as "toxic" was determined by a two step statistical approach comparing test samples to laboratory controls, as described below.

To accomplish the second objective, distinguishing the most toxic stations in the region to assist in the designation and prioritization of "toxic hot spots", a relatively new statistical method was employed, termed the "reference envelope approach". This approach compared organism response (e.g. % survival) from

an individual test sample with mean organism response from a group of reference sites presumed to represent optimal ambient conditions in the San Diego Bay region. Optimal ambient conditions are defined as indicative of conditions that can be found within the study area at sites that have relatively low pollutant concentrations and relatively undisturbed benthic communities. This method was intended to refine the definition of sample toxicity in order to identify a subset of toxic sites that were of greatest concern. This method is also described in detail below.

It should be noted that the EMAP approach and the reference envelope approach are distinctly different, yet complementary, statistical methods for determining toxicity. The intent of using two approaches is to identify non-toxic, significantly toxic and highly toxic locations based on multiple analyses of the data, for ranking toxicity results in a tiered approach.

EMAP Approach for Determining Spatial Extent of Toxicity

The "San Diego Bay Region" incorporates three non-connecting water bodies: San Diego Bay, Mission Bay and Tijuana Slough. Ideally these water bodies should be treated as discrete areas and analyzed separately to determine percent area toxic for each. However, the number of samples from Mission Bay and Tijuana Slough were 13 and 6, respectively, and these were considered too few to accurately represent toxicity in a frequency distribution.

Consequently, data from all three water bodies were combined in this report to determine the percentage of total area that was toxic.

In this analysis, sample toxicity was determined using procedures described by Schimmel *et al.* (1991); a method used in the EPA Environmental Monitoring Assessment Program (EMAP) and in similar NOAA studies nationwide (e.g., Long *et al.*, 1994). Using the EMAP approach, samples were defined as toxic if the following two criteria were met: (1) there was a significant difference in mean organism response (e.g. percent survival) between a sample and the control as determined using a t-test, and (2) mean organism response in the toxicity test was less than 80% of the laboratory control value. The t-test generates a t statistic by dividing the difference between control and test sample response by an expression of the variance between laboratory replicates. If the variation between control and test sample is sufficiently greater than the variation among laboratory replicates, the t-test indicates a significant difference in response. A "separate variance" t-test was used to adjust the degrees of freedom to account for variance heterogeneity among samples (SYSTAT, 1992).

The second criterion, that sample response must be less than 80% of the control value to be considered toxic, is useful in eliminating those samples that were statistically different from controls only because of a very small variance among laboratory replicates. For example, a sample that had 90 ± 2 % *Rhepoxynius* survival would be significantly different from a control with

survival of 96 ± 2 %, and would therefore be considered toxic based on a simple t-test even though the biological significance of this response would be negligible. By adding the second criterion, any sample with percent survival exceeding 80% of the controls would be considered non-toxic. The 80% level was established by examination of numerous amphipod toxicity data sets (Thursby and Schlekat, 1993). These researchers found that samples with survival less than 80% relative to controls were significantly different from controls about 90% of the time. Preliminary analyses of *Rhepoxynius* test data from the BPTCP indicate a similar level of statistical sensitivity. Based on this observation, the 80% criterion has been adopted previously (Schimmel *et al.*, 1991; USEPA/USACOE, 1991). Samples identified as toxic according to these criteria were used to estimate the percent of total area toxic within the San Diego Bay region.

Using Cumulative Distribution Frequencies to Characterize Spatial Extent

The stratified random sampling design, allowed 121 of the total 350 samples collected in this study, to be used to estimate the areal extent of toxicity. Samples collected using directed sampling (non-random sampling directed to areas of particular characteristics) were <u>not</u> included in this analysis since they may have been biased toward increased contamination. Directed non-random sampling was designed to address the State and Regional Water Quality Boards objective to identify and prioritize potential toxic hot spots. Samples were collected from randomly selected stations within 95 non-overlapping mapped blocks of known area in the San Diego Bay region (Figure 2). Total area sampled, calculated as the sum of all 95 block areas, was 40.9 km^2. The estimate of spatial toxicity was determined from cumulative distribution frequencies (CDFs) that relate toxicity response to percent of total sampled area. CDF calculations follow procedures used by both EMAP and NS&T.

CDFs were determined using calculated areas of each block normalized to the number of samples per block. Block areas were calculated using a planimeter on NOAA National Ocean Service navigation chart (means of three trials), calibrated to the scale of the charts. Because no more than two samples were collected per block, numbers of toxic samples per block ranged from 0 to 2, representing 0%, 50% or 100% of a given block area. By combining the blocks with their toxicity designations in a cumulative manner, the CDFs indicate the percentage of total area sampled that was toxic. Sample toxicity was determined from comparisons with laboratory controls as described above in the EMAP approach; each sample with a mean significantly different from, and less than 80% of, the laboratory control mean was considered toxic. Calculations used to derive percent areas determined to be toxic are shown on worksheets in Appendix F. CDFs were generated from toxicity tests using *Rhepoxynius* survival (solid phase) and *Strongylocentrotus* larval development (pore water). There were insufficient data from randomly selected sites to generate CDFs for *Haliotis*, *Mytilus* and *Neanthes* tests.

The Reference Envelope Approach for Determining Toxicity

The second objective of this study was to assist in the identification of "toxic hotspots", where adverse biological impacts are observed in areas with localized concentrations of pollutants. Identification of problem sites was an essential step in prioritizing efforts to improve sediment and water quality through regulation and remediation programs. While it was possible large areas of San Diego Bay may be degraded to some extent, logistical constraints required efforts be focused on localized areas that were significantly more toxic than optimal ambient conditions that exist in the greater portion of the bay. In this study, a "reference envelope" statistical approach was employed (Smith, 1995) to identify samples that exhibit significantly greater toxicity than expected in San Diego Bay as a whole.

The reference envelope approach uses data from "reference sites" to characterize the response expected from sites in the absence of localized pollution. Using data from the reference site population, a tolerance limit was calculated for comparison with data from test sites. Samples with toxicity values greater than the tolerance limit were considered toxic relative to the optimal ambient condition of the Bay.

This relative standard established using reference sites was conceptually different from what might be termed the absolute standard of test organism response in laboratory controls. Rather than comparing sample data to control data using t-tests, with laboratory replication used to characterize the variance component (as in the "EMAP approach" described above), the reference envelope approach compared sample data against a percentile of the reference population of data values, using variation among reference sites as the variance component. The reference envelope variance component, therefore, included variation among laboratory replicates, among field replicates, among sites, and among sampling events.

The reference stations were assumed to be a random sample from an underlying population of reference locations that serve as a standard for what we considered relatively non-impacted conditions. The toxicity measured at different reference locations will vary due to the different local conditions that can affect the toxicity results. In order to determine whether sediments from a test location were toxic, bioassay results for the test location were compared with bioassay results from the population of reference locations.

Assuming the bioassay results from the population of reference locations are normally distributed, an estimate of the probability that the test sediment is from the underlying reference station distribution can be made. For example, if the result for a test sediment was at the first percentile of the underlying reference location distribution (in the direction of toxicity), then there would be about a 1% chance that the test sediment was from the distribution of reference locations.

The toxicity level at the first percentile of the reference distribution is not known because there were only limited samples from the underlying distribution and only an estimate could be made of where the first percentile lies. If an estimate of the first percentile value was made a large number of times, using different random samples from the reference distribution, a (non-central t) distribution of estimates, with the distribution mode at the actual first percentile would be obtained (Figure 4). In Figure 4, it can be seen from the distribution of estimates that about one half of the time the estimate from the sample was above the actual first percentile. Ideally, identification of an estimated toxicity value would cover the actual first percentile for a large percentage of the estimates (say 95% of the time). Such a value can be obtained from the left tail of the distribution of estimates where 5% of the estimates are less than the chosen value. The definition of p is the percentile of interest, and alpha is the acceptable error probability associated with an estimate of the pth percentile. Thus, in this example, p=1 and alpha = .05.

The toxicity level can be computed that will cover the pth percentile 1 minus alpha proportion of the time as the lower bound (L) of a tolerance interval (Vardeman 1992) as follows.

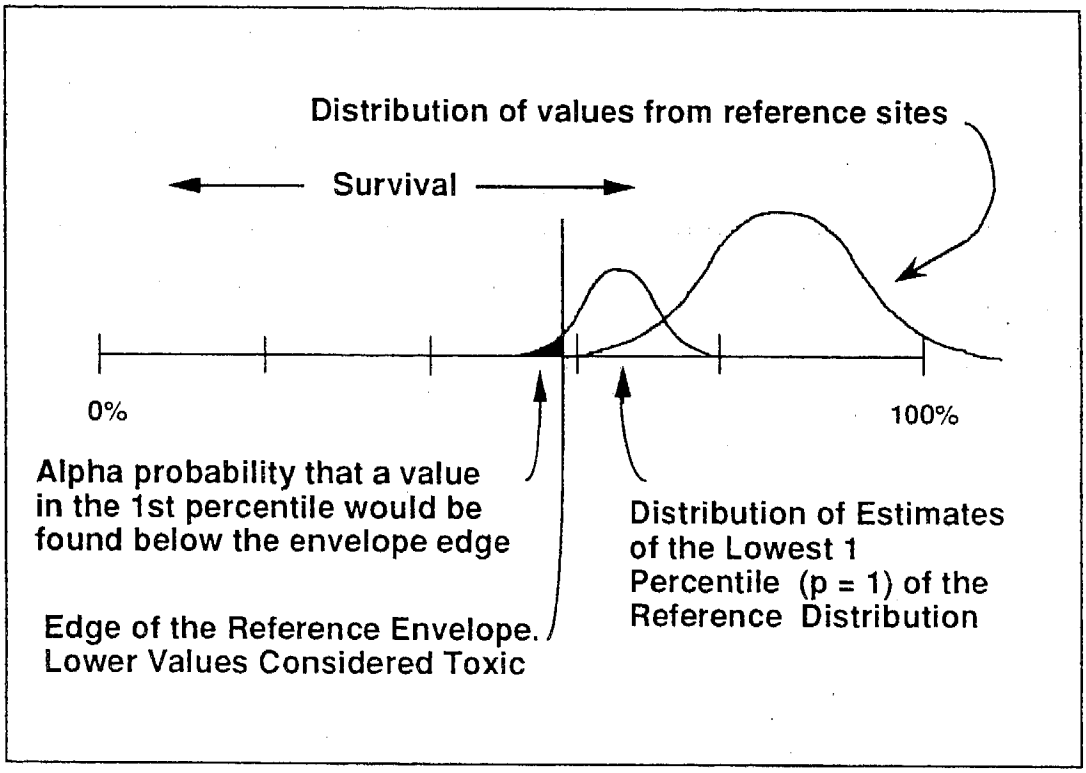
$$L = X_r - [\ g_{\alpha,p,n} * S_r\ ]$$

where X_r is the mean of the sample of reference stations, S_r is the standard deviation of the toxicity results among the reference stations, and n is the number of reference stations. The g values, for the given alpha, p, and n values, can be obtained from tables in Hahn and Meeker (1991) or Gilbert (1987). S contains the within- and between-location variability expected among reference locations. If the reference stations are sampled at different times, then S will also incorporate between-time variability. The "edge of the reference envelope" (L) represents a cutoff toxicity level used to distinguish toxic from non-toxic sediments. The value used for p will depend on the level of certainty needed for a particular regulatory situation. In this study a p value equal to 1% was chosen, to distinguish only the most toxic samples, that is, samples having a 95% certainty of being in the most toxic 1%.

Reference Station Selection for Reference Envelope

Reference stations were selected to represent optimal ambient conditions available in San Diego Bay, based on available chemistry and benthic community data. Toxicity data were not used in the selection process. Stations were selected if both of the following criteria were met: 1) the benthic communities appeared relatively undisturbed (based on indices described in the benthic community analysis section), and 2) sediment chemical concentrations were below Effects Range Median (ERM) levels (Long *et al.*, 1995) and Probable Effects levels (PELs) (McDonald, 1994). Among all stations, both randomly and non-randomly

Figure 4. Schematic illustration of the method for determining the lower tolerance interval bound (edge of the reference envelope) to determine sample toxicity relative to a percentile of the reference site distribution.



selected, a total of 75 samples were analyzed for toxicity, chemistry and benthic ecology in this study. After screening these 75 samples, eleven stations in the San Diego Bay region were selected as reference stations (Table 4). It should be noted these stations were not selected prior to the initiation of the study, but were selected after all of the analyses for the study were completed.

P450 Reporter Gene System

Summary of Methods

A subset of thirty sediment samples was sent to Columbia Analytical Services (CAS) in Kelso, Washington for extraction with methylene chloride. Extracts of 20 g sediment samples were evaporated to 1 ml and placed in small vials for shipment to the Carlsbad, CA laboratory of CAS where 2 µl samples were applied in triplicate to genetically engineered human liver cancer cells (101L cells) developed by Dr. Robert Tukey of the University of California, at San Diego. A previous study partially funded by the State Board (Anderson *et al.*, 1995) had demonstrated that low levels of dioxin, coplanar PCBs and selected PAHs could be detected by the P450-RGS response to the extracts. When this small volume of solvent (with extracted contaminants) is applied to approximately one million cells in 2 ml of medium, induction of the CYP1A1 gene leads to production of the detoxification enzyme, P450, and the luminescent enzyme, luciferase. When the cells are lysed (after 16 hours) and the centrifugate tested with luciferin, the amount of light measured in a luminometer is a function of the concentration and potency of the contaminants on the sediments. When the contents of a single well (containing ≈ one million cells) are centrifuged and placed in the luminometer the resulting measure is in Relative Light Units (RLU). The RLUs of the solvent blank are set to unity and by dividing all RLU readings for the reference toxicant and samples by the RLUs of the blank, the data are converted to Fold Induction (or times background). To make the data more relevant to environmental samples, the data are converted to Equivalents of Benzo(a)pyrene (BaPEq), a ubiquitous PAH compound of environmental concern (U.S. EPA, 1995). To convert mean fold induction to BaPEq in µg/g dry weight, the fold induction values are divided by sixty, which (based on a dose response curve) is the response of the assay to 1µg/ml of Benzo(a)pyrene (BaP). The µg of BaP per volume of extract (e.g. 10 µl) is adjusted to an initial volume of 1 ml and this product divided by the dry grams of sample contained in the 1 ml extract. This method can be used to calculate Equivilants for PAHs, from benz(a)anthracene to benzo(g,h,i)perylene (Table 4), as well as dioxins/furans and coplanar PCBs. Both sediments and tissues (marine mussel) from San Diego Bay have been analyzed for the presence of P450 inducing compounds in previous studies (Anderson *et al.* 1996, in press a). The detailed methods and results of P450-RGS testing with standards and sediment extracts are described in Postlind *et al.* (1994), and Anderson *et al.* (1995). In 1996, three publications will be available describing the specific test methods (ASTM, Standard Methods, and CRC Press).

TABLE 4
REFERENCE STATIONS SELECTED FOR REFERENCE ENVELOPE ANALYSIS

Station #	Station Name	IDORG #	Leg	% Fines	TOC	ERMQ	PELQ	BENTHICS	Amphipod Surv.	Urchin Devo.(25%)
93112.0	MISSION BAY A8 (x1)-REP 1	856	21	30.12	0.81	0.065	0.116	UNDEGRADED	96 ± 5	20.2 ± 1
93112.0	MISSION BAY A8 (x1)-REP 2	857	21	37.28	0.94	0.082	0.134	UNDEGRADED	98 ± 3	89 ± 4
93112.0	MISSION BAY A8 (x1)-REP 3	858	21	43.56	0.91	0.089	0.145	UNDEGRADED	94 ± 5	53.6 ± 49
93202.0	EAST BASIN I1 (x5)	842	21	46.28	1.11	0.238	0.362	UNDEGRADED	83 ± 6	67.2 ± 17
90013.0	37 SWARTZ (MARINA)	815	20	88.21	1.37	0.217	0.347	UNDEGRADED	81 ± 8	73.8 ± 10
93190.0	MARINA I1 (x1)	816	20	93.97	1.22	0.219	0.356	UNDEGRADED	87 ± 12	59.4 ± 9
90053.0	35 SWARTZ (CORONADO CAYS)	843	21	91.65	1.47	0.180	0.292	UNDEGRADED	75 ± 11	29 ± 25
93108.0	MISSION BAY A4 (x1)-REP 2	860	21	64.60	1.87	0.104	0.166	UNDEGRADED	69 ± 14	78.5 ± 16
93195.0	GLORIETTA BAY U1 (x2)	823	20	48.24	0.95	0.239	0.369	UNDEGRADED	81 ± 9	0 ± 0
93194.0	GLORIETTA BAY U1 (x1)	822	20	55.80	1.14	0.232	0.371	UNDEGRADED	89 ± 7	46.3 ± 7
93231.0	CARRIER BASE V2 (x6)	1000	23	57.66	1.57	0.252	0.404	UNDEGRADED	74 ± 12	0 ± 0

None of the above samples exhibited any chemical exceedance of an ERM or PEL.
None of the above samples exhibited elevated ammonia or hydrogen sulfide during toxicity testing.
Amphipod Survival value is the mean and standard deviation from 5 laboratory replicates.
Urchin Development values are the mean and standard deviation of 5 replicates in 25% porewater.
ERM and PEL summary quotients are discussed in Appendix B and the report text.

Quality Assurance/Quality Control

Summary of Methods

Summaries of quality assurance and quality control procedures are described under separate cover in the Bay Protection and Toxic Cleanup Program Quality Assurance Project Plan (QAPP). This document describes procedures within the program which ensure data quality and integrity. Quality assurance procedures follow those of the NS&T Program to ensure comparability with other NOAA survey areas nationwide. In addition, individual laboratories prepare quality assurance evaluations of each discrete set of samples analyzed and authorized by task order. These documents were submitted to the California Department of Fish and Game for review, then forwarded to the State Water Resources Control Board for further review.

RESULTS

Tabulated data for all chemical, benthic, toxicological and P450-RGS analyses are presented in Appendices B, C, D and E. The summary data presented in the following results sections were used to demonstrate significant findings from the analysis of the full data set in Appendices B, C, and D.

Distribution of Chemical Pollutants

Chemical Specific Screening Values

There have been several recent studies associating pollutant concentrations with biological responses (Long and Morgan, 1990; MacDonald, 1992). These studies provide guidance for evaluating the degree to which sediment chemical pollutants levels are responsible for effects observed in a toxicity test. Reported values are based on individual chemical pollutants within sediments. Therefore, their application may be confounded when dealing with: biological effects which could be attributed to a synergistic effect of low levels of multiple chemicals, unrecognized chemicals, or physical parameters in the sediment which were not measured.

The National Status and Trends Program has used chemical and toxicological evidence from a number of modeling, field and laboratory studies to determine the ranges of chemical concentrations which are rarely, sometimes, or usually associated with toxicity (Long and Morgan, 1992). Evaluation of available data (Long *et al.*, 1995) has led to identification of three ranges in concentration for each chemical:

1) Minimal Effects Range: The range in concentration over which toxic effects are rarely observed:

2) Possible Effects Range: The range in concentrations over which toxic effects are occasionally observed;

3) Probable-Effects Range: The range in chemical concentrations over which toxic effects are frequently or always observed.

Two slightly different methods were used to determine these chemical ranges. One method developed by NOAA (Long and Morgan, 1990; Long *et al.*, 1995) used chemical data which were associated with a toxic biological effect. These data were used to determine the lower 10th percentile of ranked data where the chemical level was associated with an effect (Effects Range-Low, or ERL). Sediment samples in which all chemical concentrations were below the 25 ERL values were not expected to be toxic. The Effects Range-Median (ERM) reflects the 50th percentile of ranked data and represents the level above which effects are expected to occur. Effects are expected to occur occasionally when chemical concentrations fall between the ERL and ERM. The probability of toxicity was expected to increase with the number and degree of exceedances of the ERM values.

Another method identifies three ranges using chemical concentration data associated with both toxic biological effects and no observed effects (MacDonald, 1992; MacDonald, 1994; MacDonald *et al., In Press*). The ranges are identified as TEL (Threshold Effects Level) and the PEL (Probable Effects Level). TEL values were derived by taking the geometric mean of the 50th percentile of the "no effects" data and the 15th percentile of the "effects" data. The PEL values were derived by taking the geometric mean of the 85th percentile of the "no effects" data and the 50th percentile of the "effects" data. Although different percentiles were used for these two methods, they are in close agreement, usually within a factor of 2. Values reported for both methods are shown in Table 5. Neither of these methods is advocated over the use of the other in this report. Instead, both are used in the following analysis to create a weight of evidence which should help explain toxicity observed from some sediments.

A cautionary note should be included; the degree of confidence which MacDonald (1994) and Long *et al.* (1995) had in their respective guidelines varied considerably among the different chemicals. For example, they express low confidence in the values derived for nickel, mercury, DDTs, chlordane, dieldrin, and endrin. When more data becomes available regarding these chemicals and their potential effects, the guidelines may be revised, probably upward for some substances.

Primary Chemicals of Concern

Figure 5 presents a summary of the chemicals and chemical groups which exceeded ERM or PEL values at the 217 stations where complete chemical analysis was performed. Copper, mercury, zinc, total chlordane, total PCBs and the PAHs were most often found to exceed ERM or PEL values and are considered the six major chemicals or chemical groups of concern in the San Diego Bay

Table 5- Comparison of Sediment Screening Levels
Developed by NOAA and the State of Florida

SUBSTANCE	State of Florida (1) TEL	State of Florida (1) PEL	NOAA (2) ERL	NOAA (2) ERM
Organics (ug/kg- dry weight)				
Total PCBs	21.550	188.79	22.70	180.0
PAHs				
Acenaphthene	6.710	88.90	16.00	500.0
Acenaphthylene	5.870	127.89	44.00	640.0
Anthracene	46.850	245.00	85.30	1100.0
Fluorene	21.170	144.35	19.00	540.0
2-methylnaphthalene	20.210	201.28	70.00	670.0
Naphthalene	34.570	390.64	160.00	2100.0
Phenanthrene	86.680	543.53	240.00	1500.0
Total LMW-PAHs	311.700	1442.00	552.00	3160.0
Benz(a)anthracene	74.830	692.53	261.00	1600.0
Benzo(a)pyrene	88.810	763.22	430.00	1600.0
Chrysene	107.710	845.98	384.00	2800.0
Dibenz(a,h)anthracene	6.220	134.61	63.40	260.0
Fluoranthene	112.820	1493.54	600.00	5100.0
Pyrene	152.660	1397.60	665.00	2600.0
Total HMW-PAHs	655.340	6676.14	1700.00	9600.0
Total PAHs	1684.060	16770.54	4022.00	44792.0
Pesticides				
p,p'-DDE	2.070	374.17	2.20	27.0
p,p'-DDT	1.190	4.77		
Total DDT	3.890	51.70	1.58	46.1
Lindane	0.320	0.99		
Chlordane	2.260	4.79	0.50	6.0
Dieldrin	0.715	4.30	0.02	8.0
Endrin			0.02	45.0
Metals (mg/kg- dry weight)				
Arsenic	7.240	41.60	8.20	70.0
Antimony			2.00	2.5
Cadmium	0.676	4.21	1.20	9.6
Chromium	52.300	160.40	81.00	370.0
Copper	18.700	108.20	34.00	270.0
Lead	30.240	112.18	46.70	218.0
Mercury	0.130	0.70	0.15	0.7
Nickel	15.900	42.80	20.90	51.6
Silver	0.733	1.77	1.00	3.7
Zinc	124.000	271.00	150.00	410.0

(1) D.D. MacDonald, 1994

(2) Long et al., 1995

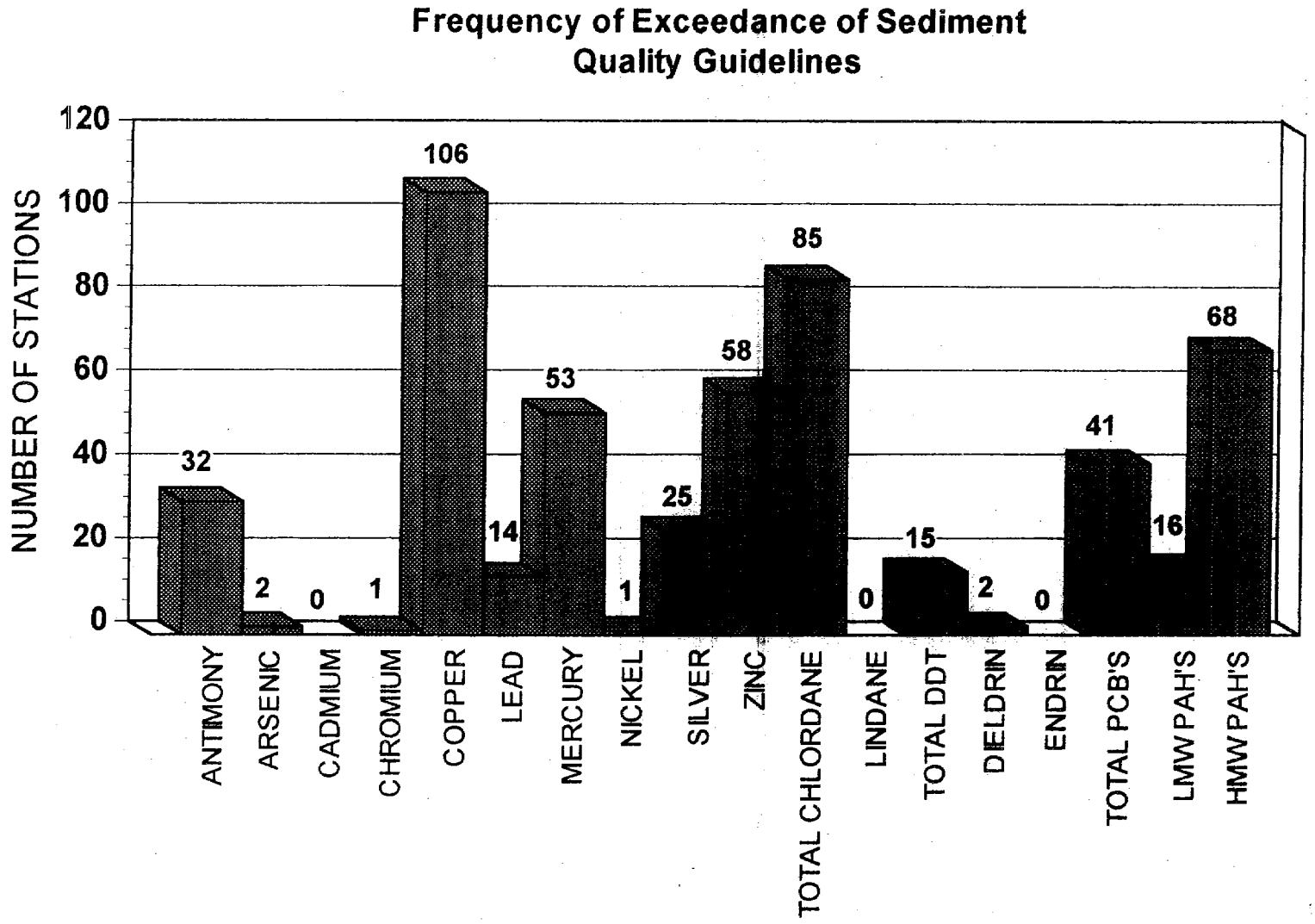


Figure 5. Number of stations which exceeded either the PEL or ERM values.

Region. MacDonald (1994) and Long *et al.* (1995) express relatively high confidence in the ERM and PEL values derived for copper, zinc, total PCBs and PAHs. Figures 6-12 map the geographical distribution of the six chemicals of concern throughout the San Diego Bay Region. Three ranges of chemical concentration are given for each chemical: (1) below the TEL, (2) between the TEL and PEL and (3) above the PEL to the maximum concentration determined.

Copper is a broad spectrum biocide which may be associated with acute and chronic toxicity, reduction in growth, and a wide variety of sublethal effects (Spear and Pierce, 1979). Elevated copper concentrations above the PEL (>108.2 mg/kg) or ERM (>270 mg/kg) were found throughout San Diego Bay (Figure 6(a-d)), with small boat harbors, commercial shipping berths and military berths most often impacted. Considering the historical use of copper based anti-fouling paint in the area, this distribution pattern is expected.

Zinc demonstrates a similar pattern of distribution, although actual exceedances of PEL levels (>271 mg/kg) or ERM levels (>410 mg/kg) only occur in the central portion of the bay, along the naval shipyard waterfront (Figure 7(a-d).

Mercury, particularly methylmercury, is highly toxic to aquatic biota. Although there is variability in sensitivity of different organisms to the substance, bioaccumulation of mercury in aquatic species has significant implications with respect to human health. PEL exceedances (> 0.696 mg/kg) and ERM exceedances (>0.71 mg/kg) of mercury were found in several small boat areas, near commercial shipping operations and predominately near naval shipyard areas (Figure 8(a-d)).

Polycyclic (polynuclear) aromatic hydrocarbons (PAHs) are base/neutral organic compounds with a fused ring structure of two or more benzene rings. They are components of crude and refined petroleum products and are also products of incomplete combustion of organic materials. Exposure to PAHs may result in a wide range of carcinogenic, teratogenic and mutagenic effects to terrestrial and aquatic organisms (Eisler, 1987). Due to their similar modes of toxic action, individual PAHs are often grouped into low and high molecular weight compounds, for concise reporting purposes. Individual PAHs used for the summations of low and high molecular weight PAHs in this report are given in Appendix B -Section VII. PAH pollution, as shown for high molecular weight PAHs in Figure 9(a-d), exceeds the PEL (>6676.14 μg/kg) or ERM (>9600 μg/kg) near commercial shipping operations and naval shipyard areas, as well as the submarine facility near the mouth of the harbor. The pattern for PEL (>1442 μg/kg) or ERM (>3160 μg/kg) exceedances of low molecular weight PAHs is similar to high molecular weight PAHs (Fig. 10(a-d)).

A significant concern is polychlorinated biphenyls (PCBs) levels found in sediments throughout San Diego Bay. PCBs are base/neutral compounds which are formed by direct chlorination of

Figure 6a
Copper Concentrations in Sediment
North San Diego Bay

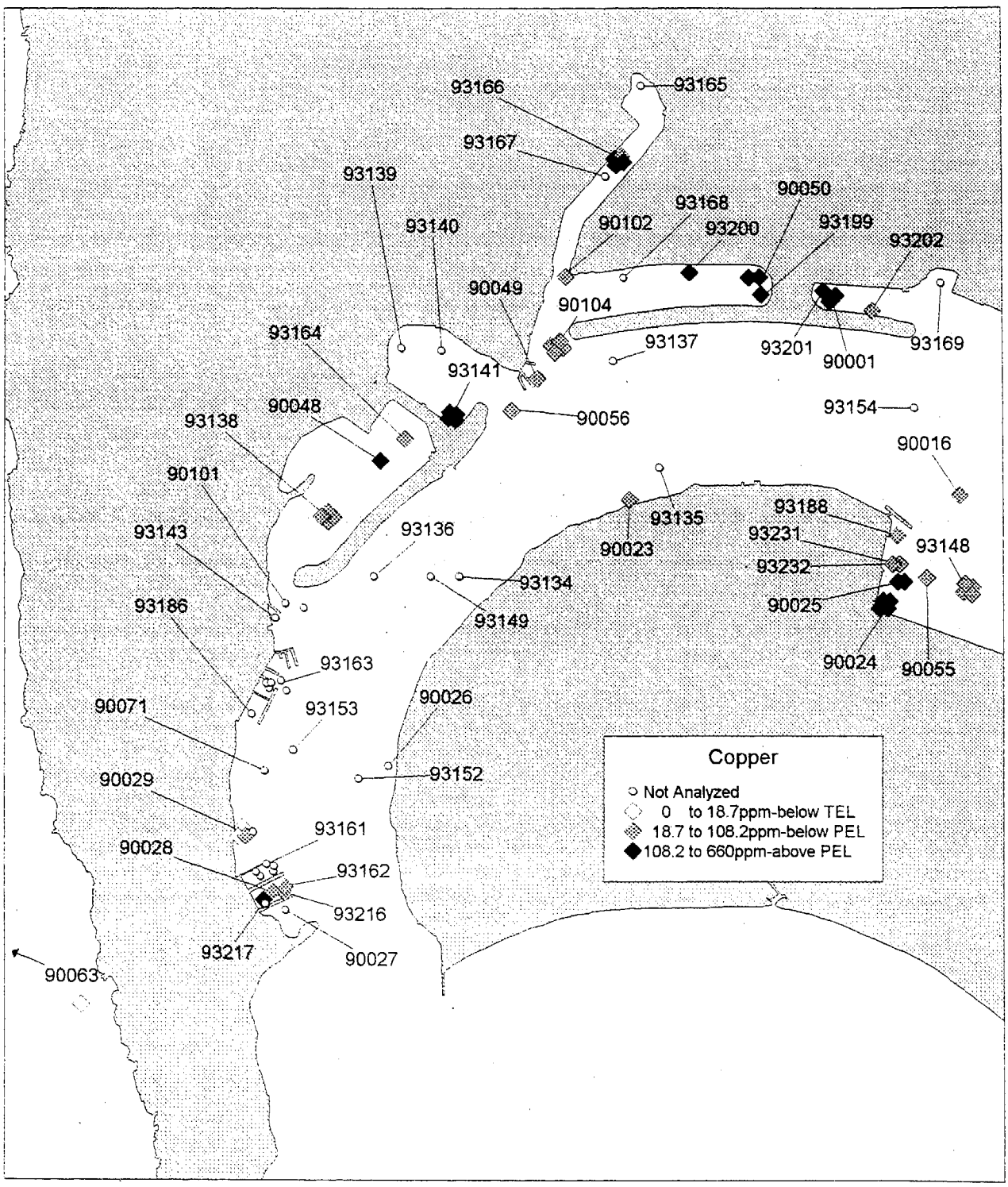


Figure 6b
Copper Concentrations in Sediment
Mid San Diego Bay

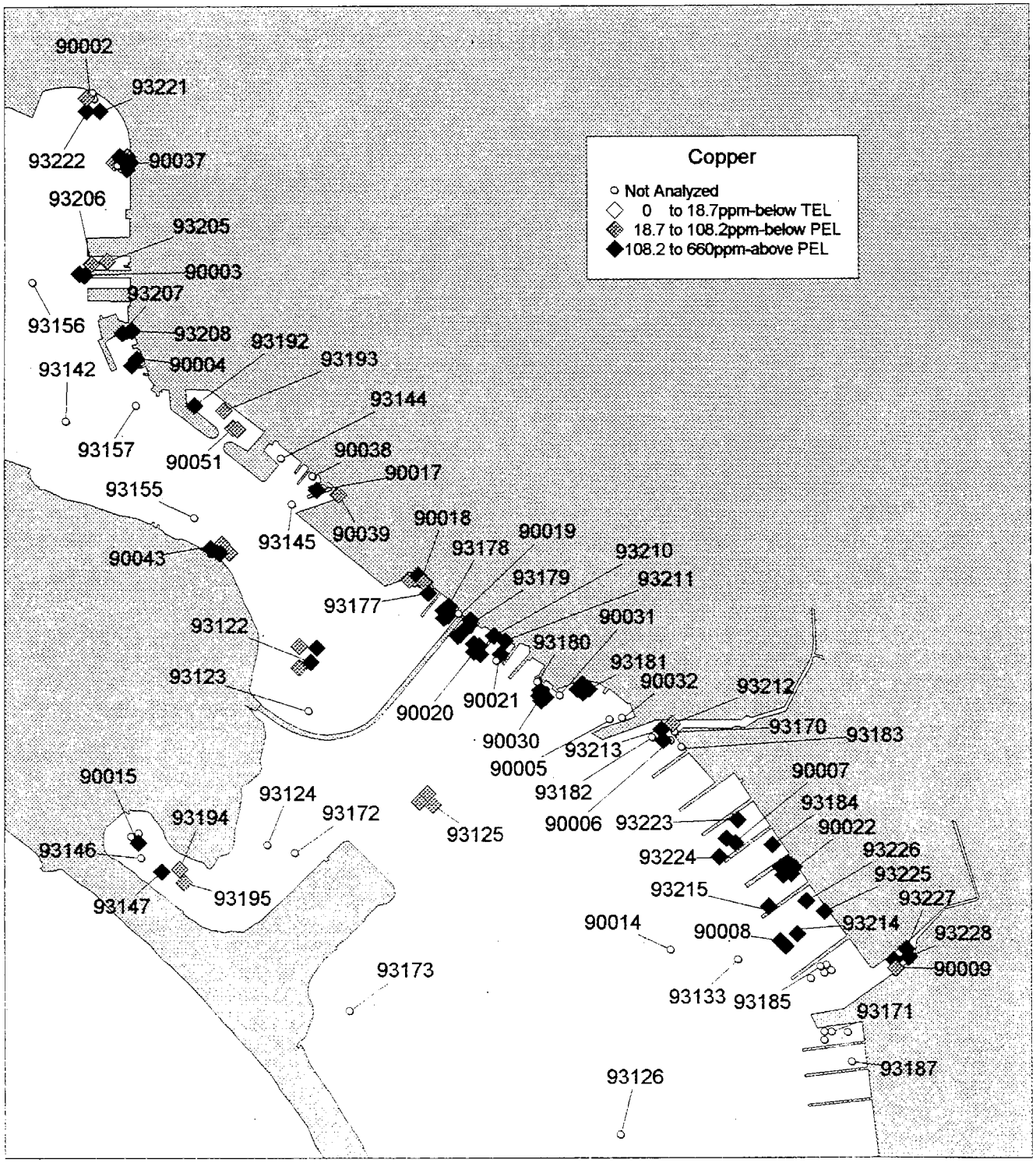


Figure 6c
Copper Concentrations in Sediment
South San Diego Bay

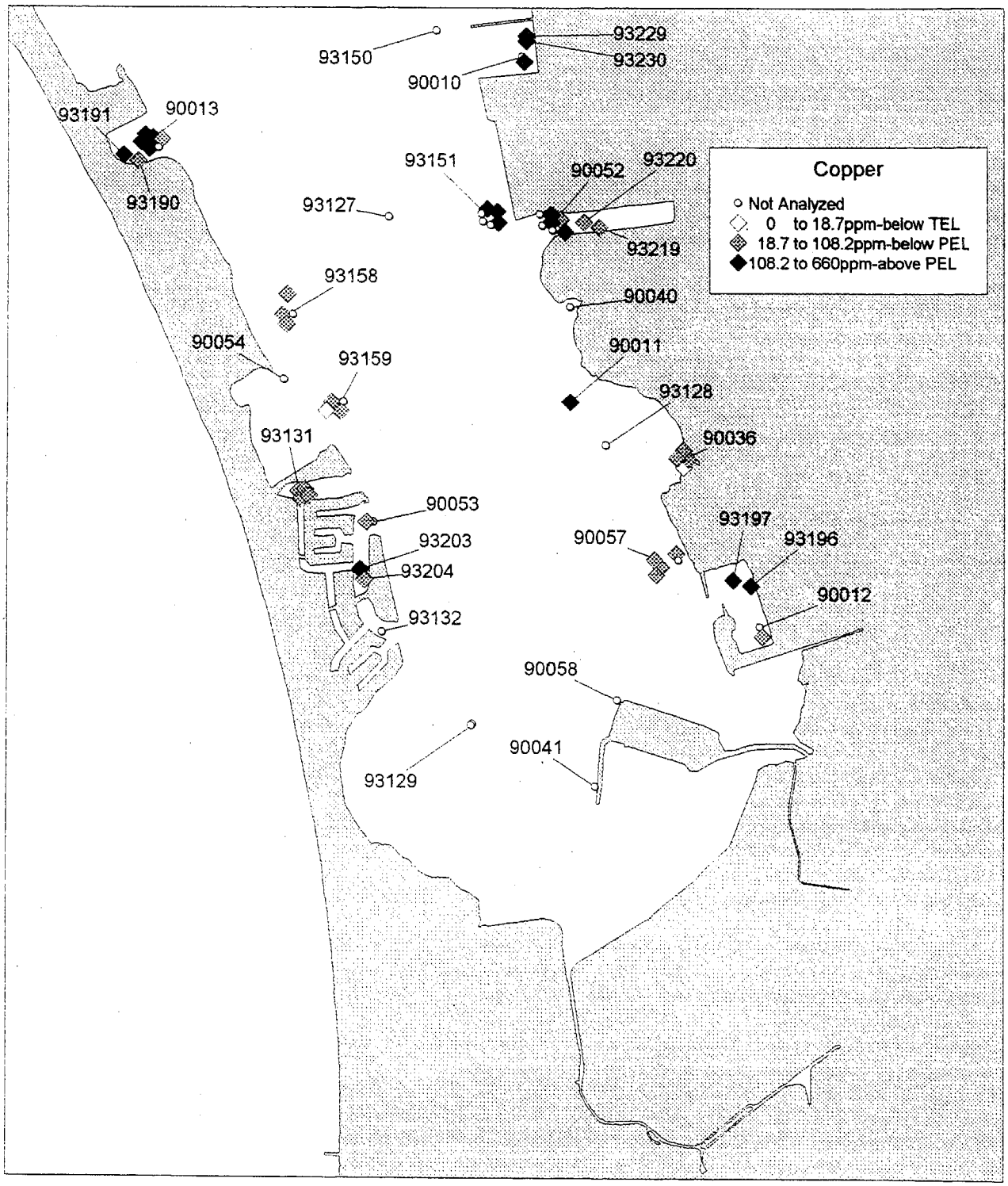
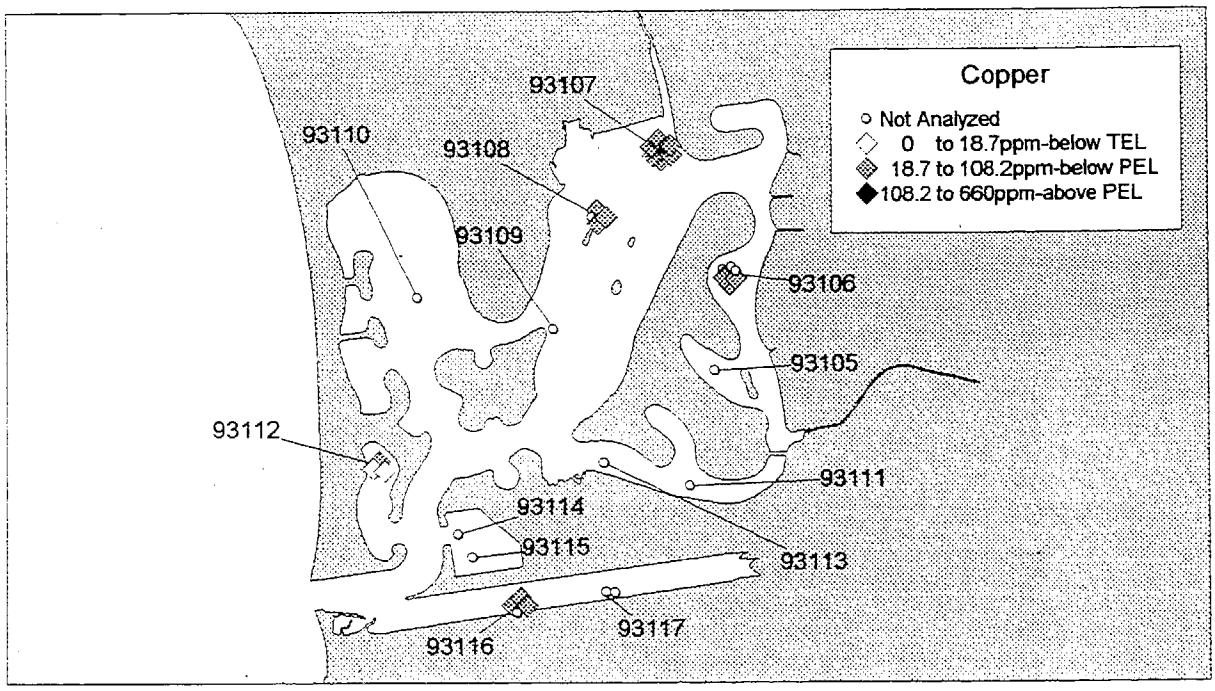


Figure 6d
Copper Concentrations in Sediment
Mission Bay and San Diego River Estuary



Tijuana River Estuary

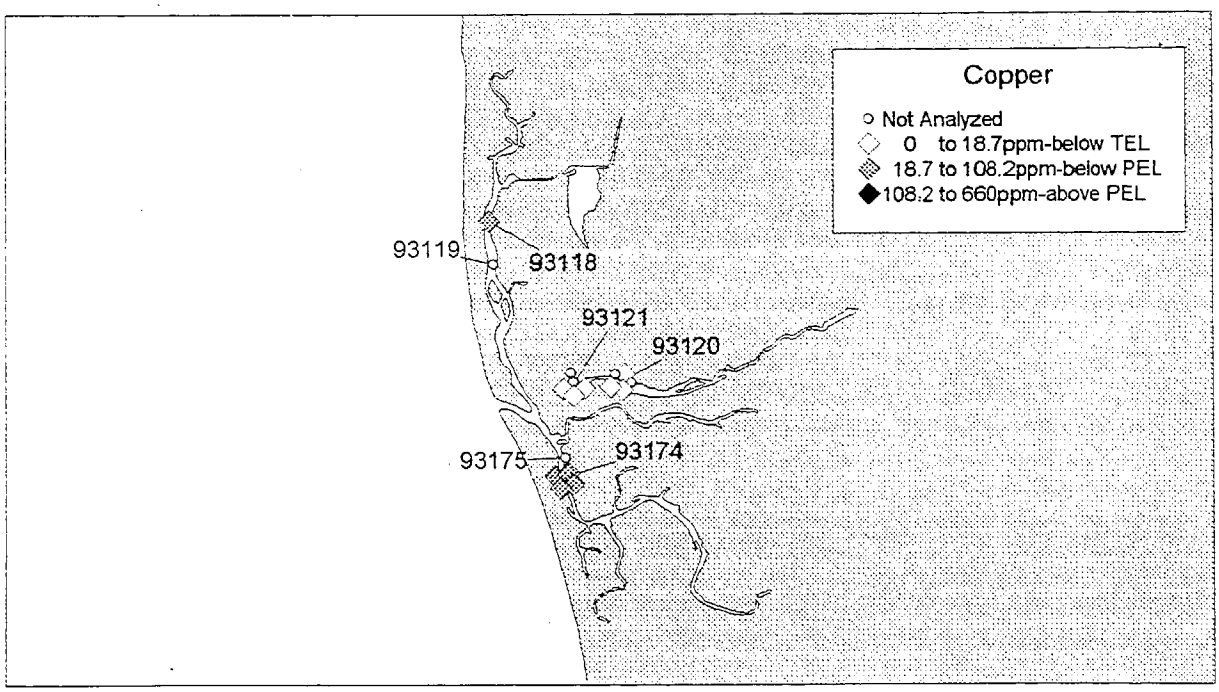


Figure 7a
Zinc Concentrations in Sediment
North San Diego Bay

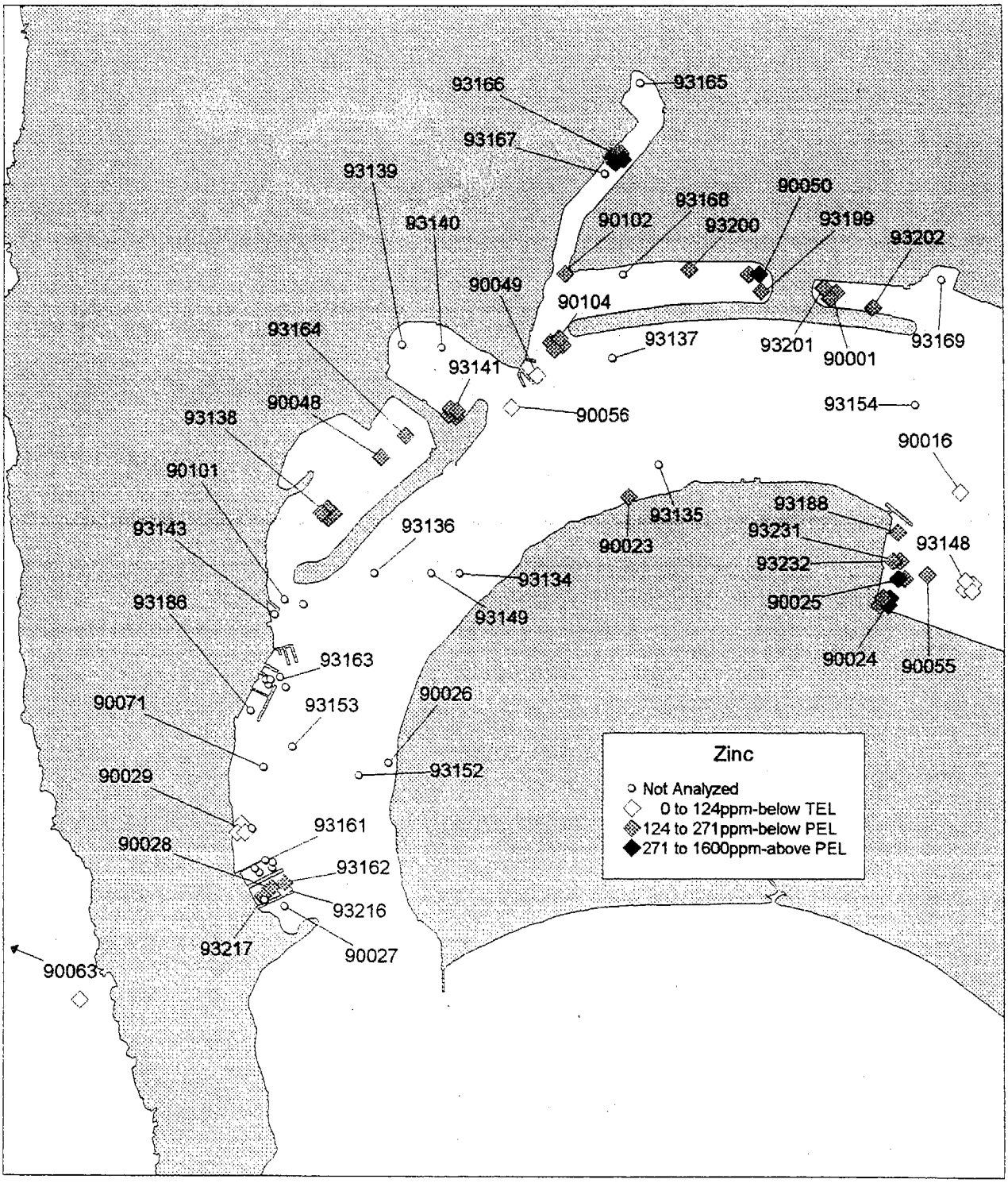


Figure 7b
Zinc Concentrations in Sediment
Mid San Diego Bay

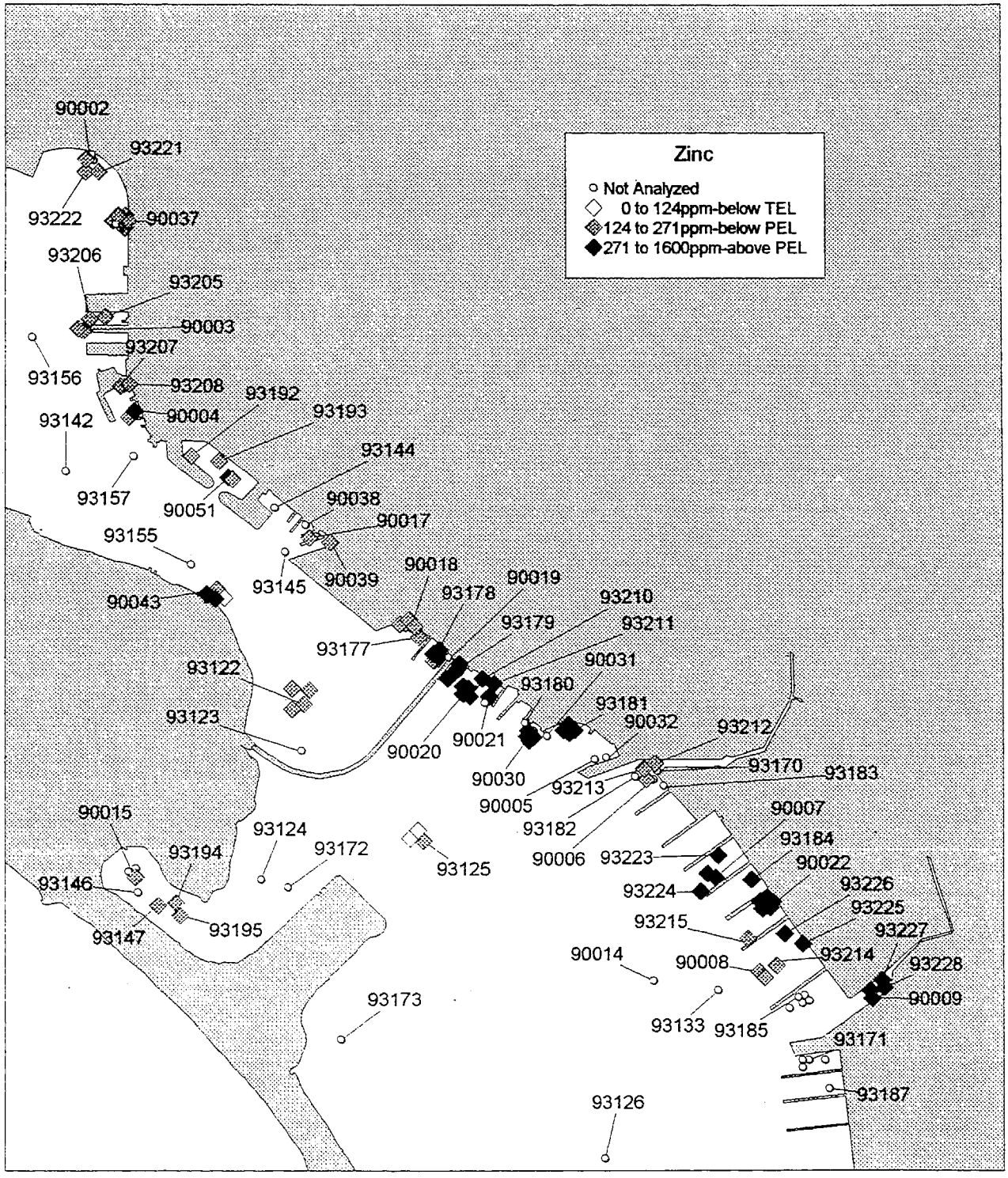


Figure 7c
Zinc Concentrations in Sediment
South San Diego Bay

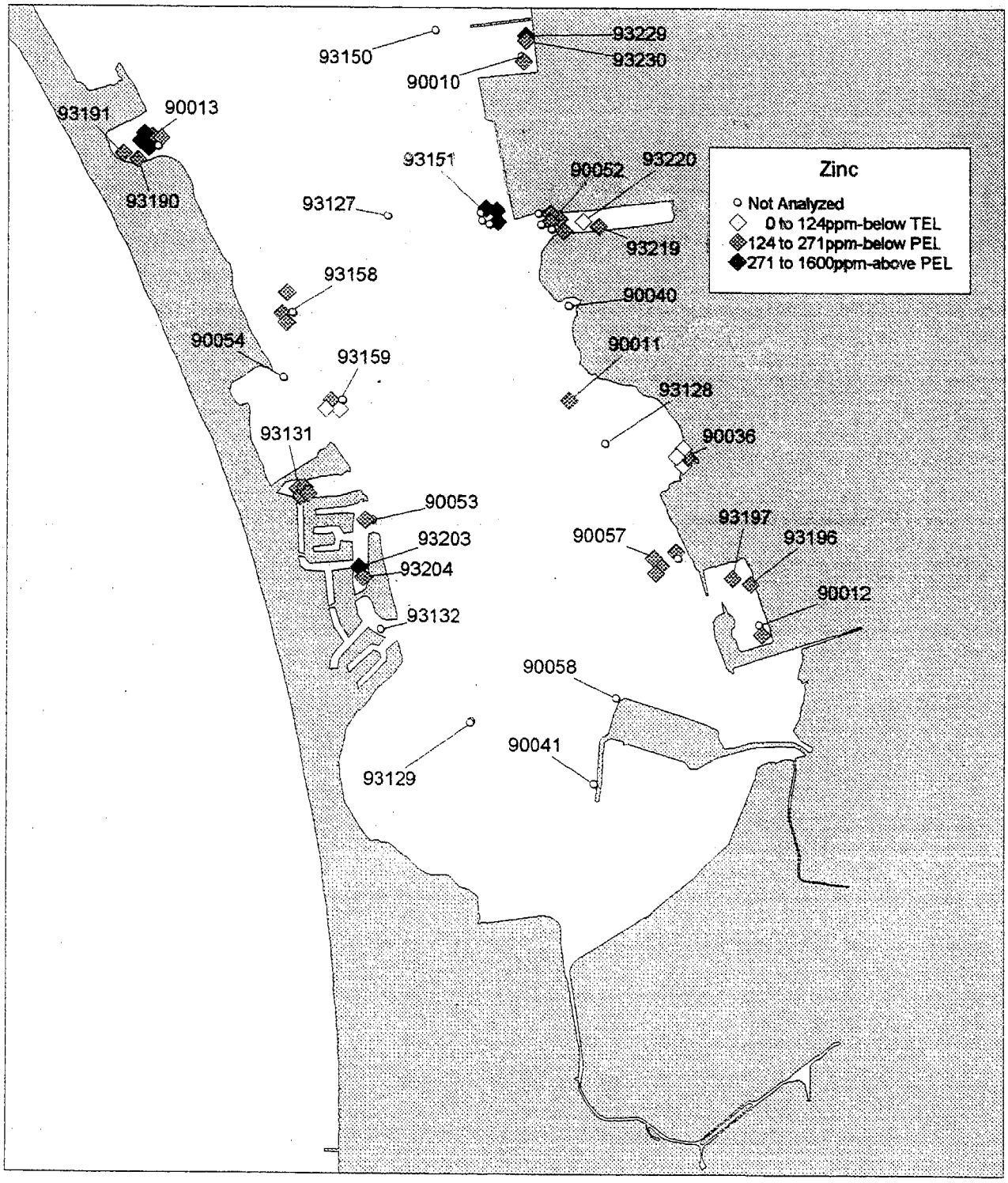
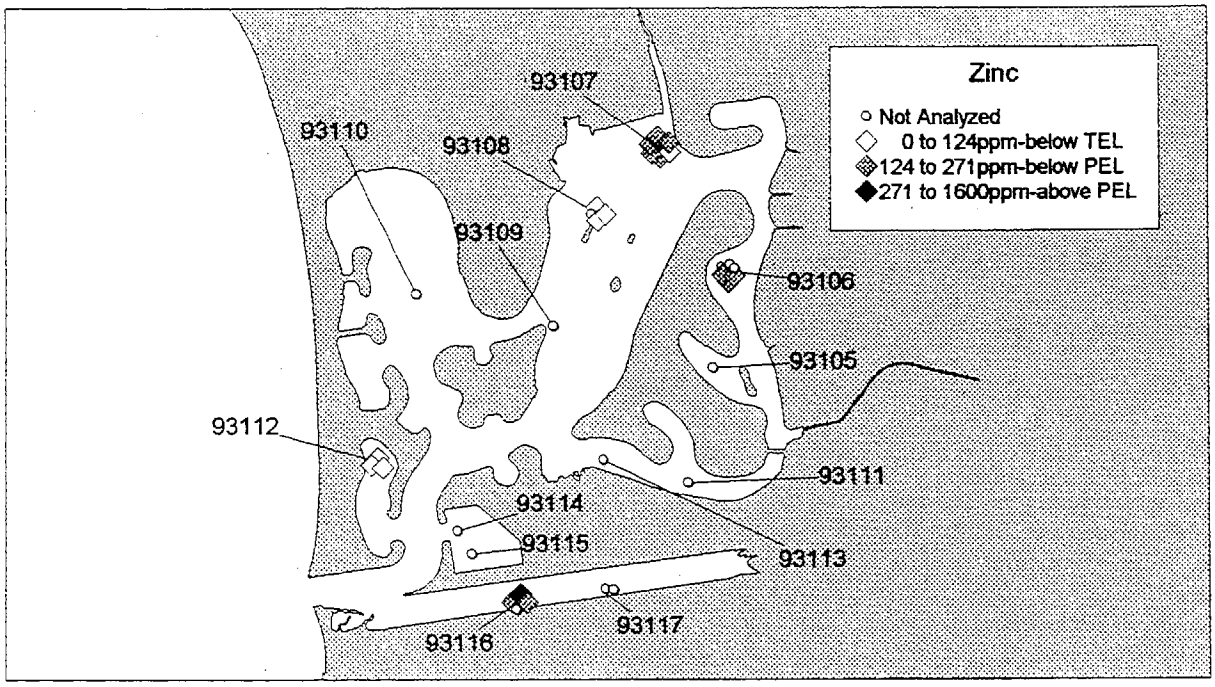


Figure 7d
Zinc Concentrations in Sediment
Mission Bay & San Diego River Estuary



Tijuana River Estuary

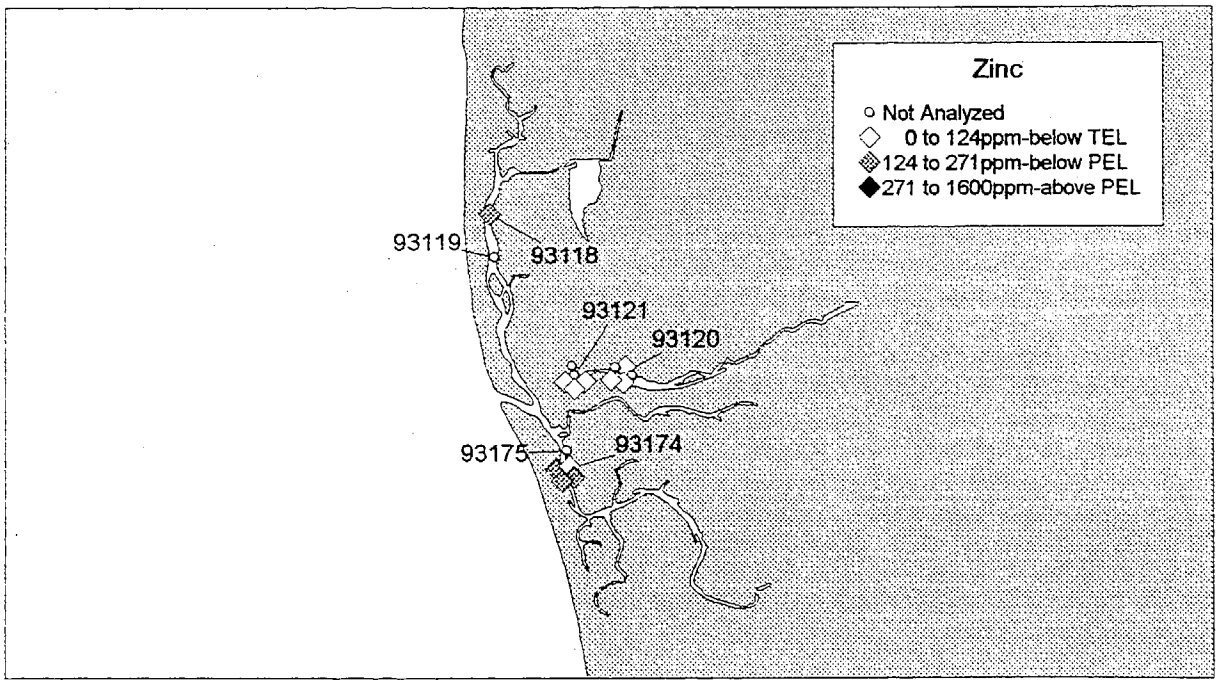


Figure 8a
Mercury Concentrations in Sediment
North San Diego Bay

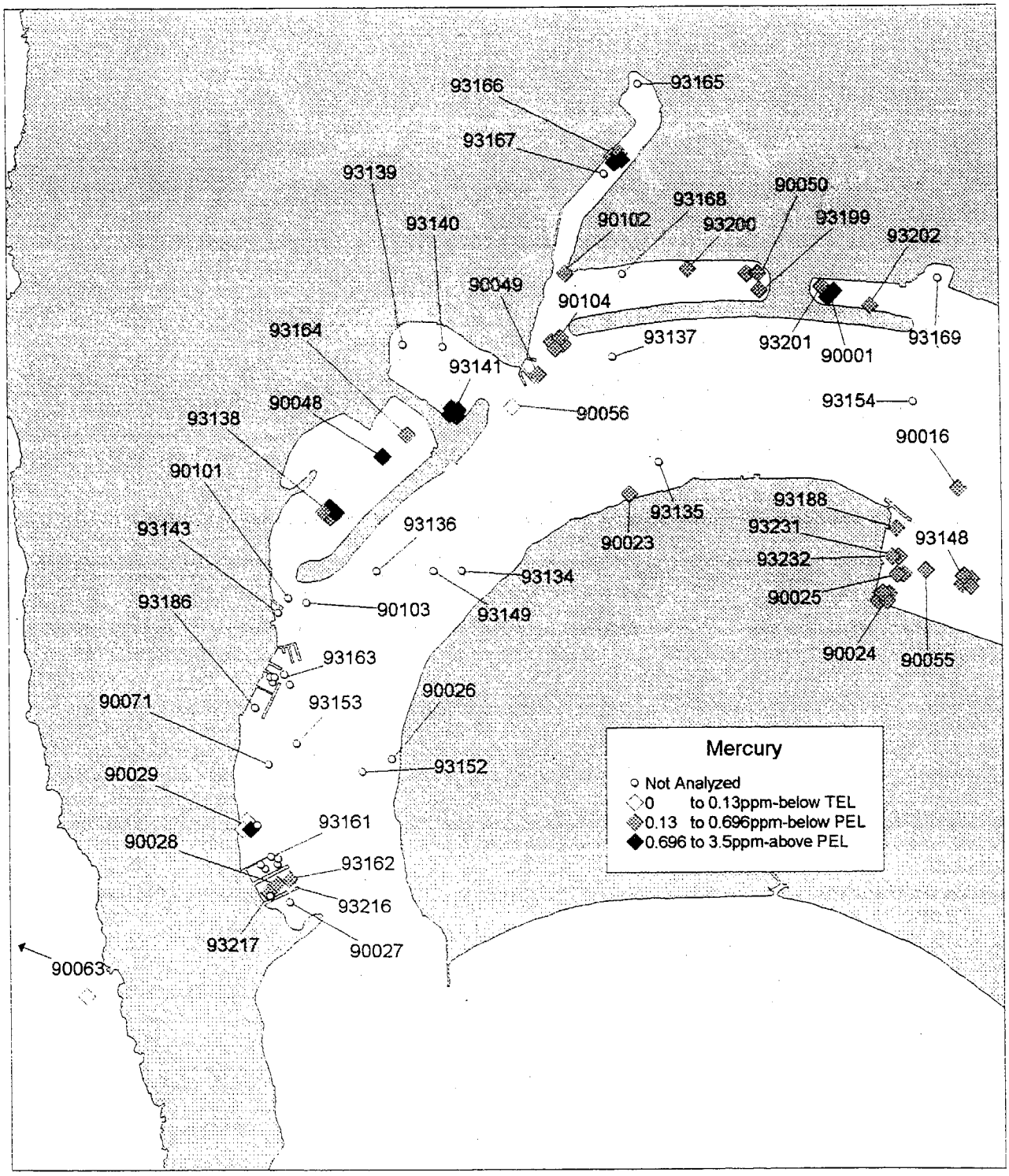


Figure 8b
Mercury Concentrations in Sediment
Mid San Diego Bay

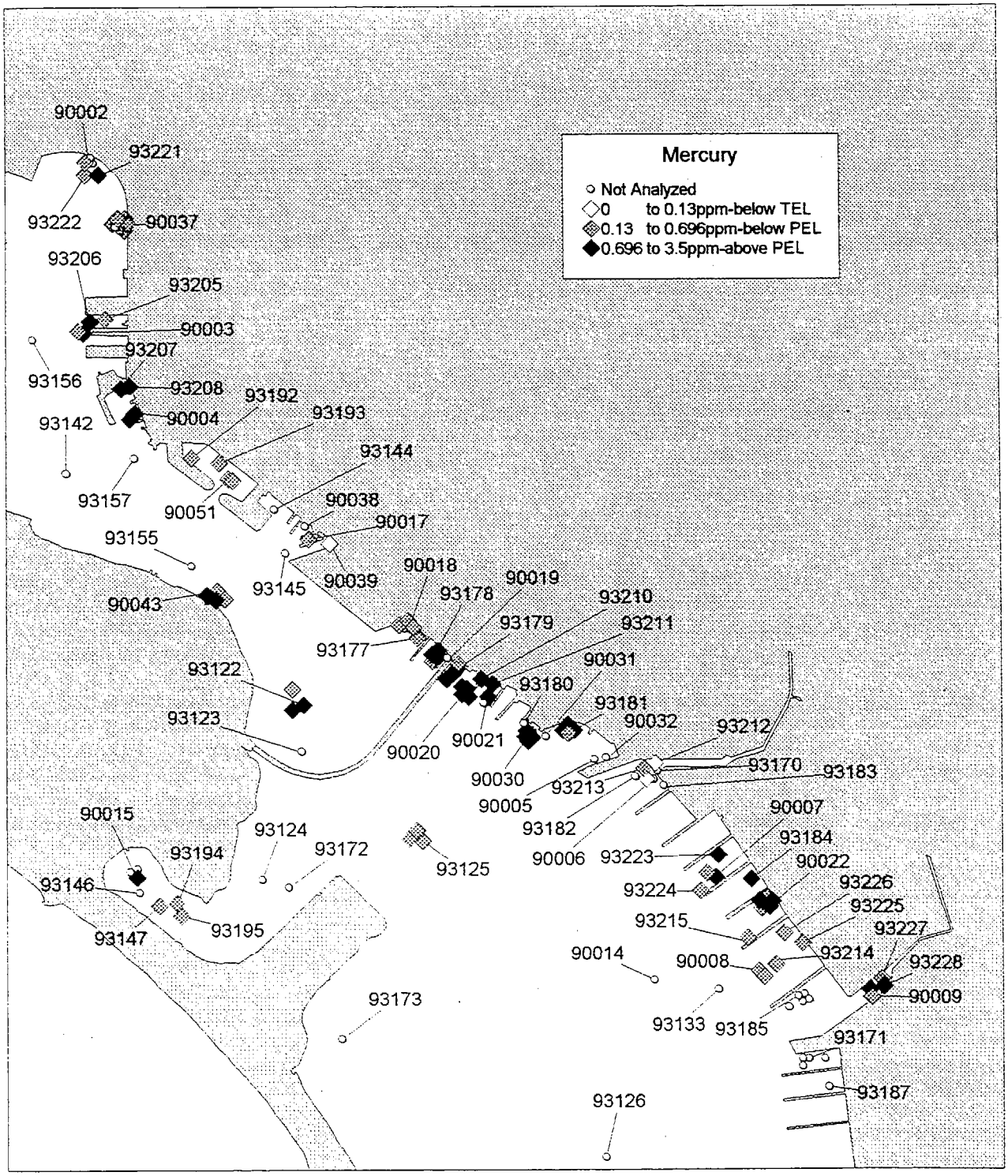


Figure 8c
Mercury Concentrations in Sediment
South San Diego Bay

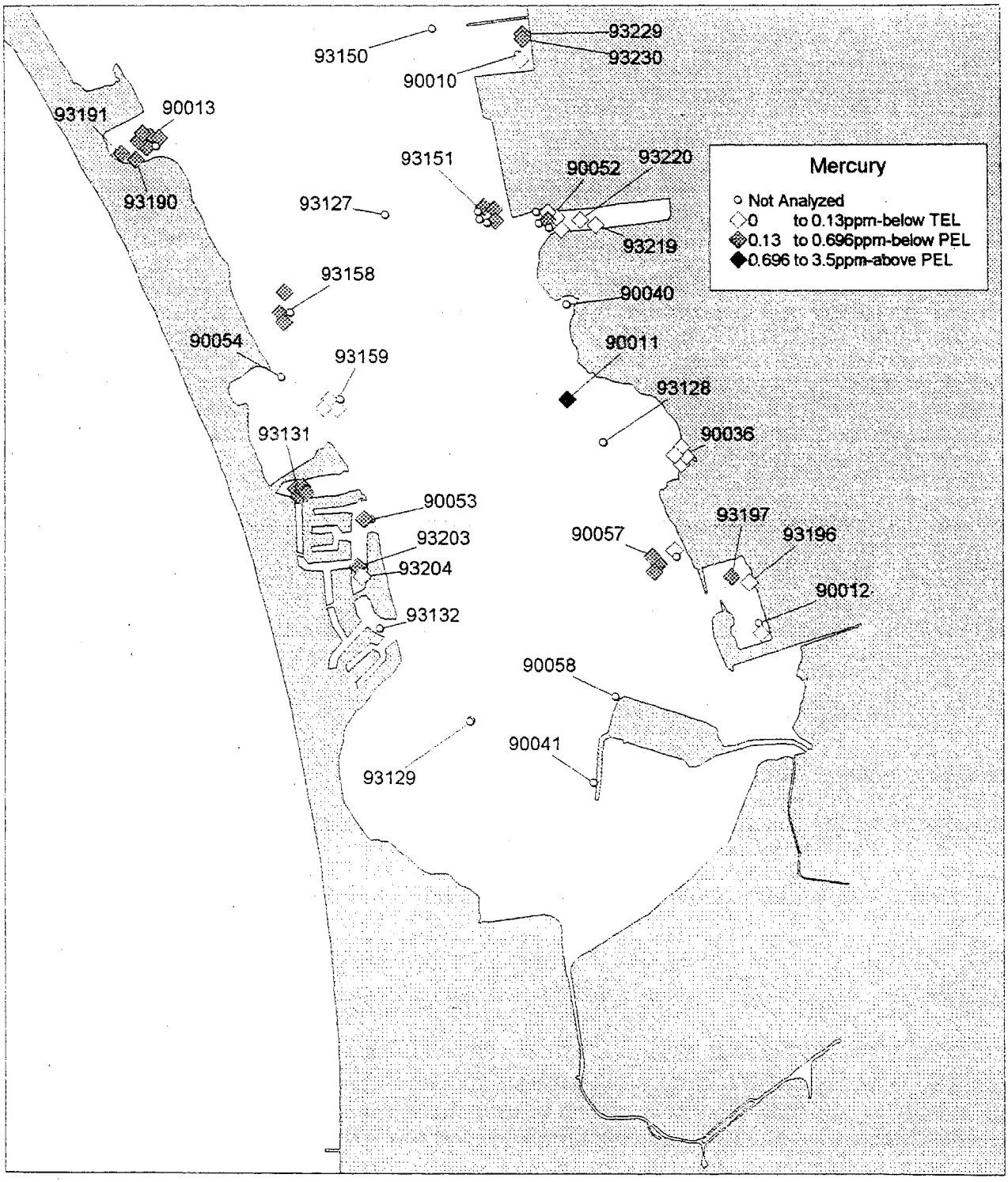
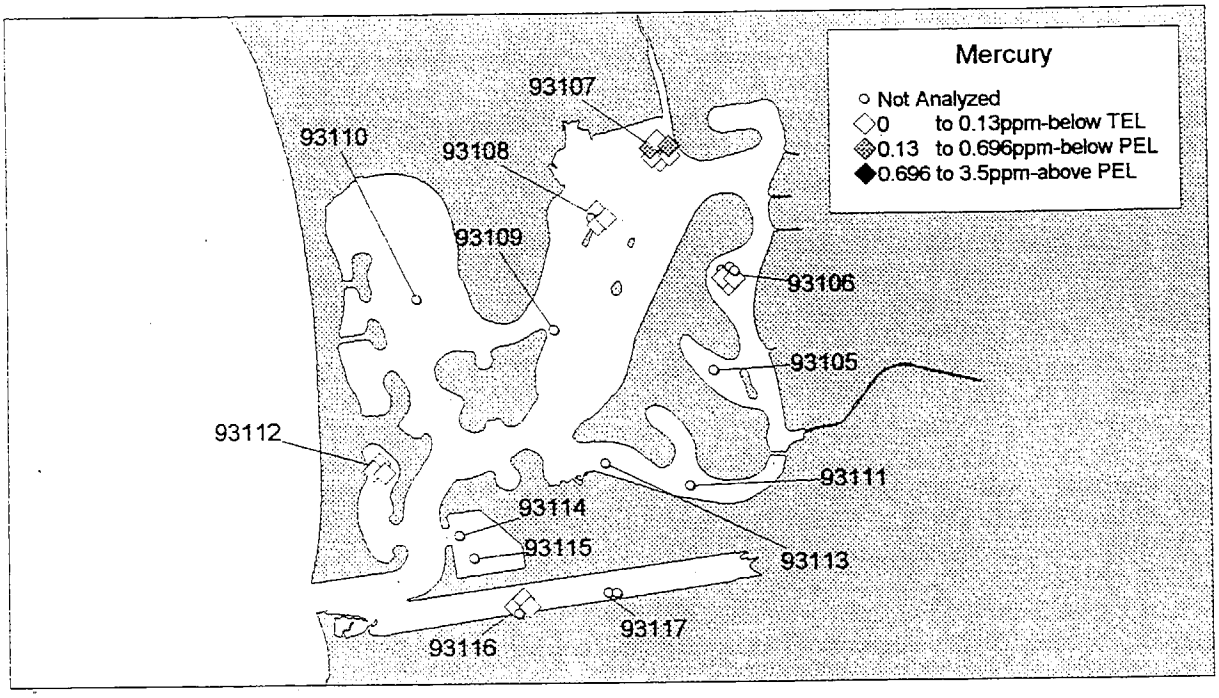


Figure 8d
Mercury Concentrations in Sediment
Mission Bay & San Diego River Estuary



Tijuana River Estuary

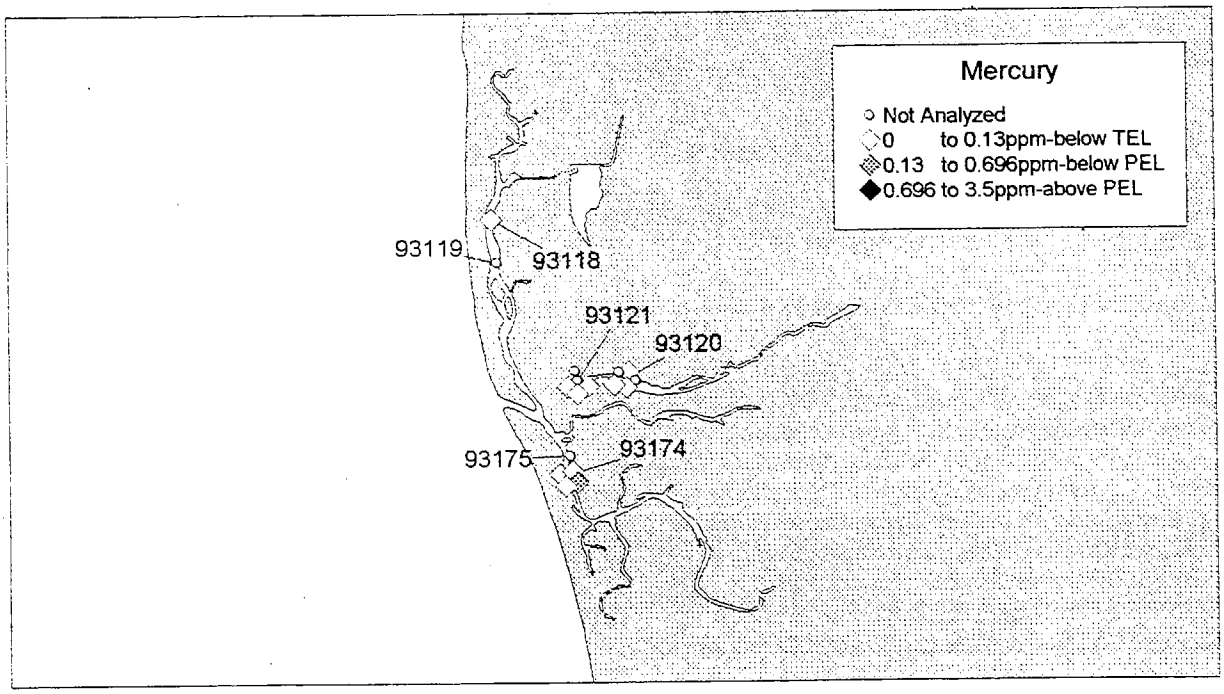


Figure 9a
High Molecular Weight PAH Concentrations in Sediment
North San Diego Bay

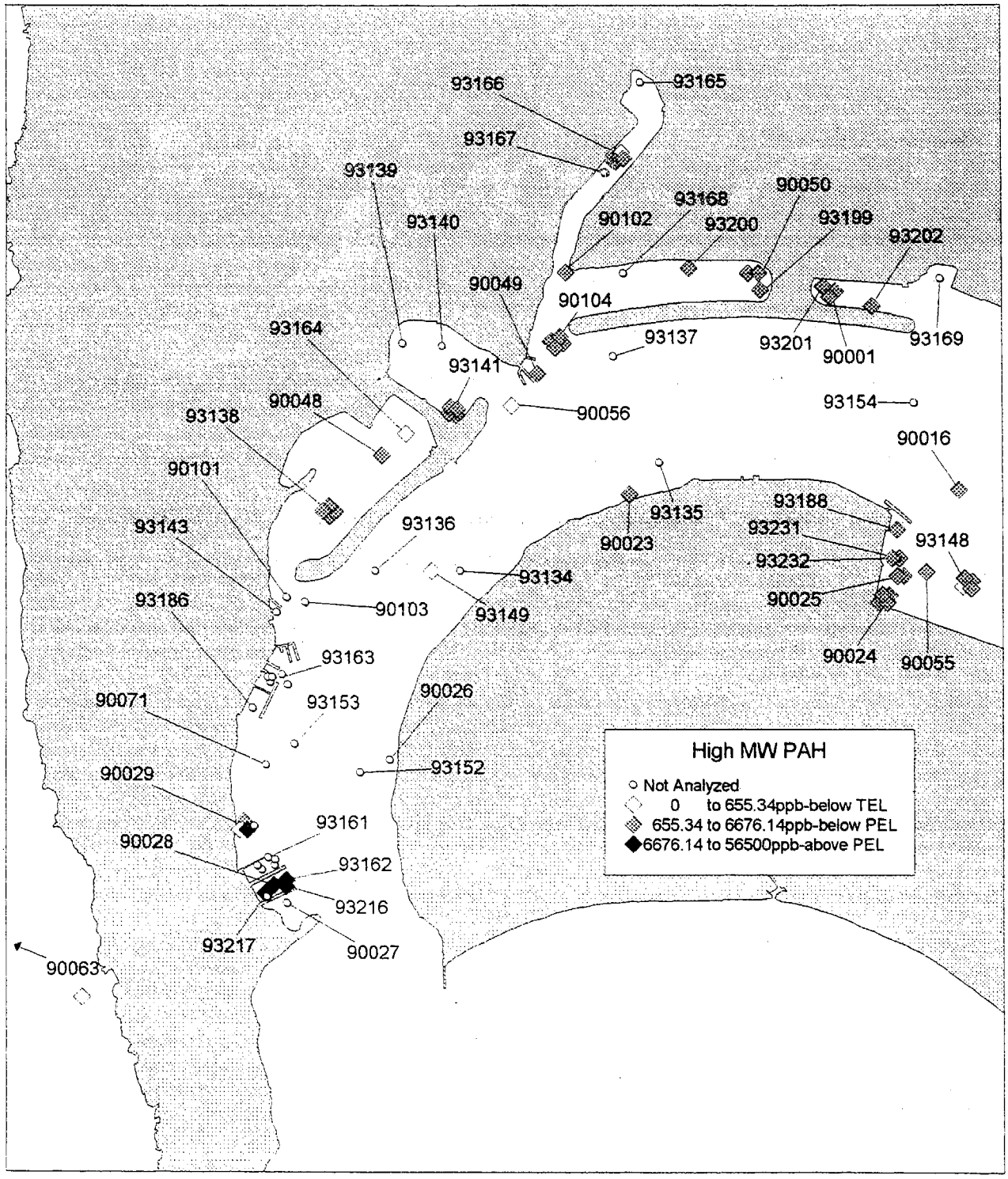


Figure 9b
High Molecular Weight PAH Concentrations in Sediment
Mid San Diego Bay

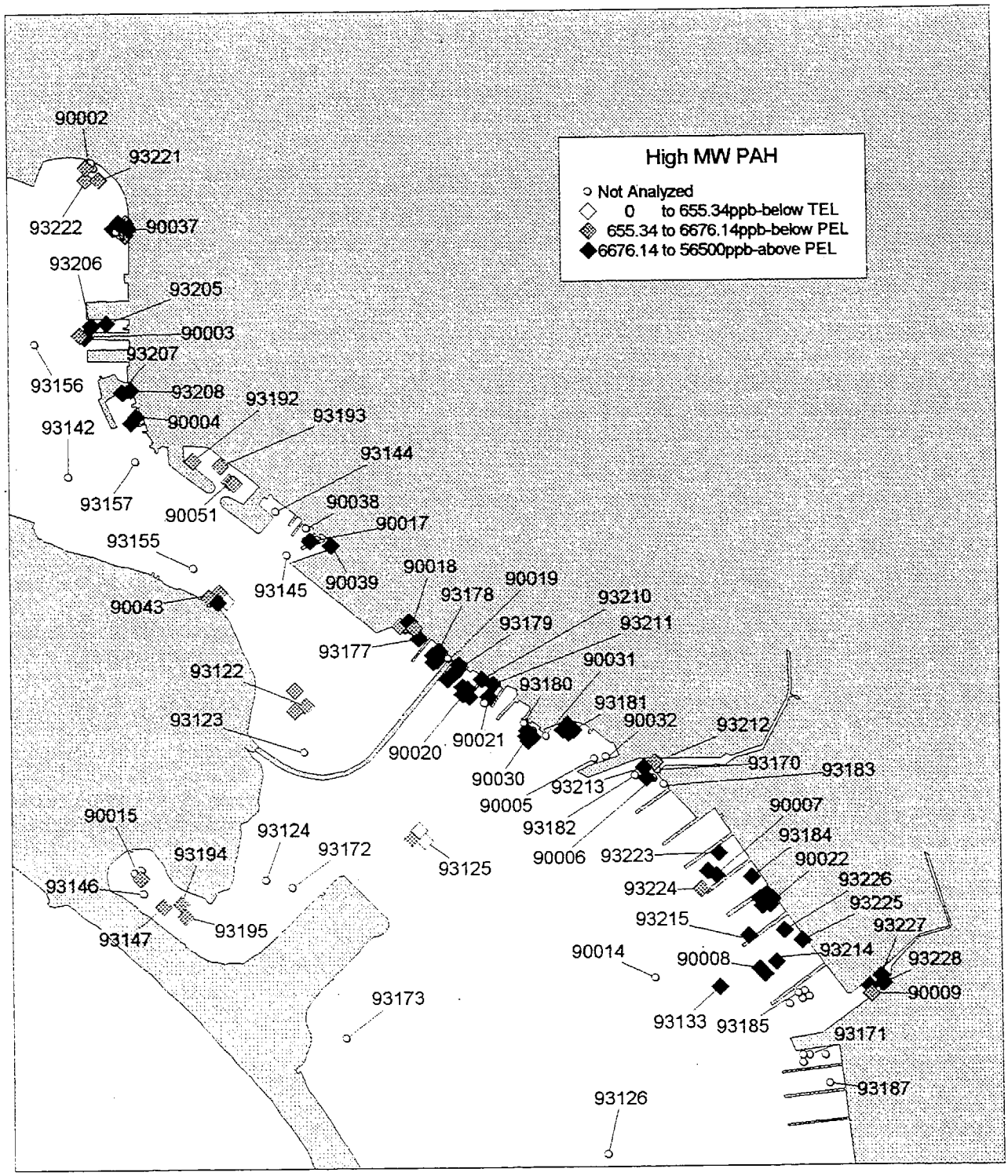


Figure 9c
High Molecular Weight PAH Concentrations in Sediment
South San Diego Bay

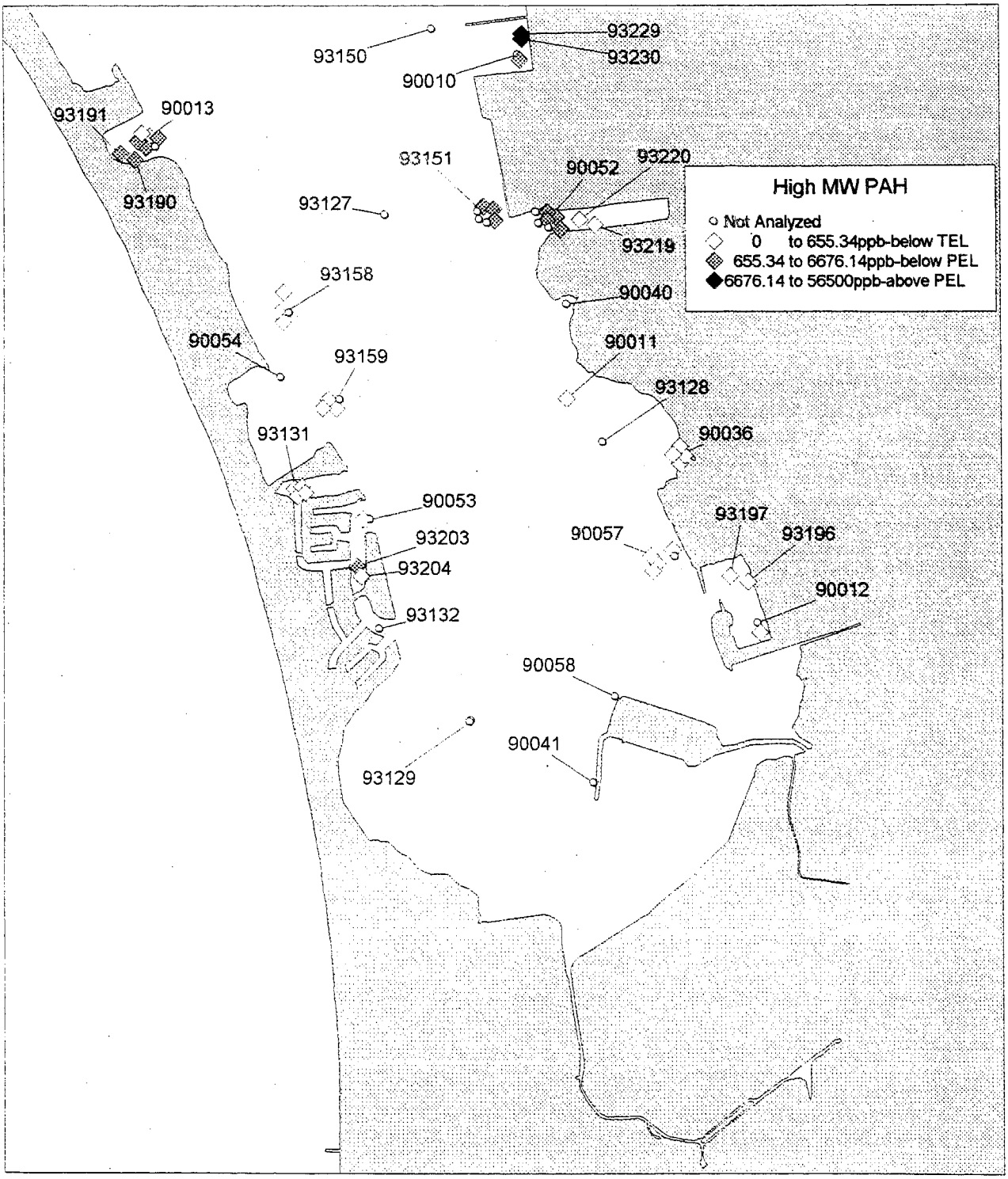
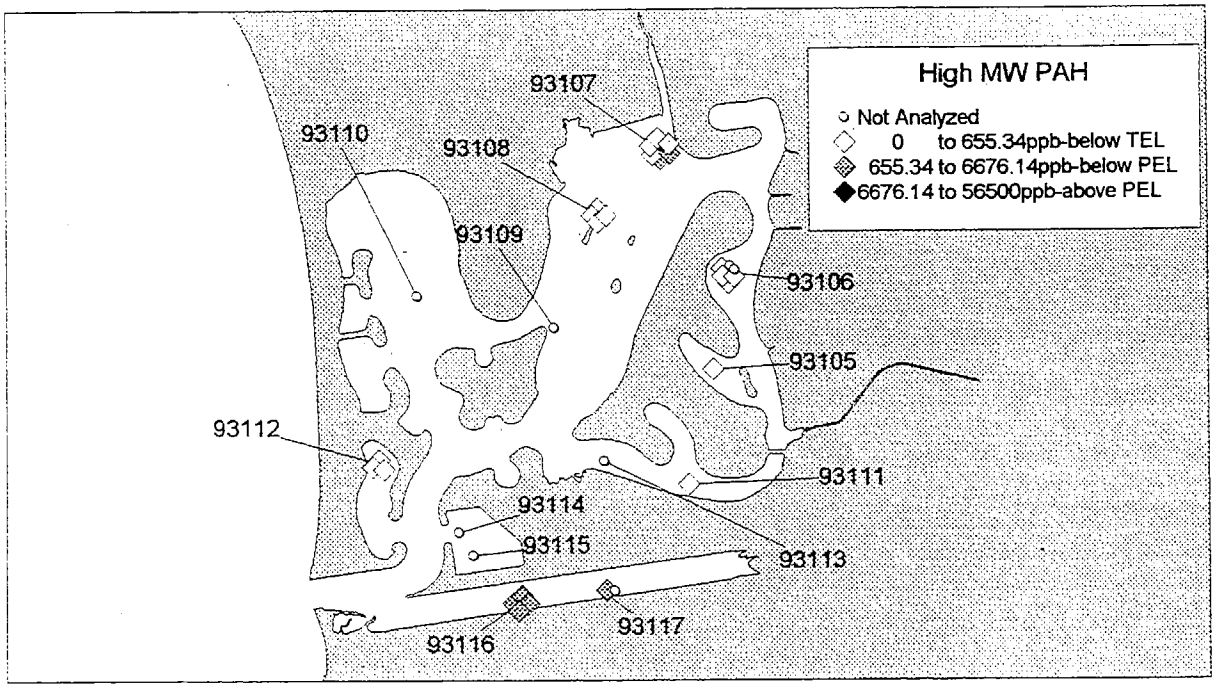


Figure 9d
High Molecular Weight PAH Concentrations in Sediment
Mission Bay and San Diego River Estuary



Tijuana River Estuary

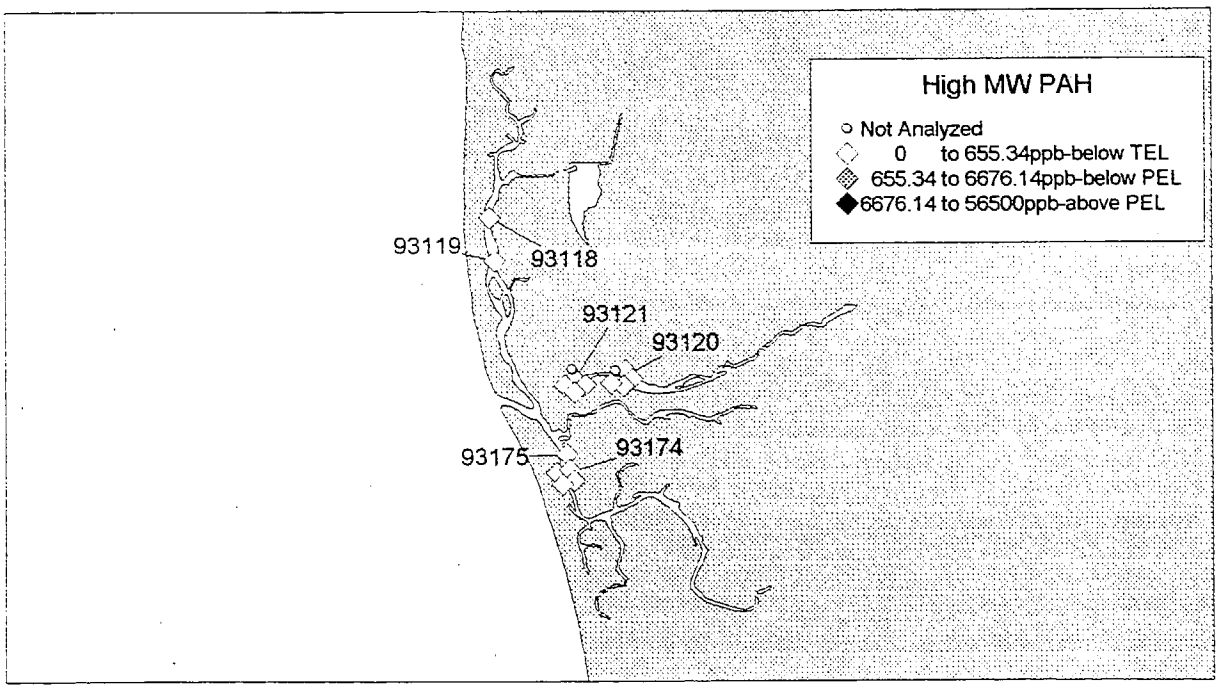


Figure 10a
Low Molecular Weight PAH Concentrations in Sediment
North San Diego Bay

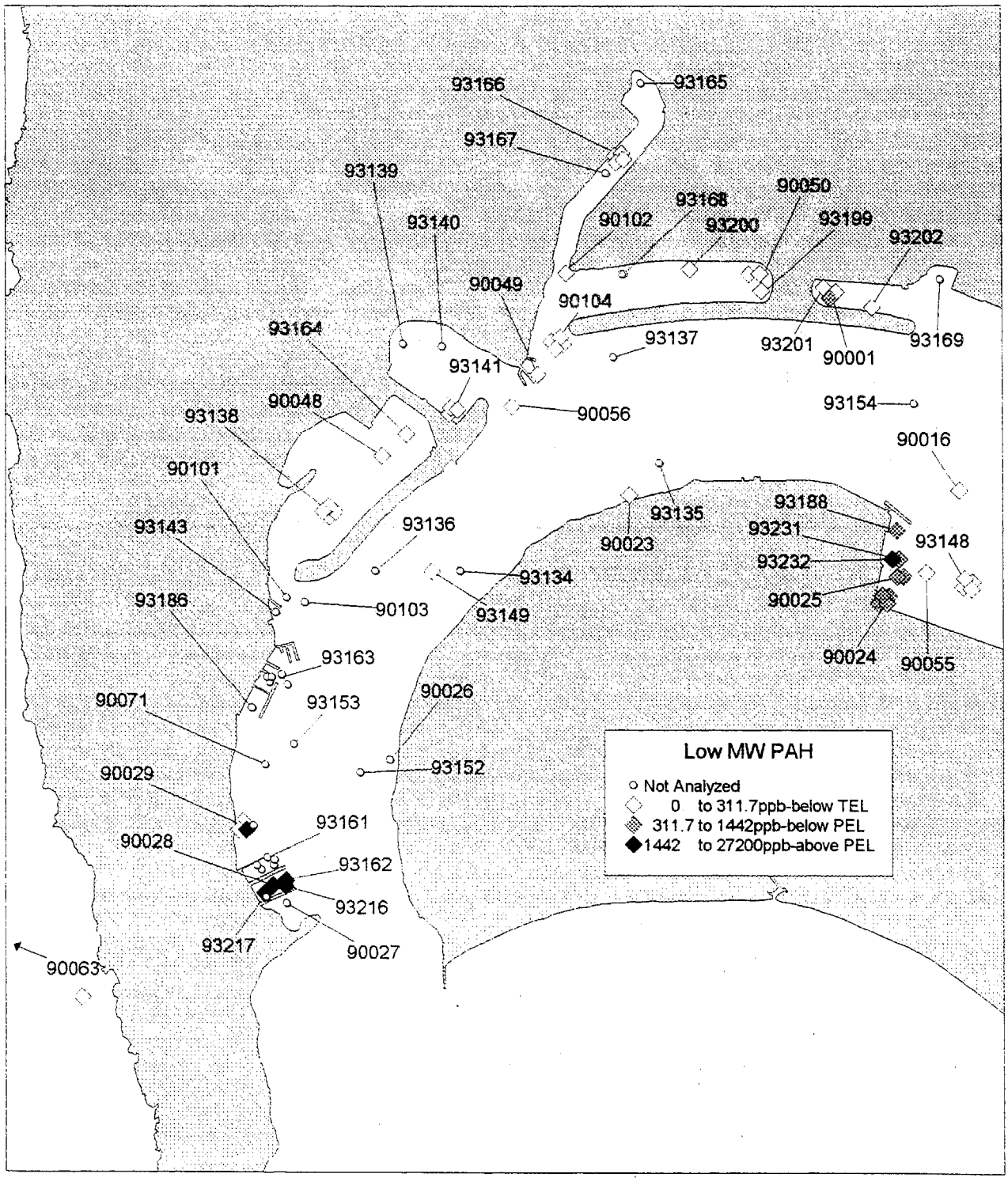


Figure 10b
Low Molecular Weight PAH Concentrations in Sediment
Mid San Diego Bay

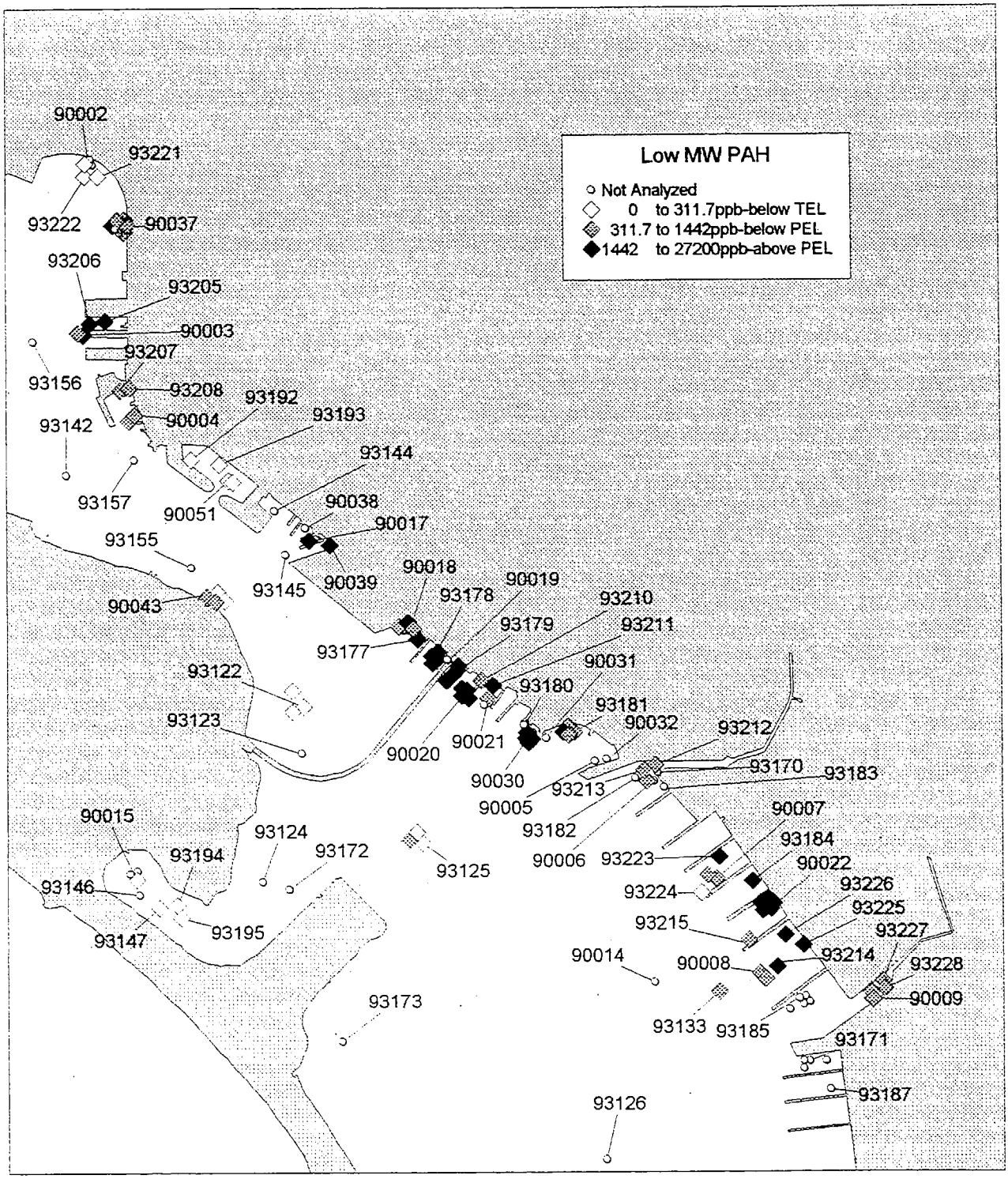


Figure 10c
Low Molecular Weight PAH Concentrations in Sediment
South San Diego Bay

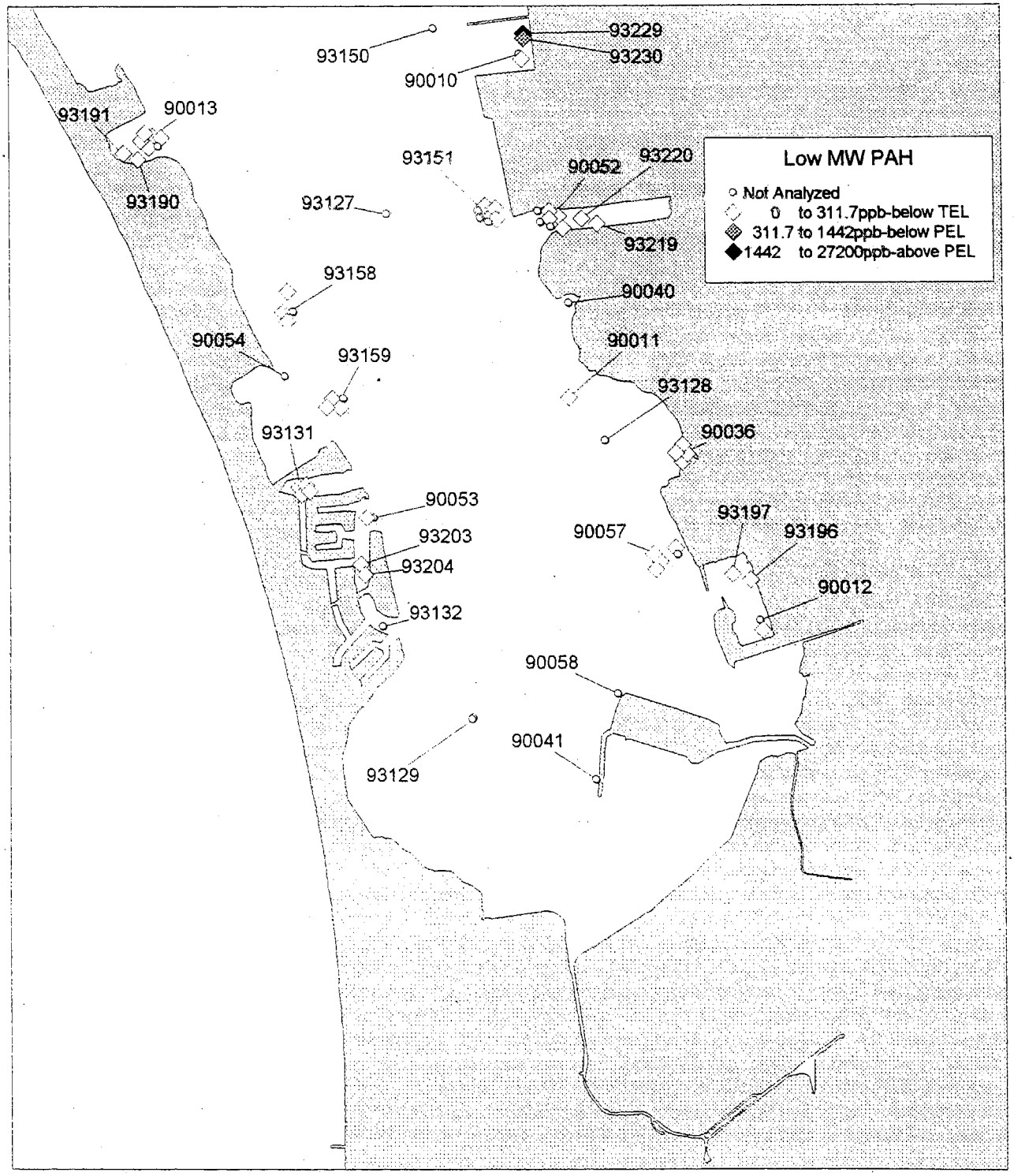
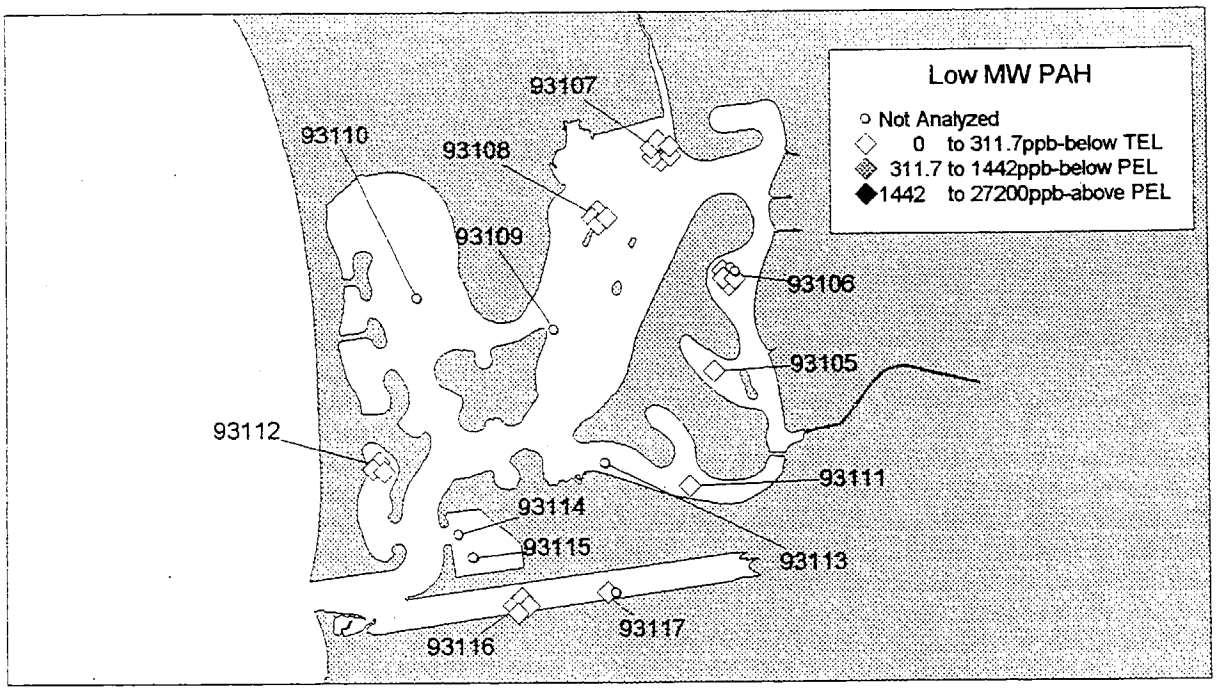
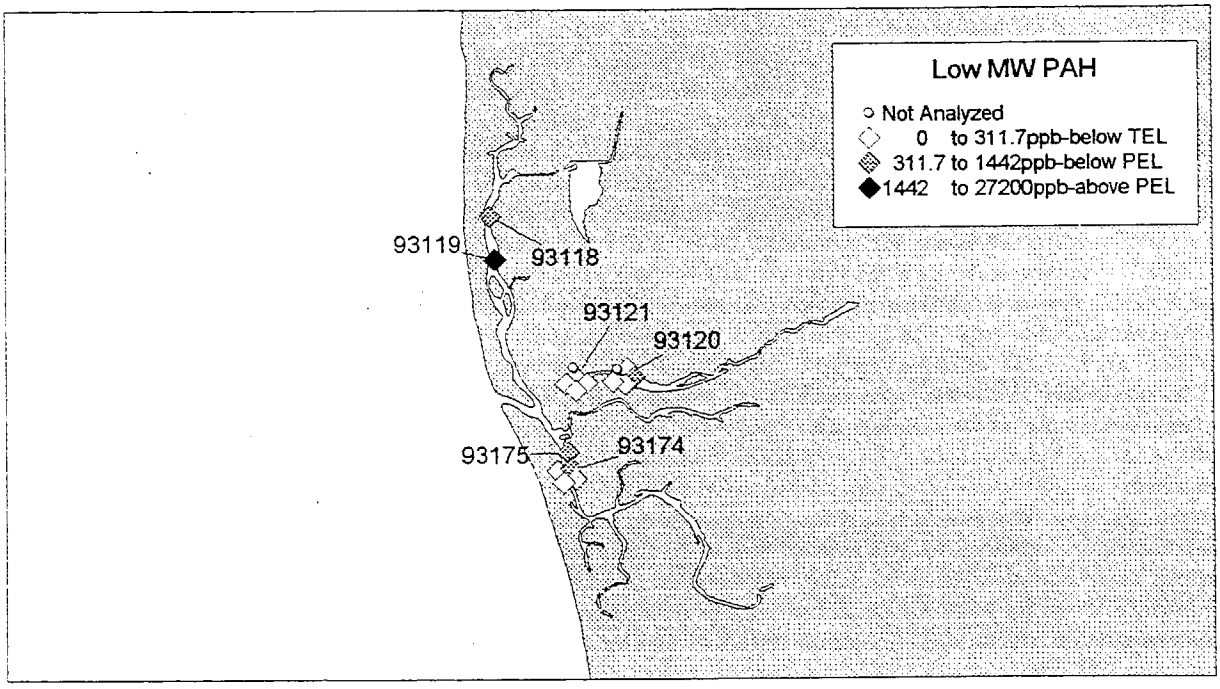


Figure 10d
Low Molecular Weight PAH Concentrations in Sediment
Mission Bay and San Diego River Estuary



Tijuana River Estuary



biphenyl. There are 209 numerically designated individual compounds, called congeners (*i.e.*, PCB #101), based on the possible chlorine substitution patterns. Mixtures of various PCB congeners have been manufactured in the U.S. since 1929 (Phillips, 1987) and are used commercially under the trade name Aroclor. Each PCB mixture has a number designation (*i.e.*, Aroclor 1254) with the last two numbers indicating the percentage of chlorine in the mixture. PCB mixtures were used extensively in the U.S. prior to 1979 for industrial applications which required fluids with thermal stability, fire and oxidation resistance and solubility in organic compounds (Hodges, 1977). PCBs have proven to be extremely persistent in the environment and have demonstrated a variety of adverse carcinogenic and non-carcinogenic effects (USEPA, 1993c). These substances have a high potential to accumulate in the tissues of aquatic organisms and can represent significant hazards to consumers of aquatic species (Moore and Walker, 1991). Total PCB (the sum of 18 congeners, Appendix B - Section VII) pollution is most prominent in sediments along the naval shipyard waterfront (Figure 11(a-d)), although several locations along the downtown waterfront and small boat harbors also show total PCB values in excess of the PEL (>188.79 µg/kg) and ERM (>180 µg/kg).

Chlordane is a multipurpose insecticide which has been used extensively in home and agricultural applications for the control of termites and other insects. Although use of this compound ended in the mid-70s, its persistence in sediments of the region is apparent. Total chlordane is the summation of major constituents of technical grade chlordane and its metabolite (Appendix B - Section VII). Chlordane pollution is extensive along the north shore of San Diego Bay, the San Diego River, and the most northerly station in Mission Bay (Figure 12(a-d)). Areas which receive storm runoff, such as Chollas Creek, Seventh St. Channel, and urban storm drains appear to be the most heavily contaminated (PEL (>4.79 µg/kg) or ERM (>6 µg/kg)).

ERM and PEL Summary Quotients

In this report, comparisons of the data to effects-based numerical guidelines were made to assess how sediment pollution in the San Diego Bay Region compares to sediment pollution on a national scale. Additionally, these guidelines were used to identify chemicals of concern for sediment quality management within the San Diego Bay Region. Rankings and comparisons were made in this report using summary ERM-quotients (ERMQ) and PEL-quotients (PELQ). Summary quotients are summations of chemical concentrations for chemicals listed in Table 5, divided by their respective ERM or PEL value, and then divided by total number of chemicals used. In samples where levels of measured chemicals were below the analytical method detection limit (MDL), a value of one-half the MDL was used for summations. Methods and analytes used for summations and averaging are given in Appendix B-Section VII. This was a simple approach for addressing overall chemical pollution where there were multiple pollutants at a station, and was in addition to the standard chemical by chemical

Figure 11a
Total PCB Concentrations in Sediment
North San Diego Bay

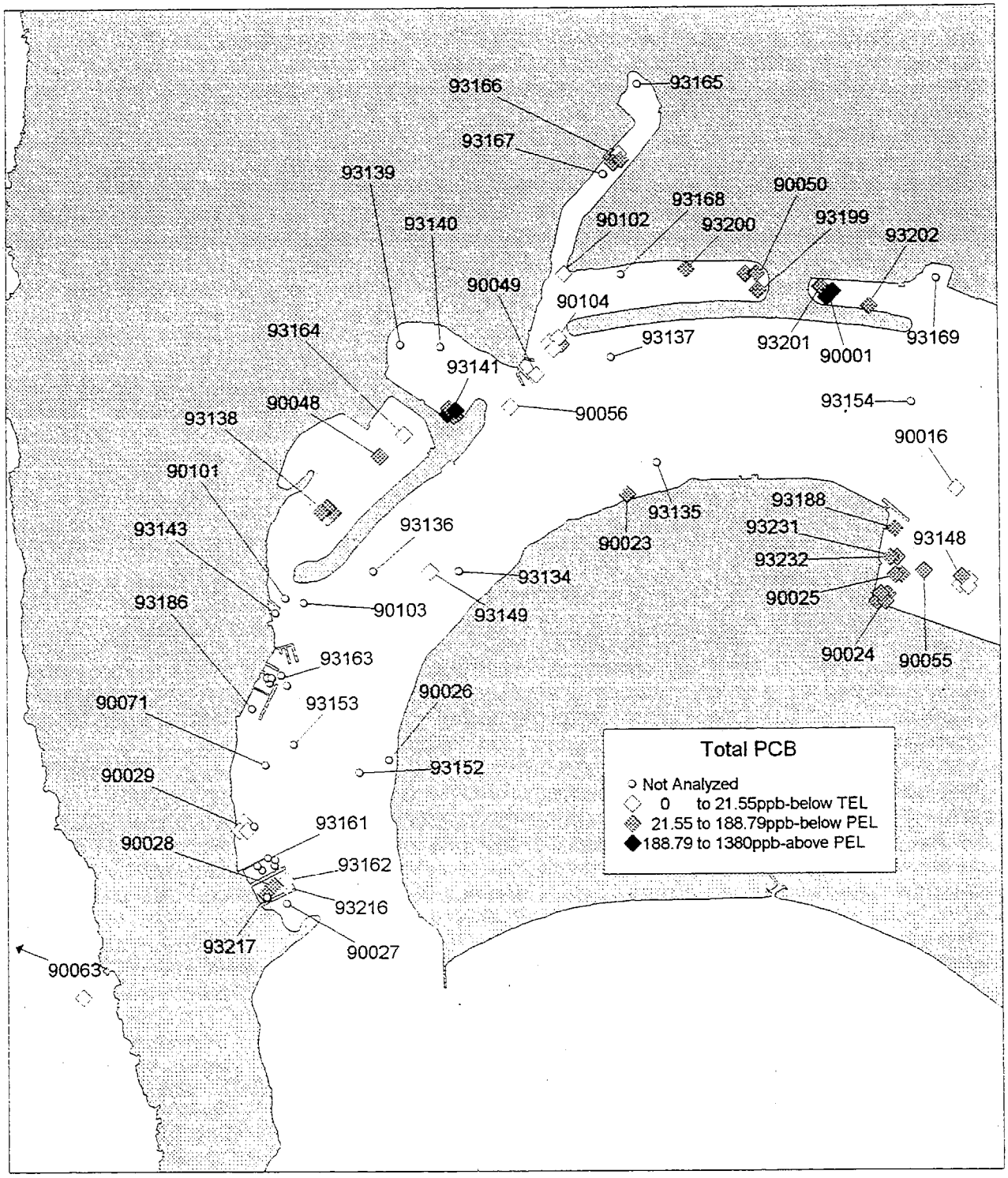


Figure 11b
Total PCB Concentrations in Sediment
Mid San Diego Bay

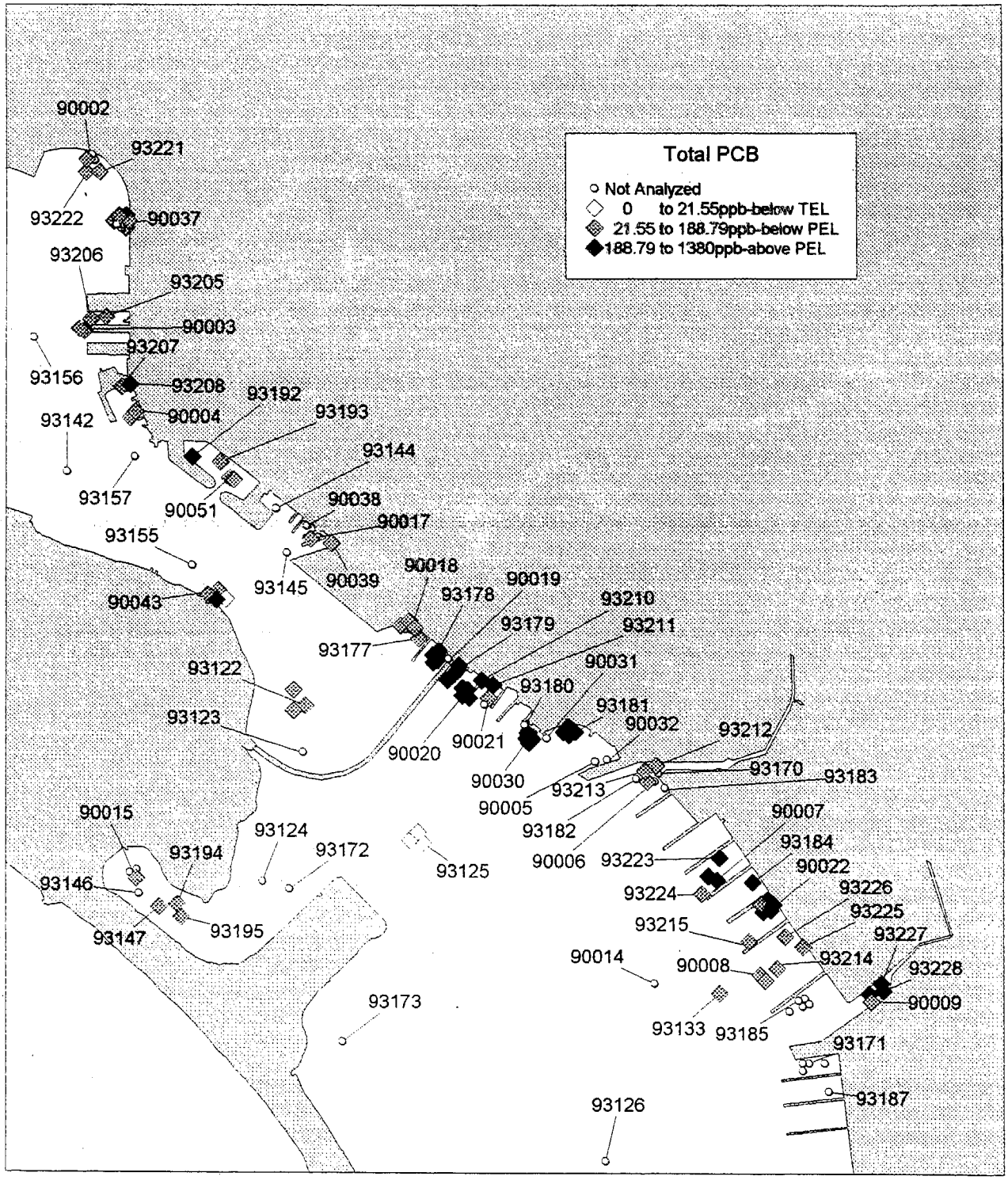


Figure 11c
Total PCB Concentrations in Sediment
South San Diego Bay

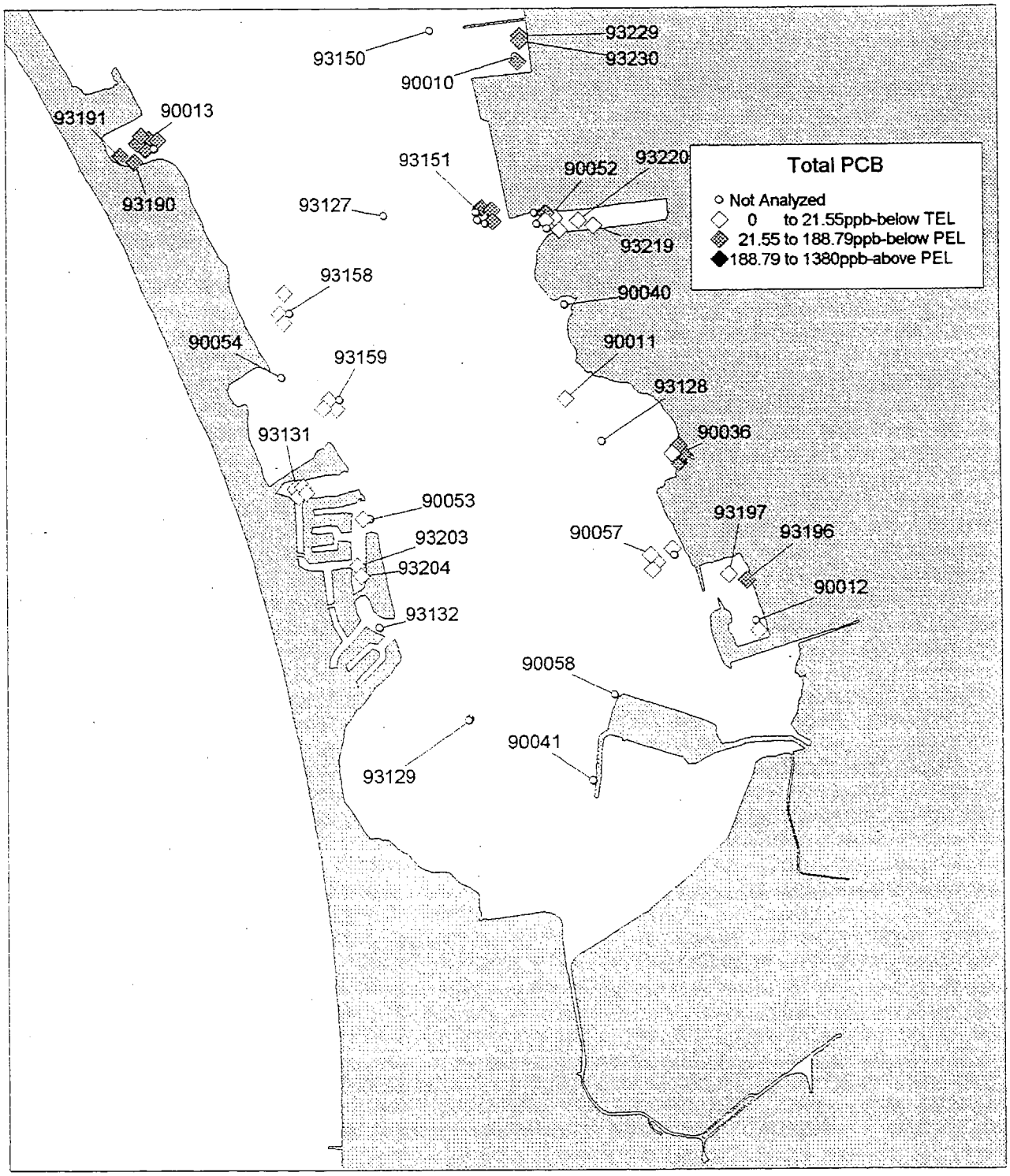
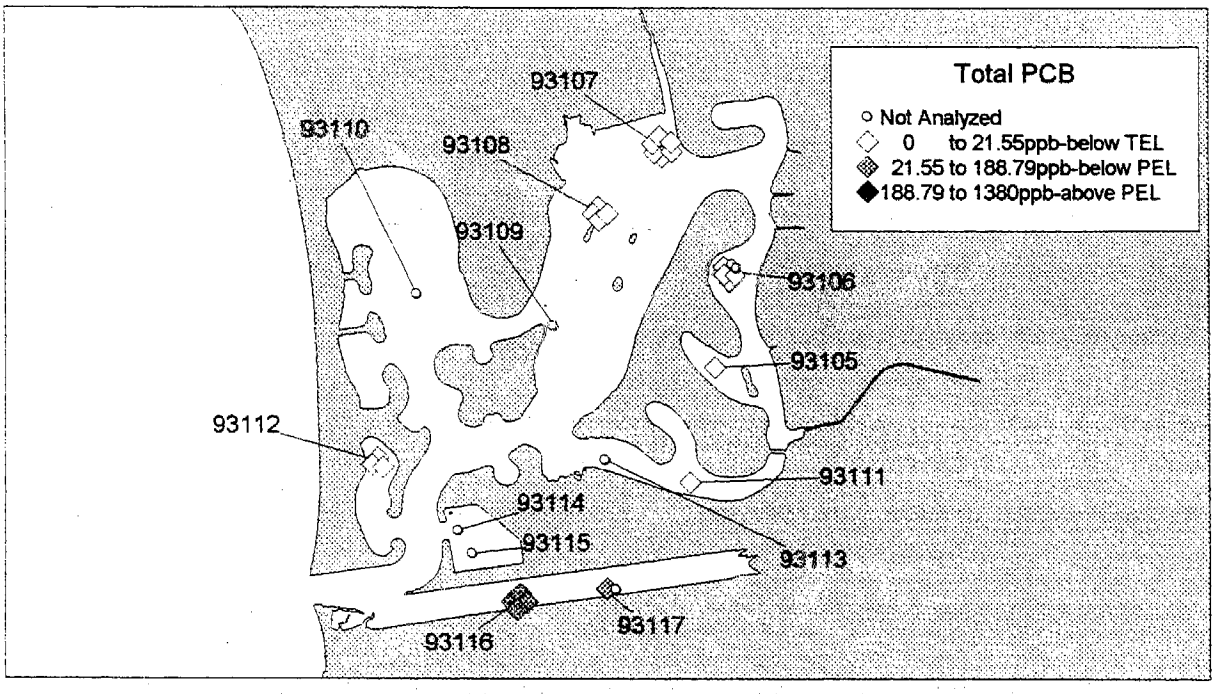


Figure 11d
Total PCB Concentrations in Sediment
Mission Bay and San Diego River Estuary



Tijuana River Estuary

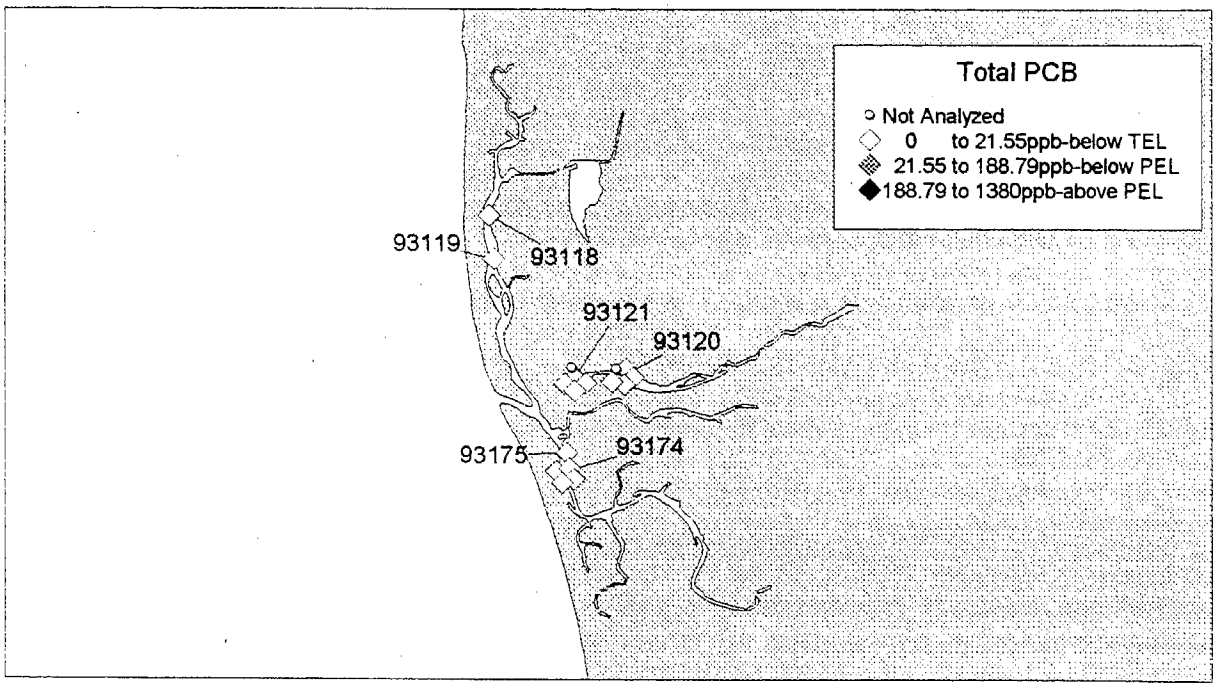


Figure 12a
Total Chlordane Concentrations in Sediment
North San Diego Bay

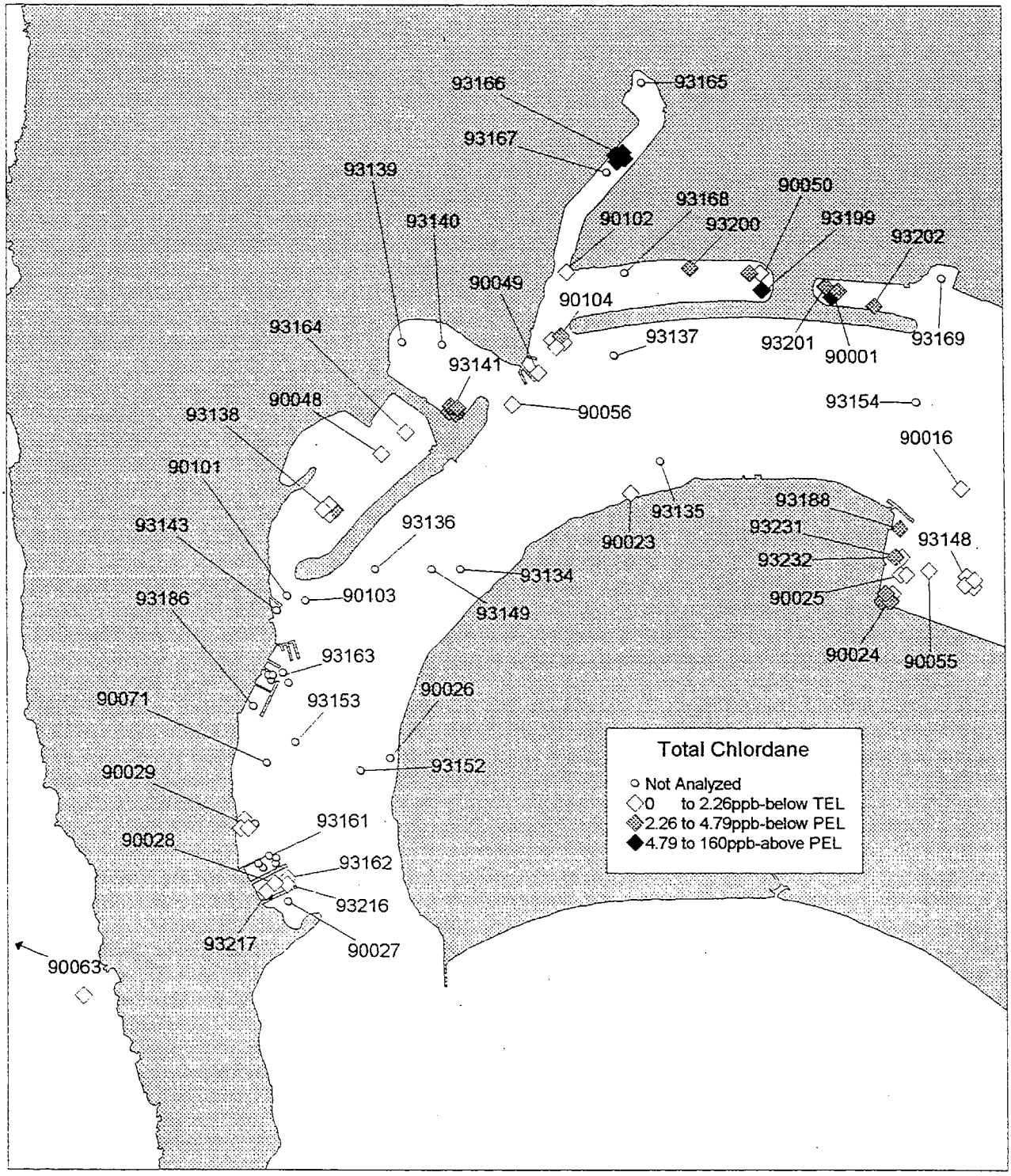


Figure 12b
Total Chlordane Concentrations in Sediment
Mid San Diego Bay

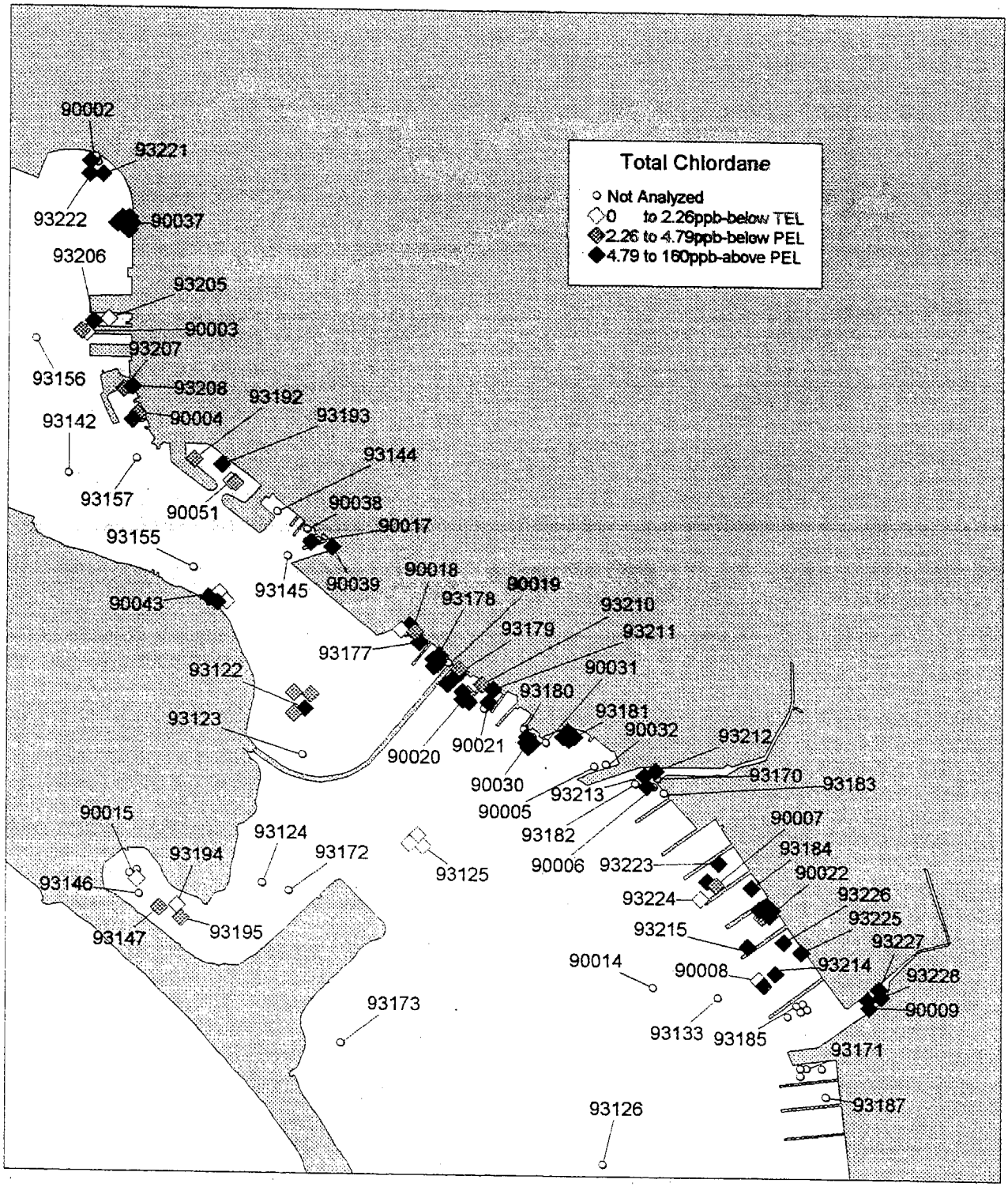


Figure 12c
Total Chlordane Concentrations in Sediment
South San Diego Bay

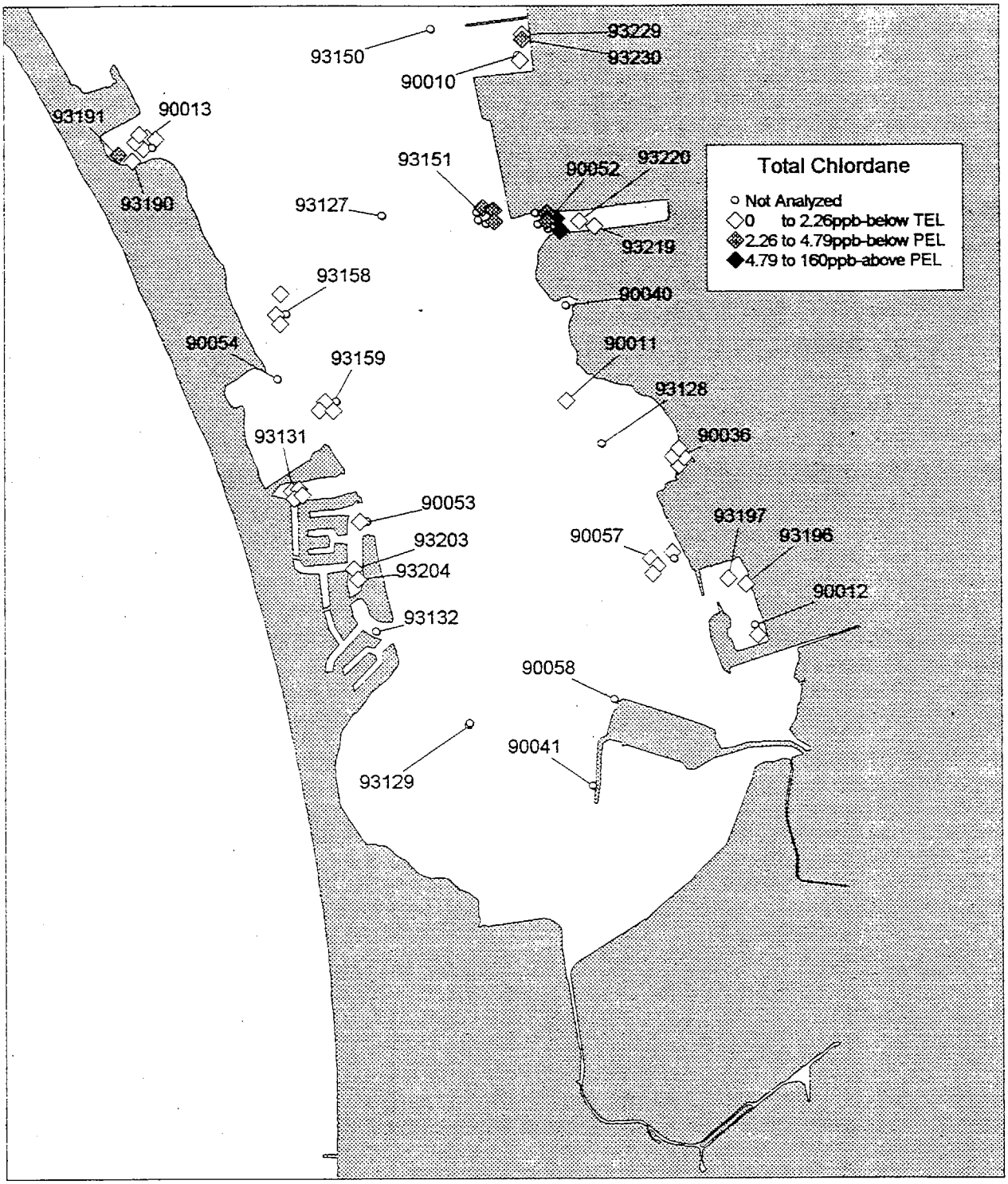
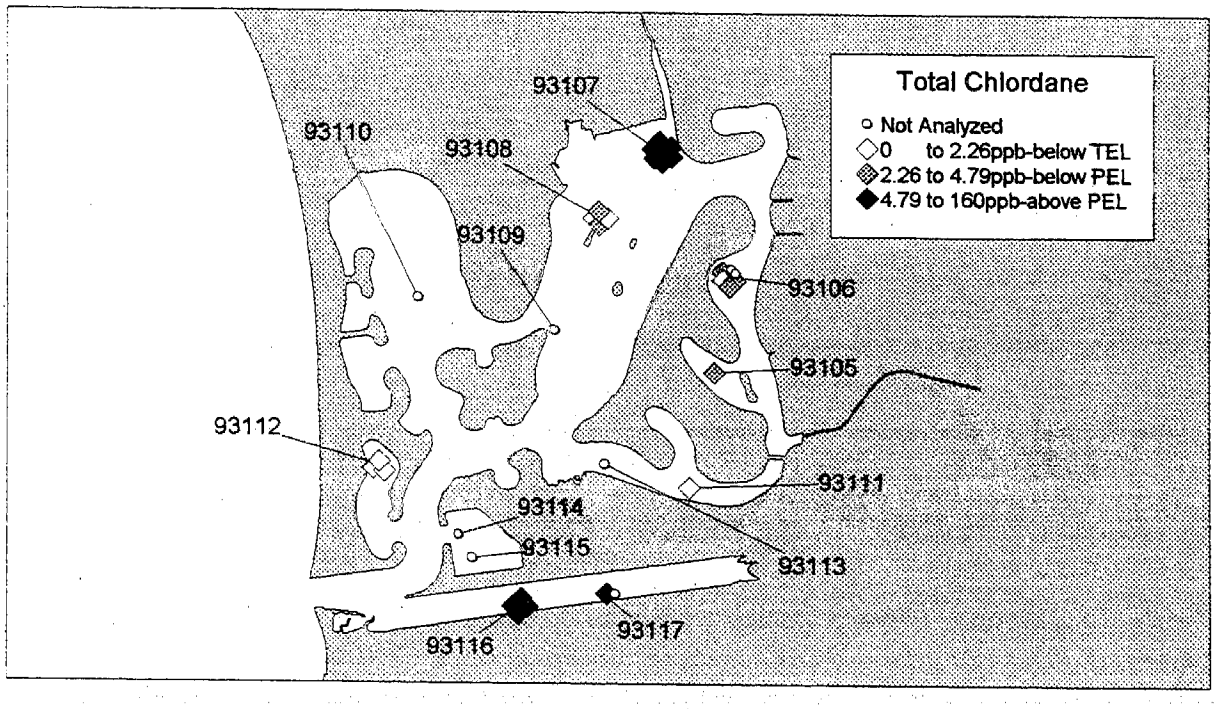
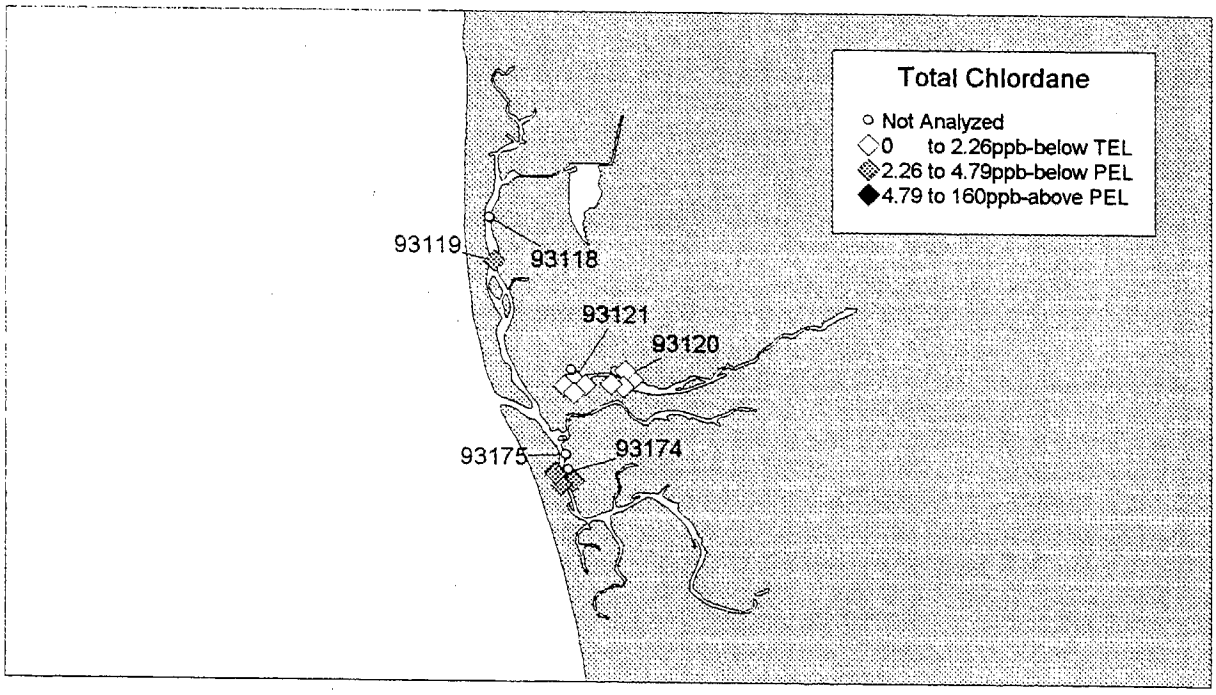


Figure 12d
Total Chlordane Concentrations in Sediment
Mission Bay and San Diego River Estuary



Tijuana River Estuary



approach discussed earlier. This approach considered not only the presence of guideline exceedances, but the number and degree of multiple exceedances.

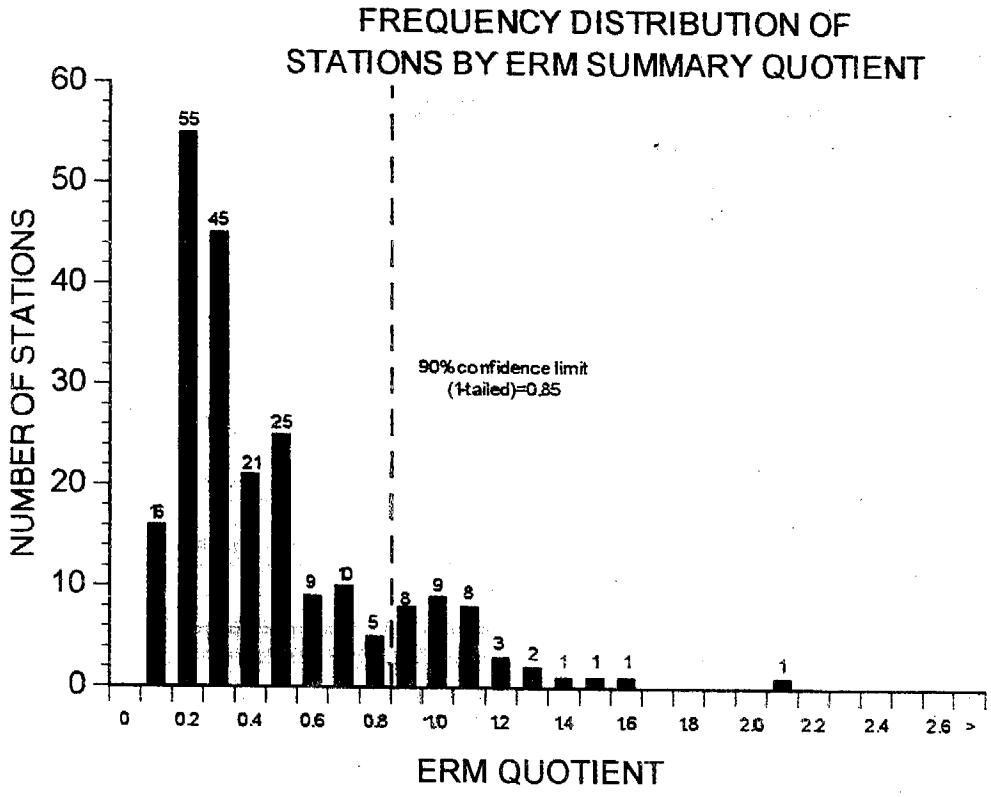
Based upon analyses of the national NS&T and EMAP database, the incidence of toxicity has been shown to increase with increasing summary ERM and PEL quotients (Long, Field and MacDonald, in prep). Synergistic effects are possible, but not implied by the quotient summations, therefore, this method should be recognized only as a ranking scheme meant to better focus management efforts on interpretation of ambient sediment chemistry data.

Interpretations using ERM and PEL summary quotients were limited to statistical analysis within this dataset because the approach has not been formally presented in other reports, therefore, outside comparisons are unavailable at this time. The 90% confidence interval from a 1-tailed t-distribution was chosen as an arbitrary threshold level for evaluating the data set. For the 220 stations on which chemical analysis was performed, stations with an ERMQ>0.85 or a PELQ>1.29 were found to fall above this confidence interval (Figure 13). Although these values of 0.85 and 1.29 cannot be considered threshold levels with proven ecological significance, they can be used for within bay comparative purposes. Forty-one stations exhibited ERM or PEL quotient levels exceeding the confidence interval cutoffs. Of these forty-one stations, twelve received benthic community analysis, all which were determined to have degraded communities in the analysis discussed later (Figure 14). All 41 stations were tested for *Rhepoxynius* toxicity, of which 29% demonstrated significant toxicity, at the 48% limit established by the reference envelope method discussed later. This difference in biological response to pollutants, between benthic community structure and bioassays, may be explained by long term exposure to pollutants in the benthic community relative to short term (10 day) pollutant exposure in bioassay tests. Use of the ERM and PEL quotients appear to give a worthwhile representation of overall chemical pollution and are used later in this report for station rankings and characterizations.

Distribution of Benthic Community Degradation

Data Analyses and Interpretation

The identification of benthic degraded and undegraded habitat (as determined by macrobenthic community structure) was conducted using a cumulative, weight-of-evidence approach. Tests were employed without prior knowledge or integration of results from laboratory exposures or chemical analyses. Analyses were performed to identify relationships between community structure within and between each station or site. This included diversity/evenness indices, analyses of habitat and species composition, construction of dissimilarity matrices for pattern testing, assessment of indicator species and development of a benthic index, cluster and ordination (multidimensional scaling) analyses. Initially, a triangular correlation matrix was produced



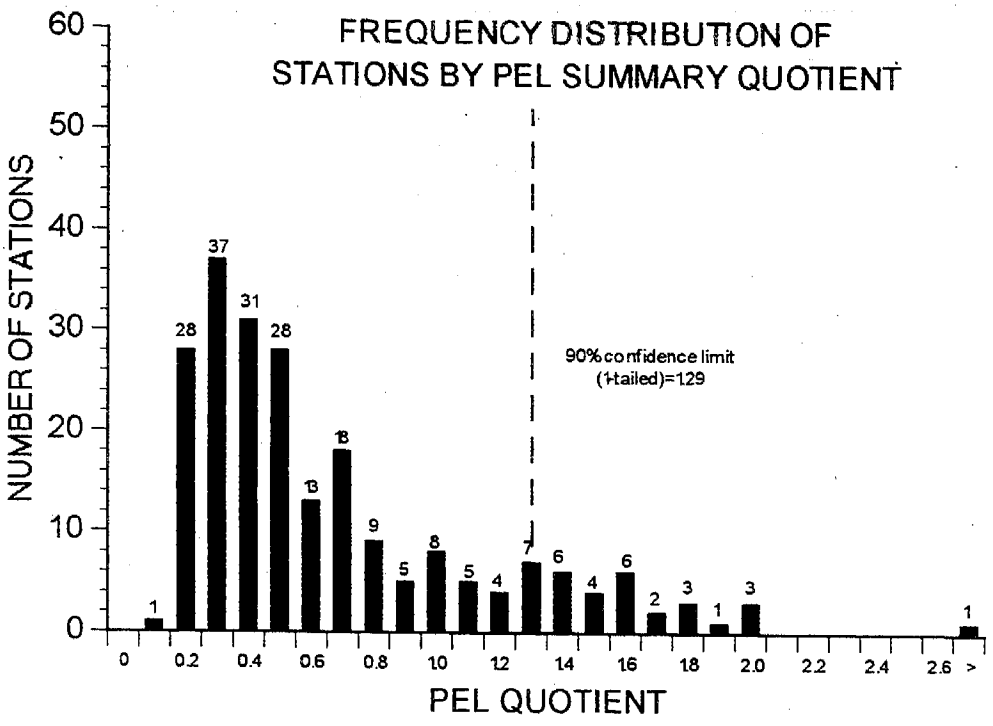


Figure 13. Histogram of the number of stations by ERM or PEL summary quotient group. Vertical dashed line indicates 90% confidence limit of the mean.

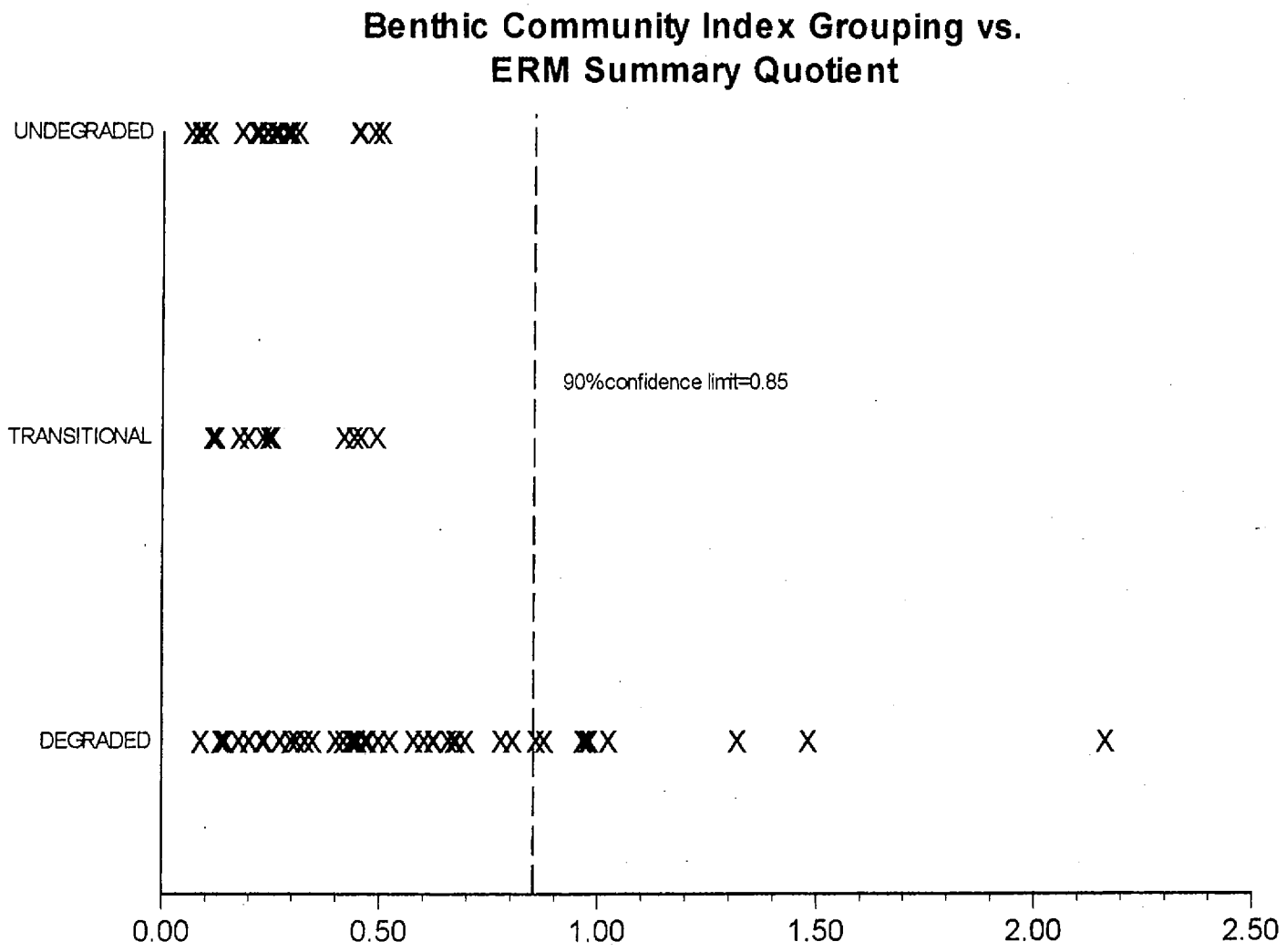


Figure 14. Benthic index grouping vs. ERM summary quotient value. Each data point represents one station (n=75).

from species density data from each site using the Systat® statistical program. From this matrix several tests for association of variables were performed. The tests employed are common in marine and estuarine benthic community analyses and are well-documented in the literature (Field *et al.*, 1982; Pearson *et al* 1983; Swartz *et al.*, 1985; Gray, 1989; Clark and Ainsworth, 1993). Classification analysis was employed to demonstrate site-related community patterns such as species dominance. Cluster analysis is a multivariate procedure for detecting natural groupings in data, and, for our purposes, data were grouped by average similarities in total composition and species abundance (Krebs, 1989). The average-linkage method calculates similarity between a pair of cluster groups as the average similarity among entities in the two groups. Species information is used to compute similarity index values. Grouped stations were clustered at a conservative distance limit of 50-60% similarity, however, this level was purely arbitrary. Because classification analyses have the tendency to force data into artificially distinct groups, another method (*e.g.*, multi-dimensional scaling) was used to confirm the validity of group clusters and site similarity. Ordination analysis was useful because it enables one to see multidimensional gradients in data rather than just groupings (Smith, personal communication).

Multi-dimensional scaling (MDS) is used extensively in the analyses of benthic communities, particularly in estuarine and marine pollution studies. MDS is a procedure for fitting a set of points in space such that the distance between points correspond to a given set of dissimilarities. This technique is more flexible than principal co-ordinate analyses when handling the large number of zero counts generally characteristic of species-samples matrices. Nonmetric MDS analyses were performed using Systat®. For a detailed account of MDS statistical procedures, see Clarke and Ainsworth (1993) and Warwick and Clarke (1993). Inferences from the resultant ordination are also presented. It is important to note that, as with cluster analyses, MDS results are not definitive and must be used in conjunction with additional ecological information. MDS results are based on total species number and numbers of individuals. Inferences from the resultant ordination are also presented.

After classification and ordination patterns were determined, the raw data were reevaluated to assess which species may have influenced the observed patterns. Indicator species were then selected on the basis of a literature review (*i.e.*, distribution, life history strategies and habitat preference), by recommendations from other experienced benthic taxonomists, and review of the raw data. Initially, community analyses were conducted as a per "site" comparison. Later, it was decided analyses also be expanded to a per "station" comparison to produce a more definitive data set for the reference pool. The extended analysis of station variability was performed using the benthic index.

Benthic assemblages have many attributes which make them reliable and sensitive indicators of the ecological condition in estuarine

environments. The following procedure summarizes the construction and application of the benthic index used to reliably discriminate between degraded and undegraded conditions at sites in the San Diego Bay Region. Although there are problems with trying to simplify complex biological communities, we attempted to develop a quantitative method which creates a partition between degraded and undegraded areas. Polluted sites can not be conclusively identified using results from benthic community analyses alone, but these analyses impartially describe "environmentally stressed" areas. This benthic index is based on species (indicators), and group (general taxa) information. The index also evaluates community parameters, such as species richness, and abundance or presence of pollution indicators, which identify the extremes of the community characteristics. Sites are ranked according to these extremes and are represented by a single value. In general, decreasing numbers of species, increasing numbers of individuals, and decreasing diversity values are common responses observed near polluted areas. These trends are incorporated into the index. One of the important restrictions with the existing method is it evaluates this limited San Diego Bay benthic data set when dividing groups for categorization. Construction and subsequent validation of this simplified benthic index are loosely based on criteria developed by several agencies, including USEPA-EMAP and SCCWRP. However, the benthic index developed by USEPA-EMAP (Weisberg *et al.*, 1993) included several environmental variables in its construction (*e.g.* dissolved O_2), while the index for San Diego Bay data used only biological parameters. Briefly, the following major steps were followed in constructing and validating this benthic index:

1. Degraded and undegraded (*i.e.*, reference condition) stations were identified on the basis of measured environmental and biological variables.

2. A list of "candidate" parameters was developed using species abundance data. The list included metrics having ecological relevance (*e.g.*, species diversity indices, etc.) which were used to discriminate between degraded and reference areas.

3. A value for each candidate parameter (*i.e.*, diversity, abundance, taxonomic composition) was calculated for each station (*e.g.*, total species per station, total individuals per station, total crustaceans species per station, total number of polychaete individuals, total amphipods per station, etc.).

4. Range of values per metric was determined (lowest to highest value).

5. Quartiles from that range were determined.

6. Ranking within quartiles were assigned: upper quartile=2, lower quartile=0, middle quartile=1. These calculations were applied to the metrics from step 3.

7. The index was defined by values of 0, 1, or 2. A value of 0 defines the degraded (detectable stress) stations(s), and 2 identifies environmentally undegraded stations(s). Stations with an index value of 1 are considered transitional communities, which are neither degraded nor reference stations. Transitional stations have species or other parameters which indicate both degraded and undegraded habitats. These stations are investigated further to determine the cause of ambiguity of the transitional status.

8. Relative abundance of indicator species (both degraded and undegraded habitat indicators) per station is assessed.

A primary concern regarding the benthic index is how well it fulfills the objective of discriminating among degraded and undegraded estuarine conditions. This simplified version forms the basis for ongoing iterative procedures involved in construction of an index. This index will include a variety of indicator values (Bascom *et al.*, 1978; Kerans *et al.*, 1994; EcoAnalysis *et al.*, 1995) for future applications of the assessment of benthic community structure. The following sections report results of benthic community analyses based solely on composition and abundance of macrobenthic species from sediment cores throughout San Diego Bay and its vicinity. Environmental parameters (*e.g.*, total organic carbon levels and sediment grain size range) and other factors capable of influencing benthic composition were examined, but not evaluated in conjunction with the data presented here. Those data are examined later in sections which address correlative analyses.

In this study, bioeffects are required to be demonstrated in relation to properly selected reference sites and to occur in association with significant pollutant levels. The following evidence for undegraded (possible reference) and degraded (possible contaminated) sites was based on benthic community "quality" at each site and station. Benthic community structure was evaluated as an indicator of environmentally degraded or undegraded areas and not as a pollution or contamination indicator. Benthic reference sites were determined predominantly by analyses of specific indicator species and groups (*e.g.*, amphipods). These species are generally not found in polluted or disturbed areas.

The intention of this section is to clearly describe the condition of macrobenthic communities from sampling areas. Definitions of degraded, transitional, and undegraded used in this section are adopted from several papers (Bascom *et al.*, 1978; Pearson and Rosenberg, 1978; Schindler, 1987; Swartz *et al.*, 1985; Underwood and Peterson, 1988). Although the boundaries set in Bascom *et al.* (1978) were based on food supply and not on toxicants, the same general principles apply to this study. In

benthic analyses, the term "degraded" does not refer to a community response to significant levels of toxic chemicals. Degraded areas are those which contain significant numbers of opportunistic species, in the absence of non-opportunistic species, and have relatively low species diversity. Correlations are later used to determine if community profiles are influenced by chemistry or by natural environmental disturbances. Sites and stations which are categorized as "undegraded" have high species diversity, high proportional abundance of amphipods and other crustaceans, while noting there are a few exceptions to this rule (*e.g., Grandidierella japonica*, etc.). Undegraded areas generally contain species which are known to be sensitive to pollutants. Transitional sites and stations are those which are not confidently partitioned into the other two categories. These areas may solicit further study. Overall, an integration of data from laboratory exposures, chemical analyses, and benthic community assessments provide strong complementary evidence of the degree of pollution-induced degradation in aquatic communities. The following data analyses were conducted on a per site basis using sample replicates (n=5) at each sampling location (Table 6). An analysis also was performed using per station data (n=1) and is presented later in this section. Tests included classification and ordination analyses, diversity measurements, construction of a benthic index, and assessment of indicator species. One cautionary note is each of the benthic community and population condition tests are subject to effects of not only the pollutants measured in this study, but many other confounding natural factors, such as depth, salinity, sediment texture, and/or predation.

Abundance and Diversity

There were 7,232 individuals, representing 198 macrobenthic species, collected from 375 benthic cores during sampling legs 20 through 23 of the San Diego Bay confirmation phase (Table 7). Mean number of species was calculated from 5 replicates per site (Table 8). Polychaetes comprised the majority of specimens in samples. Great numbers of mollusks in sites within West Basin, Downtown Piers, and Glorietta Bay were due to the bivalve *Musculista senhousei* which was collected as large aggregates. Echinoderms were found at only 6 of the 25 sites, and were significantly ($p>0.01$) greater at the Mission Bay A3 site (640.0±216.6) and the Mission Bay A8 site (213.3±53.3) compared to all other sites. Holothurians comprised the majority of echinoderms found at these sites, although ophiuroids were also present. Colonial species were not present. Diversity ranged from 9 to 46 benthic species per site in collected samples. Significant differences in species diversity were not as distinct as with other indices and no trends were obvious. Results shown in Table 9 indicate most communities in this study were relatively diverse and even. Simpson's diversity index (D') which emphasizes more common species, and Shannon-Weaver (H') which puts statistical weight on rare species, showed differences in the range of diversity values. Chula Vista Yacht Basin was the only site which showed a moderately high level of dominance as

Table 6. Benthic samples from the San Diego Bay region.

Site-Station Name	Replicate Number	Station No.	IDORG No.
10 Swartz (West Basin)	1	90050.0	837
10 Swartz (West Basin)	2	93199.0	838
10 Swartz (West Basin)	3	93200.0	839
10 Swartz (West Basin)	4	^	837.1
10 Swartz (West Basin)	5	^	837.2
11 Swartz (East Basin)	1	90001.0	840
11 Swartz (East Basin)	2	93201.0	841
11 Swartz (East Basin)	3	93202.0	842
11 Swartz (East Basin)	4	^	840.1
11 Swartz (East Basin)	5	^	840.2
12 Swartz (Downtown Anch)	1	90002.0	878
12 Swartz (Downtown Anch)	2	93221.0	879
12 Swartz (Downtown Anch)	3	93222.0	880
12 Swartz (Downtown Anch)	4	^	878.1
12 Swartz (Downtown Anch)	5	^	878.2
14 Swartz (Downtown Piers)	1	90003.0	846
14 Swartz (Downtown Piers)	2	93205.0	847
14 Swartz (Downtown Piers)	3	93206.0	848
14 Swartz (Downtown Piers)	4	^	846.1
14 Swartz (Downtown Piers)	5	^	846.2
15 Swartz (G St Pier Marina)	1	90004.0	849
15 Swartz (G St Pier Marina)	2	93207.0	850
15 Swartz (G St Pier Marina)	3	93208.0	851
15 Swartz (G St Pier Marina)	4	^	849.1
15 Swartz (G St Pier Marina)	5	^	849.2
16 Swartz (Intercont. Marina)	1	90051.0	818
16 Swartz (Intercont. Marina)	2	93192.0	819
16 Swartz (Intercont. Marina)	3	93193.0	820
16 Swartz (Intercont. Marina)	4	^	818.1
16 Swartz (Intercont. Marina)	5	^	818.2
23 Swartz (Naval Base 07)	1	90006.0	865
23 Swartz (Naval Base 07)	2	93212.0	866
23 Swartz (Naval Base 07)	3	93213.0	867
23 Swartz (Naval Base 07)	4	^	865.1
23 Swartz (Naval Base 07)	5	^	865.2
25 Swartz (Naval base/ SY 010)	1	90007.0	887
25 Swartz (Naval base/ SY 010)	2	93223.0	888
25 Swartz (Naval base/ SY 010)	3	93224.0	889
25 Swartz (Naval base/ SY 010)	4	^	887.1
25 Swartz (Naval base/ SY 010)	5	^	887.2
27 Swartz (Naval Base /SH 013)	1	90008.0	890
27 Swartz (Naval Base /SH 013)	2	93225.0	891
27 Swartz (Naval Base /SH 013)	3	93226.0	892
27 Swartz (Naval Base /SH 013)	4	^	890.1
27 Swartz (Naval Base /SH 013)	5	^	890.2
28 Swartz (7th St Channel Q1)	1	90009.0	893
28 Swartz (7th St Channel Q1)	2	93227.0	894
28 Swartz (7th St Channel Q1)	3	93228.0	895
28 Swartz (7th St Channel Q1)	4	^	893.1
28 Swartz (7th St Channel Q1)	5	^	893.2
31 Swartz (Marine Terminal R3)	1	90010.0	896
31 Swartz (Marine Terminal R3)	2	93229.0	897
31 Swartz (Marine Terminal R3)	3	93230.0	898
31 Swartz (Marine Terminal R3)	4	^	896.1
31 Swartz (Marine Terminal R3)	5	^	896.2
32 Swartz (Sweetwater Ch)	1	90052.0	875
32 Swartz (Sweetwater Ch)	2	93219.0	876
32 Swartz (Sweetwater Ch)	3	93220.0	877
32 Swartz (Sweetwater Ch)	4	^	875.1
32 Swartz (Sweetwater Ch)	5	^	875.2
34 Swartz (CV Yacht Basin)	1	90012.0	824
34 Swartz (CV Yacht Basin)	2	93196.0	825
34 Swartz (CV Yacht Basin)	3	93197.0	826
34 Swartz (CV Yacht Basin)	4	^	824.1
34 Swartz (CV Yacht Basin)	5	^	824.2
35 Swartz (Coronado Cays)	1	90053.0	843
35 Swartz (Coronado Cays)	2	93203.0	844
35 Swartz (Coronado Cays)	3	93204.0	845
35 Swartz (Coronado Cays)	4	^	843.1
35 Swartz (Coronado Cays)	5	^	843.2
37 Swartz (Marina)	1	90013.0	815
37 Swartz (Marina)	2	93190.0	816
37 Swartz (Marina)	3	93191.0	817
37 Swartz (Marina)	4	^	815.1
37 Swartz (Marina)	5	^	815.2
41 Swartz (Glorietta Bay)	1	90015.0	821
41 Swartz (Glorietta Bay)	2	93194.0	822
41 Swartz (Glorietta Bay)	3	93195.0	823
41 Swartz (Glorietta Bay)	4	^	821.1
41 Swartz (Glorietta Bay)	5	^	821.2
K Swartz (Naval Base 04)	1	90021.0	862
K Swartz (Naval Base 04)	2	93210.0	863
K Swartz (Naval Base 04)	3	93211.0	864
K Swartz (Naval Base 04)	4	^	862.1
K Swartz (Naval Base 04)	5	^	862.2
Mission Bay A4	1	93108.0	859
Mission Bay A4	2	93108.0	860
Mission Bay A4	3	93108.0	861
Mission Bay A4	4	93108.0	859.1
Mission Bay A4	5	93108.0	859.2
Mission Bay A8	1	93112.0	856
Mission Bay A8	2	93112.0	857
Mission Bay A8	3	93112.0	858
Mission Bay A8	4	93112.0	856.1
Mission Bay A8	5	93112.0	856.2
Mission Bay A3	1	93107.0	853
Mission Bay A3	2	93107.0	854
Mission Bay A3	3	93107.0	855
Mission Bay A3	4	93107.0	853.1
Mission Bay A3	5	93107.0	853.2
NSB-M1 (Sub Base C2)	1	90028.0	871
NSB-M1 (Sub Base C2)	2	93216.0	872
NSB-M1 (Sub Base C2)	3	93217.0	873
NSB-M1 (Sub Base C2)	4	^	871.1
NSB-M1 (Sub Base C2)	5	^	871.2
P Swartz (Naval Base 012)	1	90022.0	868
P Swartz (Naval Base 012)	2	93214.0	869
P Swartz (Naval Base 012)	3	93215.0	870
P Swartz (Naval Base 012)	4	^	868.1
P Swartz (Naval Base 012)	5	^	868.2
San Diego River B1	1	93116.0	881
San Diego River B1	2	93116.0	882
San Diego River B1	3	93116.0	883
San Diego River B1	4	93116.0	881.1
San Diego River B1	5	93116.0	881.2
SDNI- N5 (Carrier Base V2)	1	90025.0	899
SDNI- N5 (Carrier Base V2)	2	93231.0	1000
SDNI- N5 (Carrier Base V2)	3	93232.0	1001
SDNI- N5 (Carrier Base V2)	4	^	899.1
SDNI- N5 (Carrier Base V2)	5	^	899.2
Stormdrain EM (Grape St.)	1	90037.0	827
Stormdrain EM (Grape St.)	2	90037.0	828
Stormdrain EM (Grape St.)	3	90037.0	829
Stormdrain EM (Grape St.)	4	90037.0	827.1
Stormdrain EM (Grape St.)	5	90037.0	827.2
Long Beach Outer Harbor	1	40018.3	884
Long Beach Outer Harbor	2	40018.3	885
Long Beach Outer Harbor	3	40018.3	886
Long Beach Outer Harbor	4	40018.3	884.1
Long Beach Outer Harbor	5	40018.3	884.2
Lower Main Channel	1	40004.2	830
Lower Main Channel	2	40004.2	831
Lower Main Channel	3	40004.2	832
Lower Main Channel	4	40004.2	830.1
Lower Main Channel	5	40004.2	830.2
Off Cabrillo Beach	1	40010.0	1006
Off Cabrillo Beach	2	40010.0	1007
Off Cabrillo Beach	3	40010.0	1008
Off Cabrillo Beach	4	40010.0	1006.1
Off Cabrillo Beach	5	40010.0	1006.2
Palos Verdes (Swartz 6)	1	40031.2	1002
Palos Verdes (Swartz 6)	2	40031.2	1003
Palos Verdes (Swartz 6)	3	40031.2	1004
Palos Verdes (Swartz 6)	4	40031.2	1002.1
Palos Verdes (Swartz 6)	5	40031.2	1002.2
West Basin Entrance	1	40009.1	834
West Basin Entrance	2	40009.1	835
West Basin Entrance	3	40009.1	836
West Basin Entrance	4	40009.1	834.1
West Basin Entrance	5	40009.1	834.2

Table 7. Species list of macroinvertebrates from the San Diego Bay region benthic samples

Acmira catherinae	Gastropoda	*Fabricinuda limicola*	Polychaeta	*Orchomene pacifica*	Gammaridea
Acmira horikoshii	Gastropoda	*Glycera americana*	Polychaeta	*Orchomene sp.*	Gammaridea
Acuminodeutopus heteruropus	Amphipoda	*Glycera nana*	Polychaeta	*Paracerceis sculpta*	Isopoda
Aglaja sp.	Gastropoda	*Gnathia crenulatifrons*	Isopoda	*Paradexamine sp.*	Amphipod
Alpheus californiensis	Decapoda	*Goniada brunnea*	Polychaeta	*Paramage scutata*	Polychaeta
Amaeana occidentalis	Polychaeta	*Goniada sp(p).*	Polychaeta	*Paranthura elegans*	Isopoda
Ampelisca brevisimulata	Gammaridea	*Grandidierella japonica*	Gammaridea	*Paraprionospio pinnata*	Polychaeta
Ampelisca cristata	Gammaridea	*Harmothoe hirsuta*	Polychaeta	*Parasterope barnesi*	Ostracoda
Ampelisca hancocki	Gammaridea	*Harmothoe imbricata*	Polychaeta	*Parougia caeca*	Polychaeta
Ampharete labrops	Polychaeta	*Heptacarpus cf taylori*	Decapoda	*Parvilucina tenuisculpta*	Bivalvia
Amphicteis scaphobranchiata	Polychaeta	*Heptacarpus sp. A*	Decapoda	*Pectinaria californiensis*	Polychaeta
Amphideutopus oculatus	Amphipoda	*Hesperonoe sp(p).*	Polychaeta	*Pennatulacea*	Anthozoa
Amphilochidae	Gammaridea	*Heterophoxus oculatus*	Gammaridea	*Pherusa capulata*	Polychaeta
Ampithoe sp.	Gammaridea	unidentified holothuroid	Holothuroidea	*Pherusa sp(p).*	Polychaeta
unid. anemone	Anthozoa	*Hyale frequens*	Gammaridea	*Pholoe glabra*	Polychaeta
Aphelochaeta monilaris	Polychaeta	*Hydroides pacificus*	Polychaeta	unidentified phoronida	Phoronida
Aphelochaeta multifilis	Polychaeta	*insect larva*	Arthropoda	*Photis sp.*	Gammaridea
Aphelochaeta sp(p).	Polychaeta	*Laevicardium substriatum*	Bivalvia	*Pista alata*	Polychaeta
Apistobranchus sp(p).	Polychaeta	*Laonice cirrata*	Polychaeta	*Pista sp(p).*	Polychaeta
Apoprionospio pygmaea	Polychaeta	*Leitoscoloplos pugettensis*	Polychaeta	*Pleustidae*	Gammaridea
Armandia brevis	Polychaeta	*Lembos sp.*	Gammaridea	*Podarkeopsis glabra*	Polychaeta
Asteropella slatteryi	Ostracoda	*Leptochelia dubia*	Tanaidacea	*Podarkeopsis perkinsi*	Polychaeta
Autolytus sp(p).	Polychaeta	*Leptognathia sp.*	Tanaidacea	*Podocerus cristatus*	Gammaridea
unidentified bivalve	Bivavia	*Levinsenia gracilis*	Polychaeta	*Poecilochaetus johnsoni*	Polychaeta
Brania brevipharyngea	Polychaeta	*Listriella goleta*	Gammaridea	*Polydora cornuta*	Polychaeta
Bulla sp.	Gastropoda	*Lophopanopeus bellus diegensis*	Decapoda	*Polydora nuchalis*	Polychaeta
Campylaspis rubromaculata	Cumacea	Lumbrineridae, unident.	Polychaeta	*Polydora socialis*	Polychaeta
Capitella capitata complex	Polychaeta	*Lyonsia californica*	Bivalvia	*Polyophthalmus pictus*	Polychaeta
Caprella californica	Caprellida	*Lysippe labiata*	Polychaeta	*Pontogeneia rostrata*	Gammaridea
Caulleriella sp(p).	Polychaeta	*Macoma cf yoldiformis*	Bivalvia	*Praxillella pacifica*	Polychaeta
Chaetozone corona	Polychaeta	*Macoma nausta*	Bivalvia	*Prionospio heterobranchia*	Polychaeta
Chone mollis	Polychaeta	*Macoma sp.*	Bivalvia	*Prionospio lighti*	Polychaeta
Cirratulidae, unident.	Polychaeta	*Mactra californica*	Bivalvia	*Prionospio sp(p).*	Polychaeta
Cirratulus sp(p).	Polychaeta	*Malmgreniella macginitiei*	Polychaeta	*Prionospio steenstrupi*	Polychaeta
Cirriformia luxuriosa	Polychaeta	*Marphysa disjuncta*	Polychaeta	*Pseudopolydora paucibranchiata*	Polychaeta
Collisela depicta	Gastropoda	*Mayerella banksia*	Amphipoda	*Rhynchospio glutaea*	Polychaeta
Compsomyax subdiaphana	Bivalvia	*Mediomastus californiensis*	Polychaeta	*Rudilemboides stenopropodus*	Amphipoda
Cooperella subdiaphana	Bivalvia	*Megalomma pigmentum*	Polychaeta	*Scleroplax granulata*	Decapoda
Corophium acherusicum	Gammaridea	*Melinna oculata*	Polychaeta	*Scolelepis quinquedentata*	Polychaeta
Corophium heteroceratum	Gammaridea	*Metasychis disparidentata*	Polychaeta	*Scoletoma erecta*	Polychaeta
Cossura candida	Polychaeta	*Microjassa litotes*	Gammaridea	*Scoletoma tetraura*	Polychaeta
Crepidula fornicata	Gastropoda	*Monoculodes hartmanae*	Gammaridea	*Scoloplos acmeceps*	Polychaeta
Crucibulum spinosum	Gastropoda	*Monticellina dorsobranchialis*	Polychaeta	*Scyphoproctus sp(p).*	Polychaeta
Cryptomya californica	Bivalvia	*Monticellina sp. C*	Polychaeta	*Serolis carinata*	Isopoda
Cylichnella inculta	Gastropoda	*Monticellina tesselata*	Polychaeta	*Sigambra tentaculata*	Polychaeta
Cylichnella sp.	Gastropoda	*Munnogonium californiensis*	Isopoda	*Siliqua lucida*	Bivalvia
Diastylis sp.	Cumacea	*Musculista senhousei*	Bivalvia	unidentified spionid	Polychaeta
Diopatra sp(p).	Polychaeta	*Myriochele sp. M*	Polychaeta	*Spiophanes berkeleyorum*	Polychaeta
Diopatra tridentata	Polychaeta	*Mysella sp.*	Bivalvia	*Spiophanes missionensis*	Polychaeta
Diplocirrus sp(p).	Polychaeta	unidentified mysid	Mysidacea	*Sthenelais tertiaglabra*	Polychaeta
Dorvillea longicornis	Polychaeta	*Nassarius perpinguis*	Gastropoda	*Sthenelanella uniformis*	Polychaeta
Drilonereis falcata minor	Polychaeta	*Neanthes acuminata*	Polychaeta	*Streblosoma sp. B*	Polychaeta
Elasmopus rapax	Amphipoda	*Neastacilla californica*	Isopoda	*Streblospio benedicti*	Polychaeta
Eranno lagunae	Polychaeta	*nemertean*	Nemertea	*Sulcoretusa xystrum*	Gastropoda
Eteone californica	Polychaeta	*Neotrypaea californiensis*	Decapoda	*Synchelidium rectipalmum*	Gammaridea
Eteone sp(p).	Polychaeta	*Nephtys caecoides*	Polychaeta	*Synchelidium sp.*	Gammaridea
Euchone limnicola	Polychaeta	*Nephtys cornuta*	Polychaeta	*Tagelus subteres*	Bivalvia
Euclymeninae spp. indet.	Polychaeta	Nereididae, unident.	Polychaeta	*Tellina modesta*	Bivalvia
Eudorella pacifica	Cumacea	*Nereis procera*	Polychaeta	*Tenonia priops*	Polychaeta
Euphilomedes carcharodonta	Ostracoda	*Notomastus tenuis*	Polychaeta	Terebellidae, unident.	Polychaeta
Euphilomedes producta	Ostracoda	*Nuculana taphria*	Bivalvia	*Terebellides californica*	Polychaeta
Eupolymnia sp(p).	Polychaeta	*Odontosyllis phosphorea*	Polychaeta	*Theora fragilis*	Bivalvia
Exogone lourei	Polychaeta	*Odostomia sp.*	Gastropoda	*Trachycardium quadragenarium*	Bivalvia
Exogone molesta	Polychaeta	*oligochaeta*	Oligochaeta	*Turbonilla sp.*	Gastropoda
Exogone sp(p).	Polychaeta	*Olivella baetica*	Gastropoda	*Urocaris infraspinis*	Decapoda
Exogone uniformis	Polychaeta	unidentified ophiuroid	Ophioroidea	*Zeuxo normani*	Tanaidacea