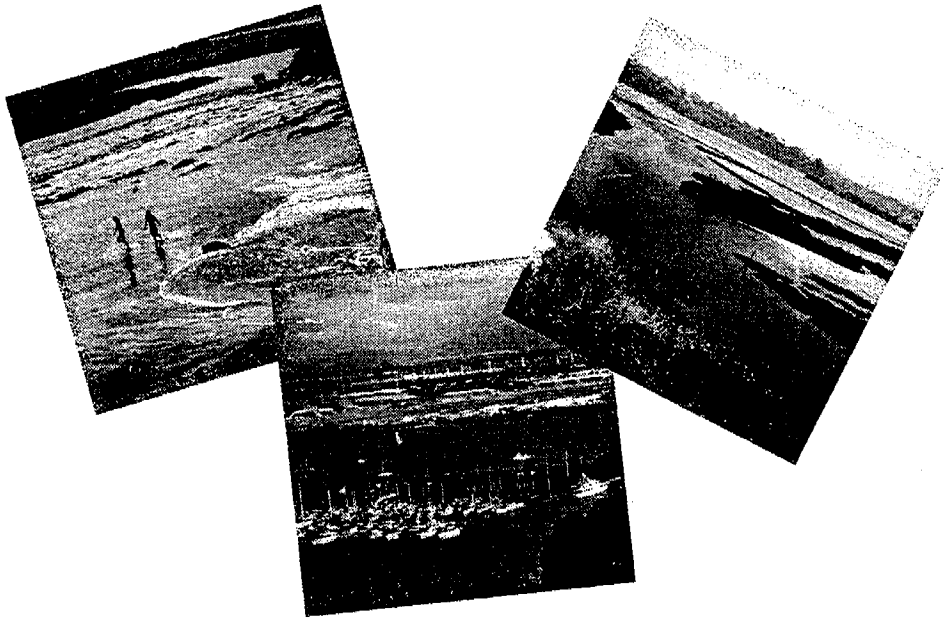

Chemistry, Toxicity and Benthic Community Conditions in Sediments of Selected Southern California Bays and Estuaries

May, 1997

California State Water Resources Control Board

U.S. Environmental Protection Agency

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EXECUTIVE SUMMARY

1. Using a weight-of-evidence approach based on the Sediment Quality Triad, measures of chemical contamination, toxicity, and benthic community structure were completed at 43 stations to determine the relative degradation in selected Southern California bays, estuaries and lagoons. Degree of chemical contamination was assessed using two sets of sediment quality guidelines: the ERL/ERM guidelines developed by NOAA (Long et al., 1995), and the TEL/PEL guidelines developed for the State of Florida (MacDonald, 1996). Relative to these guidelines, Total DDT, Total Chlordane, Copper, Mercury, and Zinc were found to be the chemicals or chemical groups of greatest concern. Chemical contamination was considered to be moderate relative to more highly industrialized areas.

2. In this study, 30 of the 43 stations sampled were selected using a stratified random (EMAP) sampling design intended to assess the spatial extent of toxicity. The remaining 13 samples were selected using a directed point sampling design intended to investigate potential toxic hotspots. Percent area contaminated and percent area toxic was calculated from the 30 randomly selected samples. When DDT was excluded from consideration, 52% of the randomly-sampled study area was considered to be contaminated as represented by samples having at least 1 PEL exceedance; 89% of the randomly-sampled study area had at least 1 TEL exceedance (after MacDonald, 1996). When samples having DDT exceedances were included in the calculations, 67% of the randomly-sampled study area had at least 1 PEL exceedance; 94% of the randomly-sampled study area had at least 1 TEL exceedance (after MacDonald, 1996).

Using toxicity information from the randomly selected stations, 58% of the total randomly-sampled study area was significantly toxic to amphipods *Rhepoxynius abronius*. With the sea urchin development test, 91, 83, and 51% of the randomly-sampled study area was significantly toxic using 100, 50, and 25% pore water concentrations, respectively. Forty-three percent of the randomly-sampled study area was toxic to sea urchin fertilization using 100% pore water.

3. Determinations of the statistical significance of toxicity test results was assessed using two approaches: the t-test-control approach compared sample toxicity to a laboratory negative control; the Reference Envelope Approach compared sample toxicity to a reference population. Using the t-test-control approach, 53% of the 43 solid-phase samples tested with the amphipod *Rhepoxynius abronius* were significantly different from controls. Using the t-test-control approach, 81% and 53% of the 43 interstitial water samples tested were significantly different from controls using sea urchin (*Strongylocentrotus purpuratus*) development and fertilization, respectively. The reference

envelope approach was a more conservative indicator of toxicity. Using this approach 12% of the 43 solid-phase samples tested with the amphipod *Rhepoxynius abronius* were significant, and 47% of the 43 interstitial water samples tested were significant in tests using sea urchin fertilization.

4. The Biomarker P450 RGS, which responds to coplanar compounds in extracts of sediments, was highly correlated ($p = 0.001$) with the presence of total PAHs, and Aroclors 1254 and 1260 in the samples. There were weak negative associations between toxicity test results and some chemical compounds measured in bulk-phase samples. Survival of the amphipod (*Rhepoxynius abronius*) was negatively associated with DDE, PCB52, un-ionized ammonia, two metals, fine-grained sediments, and P450 RGS. *Ampelisca* survival was negatively associated with PCBs and several metals. Sea urchin embryo development in 100% pore water was highly correlated ($p = 0.001$) with P450 RGS responses to sediment extracts, and development in 50 % pore water was also significantly correlated ($p = 0.01$) with this biomarker. Sea urchin embryo development was negatively associated with two metals, chlordanes, and DDT compounds. There was a strong negative correlation between sea urchin embryo development and pore water un-ionized ammonia concentrations. Other than the correlations of *Rhepoxynius* survival and sea urchin development with P450 RGS, there were no other significant correlations between any of the toxicity test results.

5. Benthic community structure was assessed using a Benthic Index, calculated based on measures of the Total Number of Fauna, Number of Crustacean Species, and Numbers of Positive and Negative Indicator Species. Based upon this index, 15 of the 43 stations sampled (35%) were considered to be significantly degraded; 10 of the 15 degraded stations were located in 4 of the coastal lagoons sampled. Benthic community degradation was not significantly correlated with individual or mixtures of measured bulk-phase chemicals. The Benthic Index was negatively correlated with pore water hydrogen sulfide concentrations, possibly indicating that anoxia influenced benthic community structure, particularly in the coastal lagoons. The Benthic Index was significantly correlated with results of the sea urchin fertilization test, but not with results of any of the other toxicity tests.

6. Interlaboratory comparisons of solid-phase samples between the Marine Pollution Studies Laboratory (MPSL) and the Southern California Coastal Water Research Project (SCCWRP) using the amphipod *Ampelisca abdita* demonstrated comparable results for all but one sample. Interlaboratory comparisons of pore water toxicity using the sea urchin development test with *Strongylocentrotus purpuratus* were less consistent. Higher toxicity in the samples tested at SCCWRP was apparently associated with greater un-ionized ammonia concentrations.

7. Comparisons of the two amphipod tests performed with *Rhepoxynius abronius* and *Ampelisca abdita* using the 30 randomly selected samples showed lower overall survival with *Rhepoxynius*. While 12% of the samples tested were significantly toxic to *Ampelisca*, 40% of the samples were significantly toxic to *Rhepoxynius*.

8. Results using the 30 stratified random samples generally demonstrated greater toxicity but comparable benthic community degradation when compared to the 13 samples selected using the directed point sampling design. Samples having the greatest chemical contamination were selected using the directed point sampling design.

9. All measures of sediment contamination and degradation proved useful in this study. Stations recommended for further investigation were prioritized to help direct future investigations by State and Regional Water Board staff. Each station receiving a high, moderate or low priority ranking met one or more of the criteria under evaluation for determining hotspot status in the Bay Protection Toxic Cleanup Program. Those meeting all of the criteria were designated with the highest priority for future investigation.

Four stations were given the highest priority ranking: two were in Newport Bay and one each was designated with the highest ranking in Dana Point Harbor and San Dieguito Lagoon. Twenty-one stations were designated with moderate rankings, and 17 stations were designated with the lowest ranking. One station was not ranked because it was considered to require more information.

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LIST OF ABBREVIATIONS

AA	Atomic Absorption
ASTM	American Society for Testing Materials
SEM-AVS	Simultaneously Extracted Metals-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
EPA	Environmental Protection Agency
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
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
RWQCB	Regional Water Quality Control Board
SJSUF	San Jose State University Foundation
SCCWRP	Southern Calif. Coastal Waters Research Project

LIST OF ABBREVIATIONS (continued)

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

Units
1 part per thousand (ppt) = 1 mg/g
1 part per million (ppm) = 1 mg/kg, 1 µg/g sed
1 part per billion (ppb) = 1 µg/kg, 1 ng/g sed

INTRODUCTION

Purpose

In 1992, the State Water Resources Control Board (SWRCB) and the National Oceanic and Atmospheric Administration (NOAA) entered into a multi-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 phased multi-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 (Sapudar et al., 1994). In the second phase, data were collected in the San Diego Bay area (Fairey et al., 1996).

In this, the third phase, the SWRCB and NOAA combined resources with the U. S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) to continue sediment assessments in selected bays and estuaries between San Diego Bay and Newport Bay. For the present study (Figure 1), data were collected in five lagoons and estuaries in San Diego County (Los Peñasquitos Lagoon, San Dieguito Lagoon, San Elijo Lagoon, Agua Hedionda, Santa Margarita River Estuary) as well as three larger marinas in San Diego and Orange Counties (Oceanside Harbor, Dana Point Harbor, and Newport Bay).

The objectives of the present study were:

1. Estimate with known confidence the percent of the study area that was degraded based upon several critical threshold values of chemistry, toxicity, and benthic community structure.

2. Identify spatial patterns in sediment quality.

3. Identify potential toxic hotspots and reference sites which may be revisited during confirmation studies.

4. Assess the effectiveness of stratified random and directed point sampling designs for locating potential toxic hotspots.

5. Assess concordance of two solid phase toxicity tests (*Ampelisca* and *Rhepoxynius*) using samples with varying contaminants and physical characteristics.

6. Develop a benthic index for interpretation of benthic community data and identify samples with degraded benthos based upon this index.

7. Identify which of the measured toxicants are most associated with toxic responses.

8. Evaluate the reproducibility and comparability of toxicity tests using interlaboratory comparisons of solid-phase and interstitial water samples.

Programmatic Background and Needs

This study was part of a cooperative agreement between NOAA and SWRCB and 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 correlate the presence of pollutants with toxicity and/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.

For the present study, the cooperative agreement between NOAA and the SWRCB was expanded to include EPA's EMAP. The cooperative study was designed to investigate the environmental effects of human activities on benthic ecosystems by evaluating the biological and chemical state of Southern California bay and estuary sediments. The methods used to assess environmental impacts include sediment and interstitial water bioassays, sediment chemistry analysis, and benthic community analysis. Together, these measures comprise a weight-of-evidence approach to environmental assessment, often referred to as the Sediment Quality Triad (Chapman et al. 1987).

The EMAP was designed to respond to increasing requirements for information characterizing the condition of the Nation's environment. The EMAP was created in response to an EPA Science Advisory Board recommendation and stresses long-term assessment to detect regional environmental degradation using probability sampling and multiple indicators. The estuaries component of EMAP (EMAP-E) is a joint EPA/NOAA program that is designed to complement

NOAA's National Status and Trends (NS&T) Program. The goals of EMAP are as follows:

1. Provide a quantitative assessment of the regional extent of estuarine environmental problems by measuring pollution exposure and ecological condition.

2. Measure changes in the regional extent of environmental problems for the nation's estuaries.

3. Identify and evaluate associations between ecological condition of the nation's estuarine ecosystems and pollutant exposure, as well as other factors known to effect ecological condition.

4. Assess the effectiveness of pollutant control actions and environmental policies on a national and regional scale.

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.

Southern California Bays and Estuaries Pilot Project

Field and laboratory work was accomplished under interagency agreement with, and under the direction of, the CDFG. Sample collection, sample processing, and data management were performed by staff of the San Jose State University Foundation at Moss Landing Marine Laboratories (MLML). 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. Under funding from the Bioeffects Assessment Branch of NOAA,

Columbia Analytical Services in Carlsbad, California utilized a screening biomarker assay (P450 RGS) to test the responses of human cells to organic extracts of sediments from 30 (R) of the 43 stations. Trace metals analyses were performed by CDFG personnel at the trace metal facility at MLML. 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.

Study Area

Coastal bays and estuaries are among the most productive ecosystems on earth (Kennish 1991). In California, most of these areas have undergone dramatic reductions over the past century (California Coastal Conservancy, 1989). The eight bays, estuaries, and lagoons included in this study represent diverse systems from highly developed urban marinas to relatively un-developed river estuaries. The study sites were selected because levels and effects of sediment contaminants in these areas were considered to be poorly characterized. A map of the entire study area is provided in **Figure 1**. These water bodies are separated physically, and are quite different in character. Descriptions of the specific water bodies are provided below. Much of the information on the southern lagoons came from a California Coastal Conservancy information booklet (California Coastal Conservancy, 1989). Information on Newport Bay, and Dana Point and Oceanside Harbors came from Regional Water Quality Control Board 8 & 9 watershed management plans and through discussions with Regional Board staff.

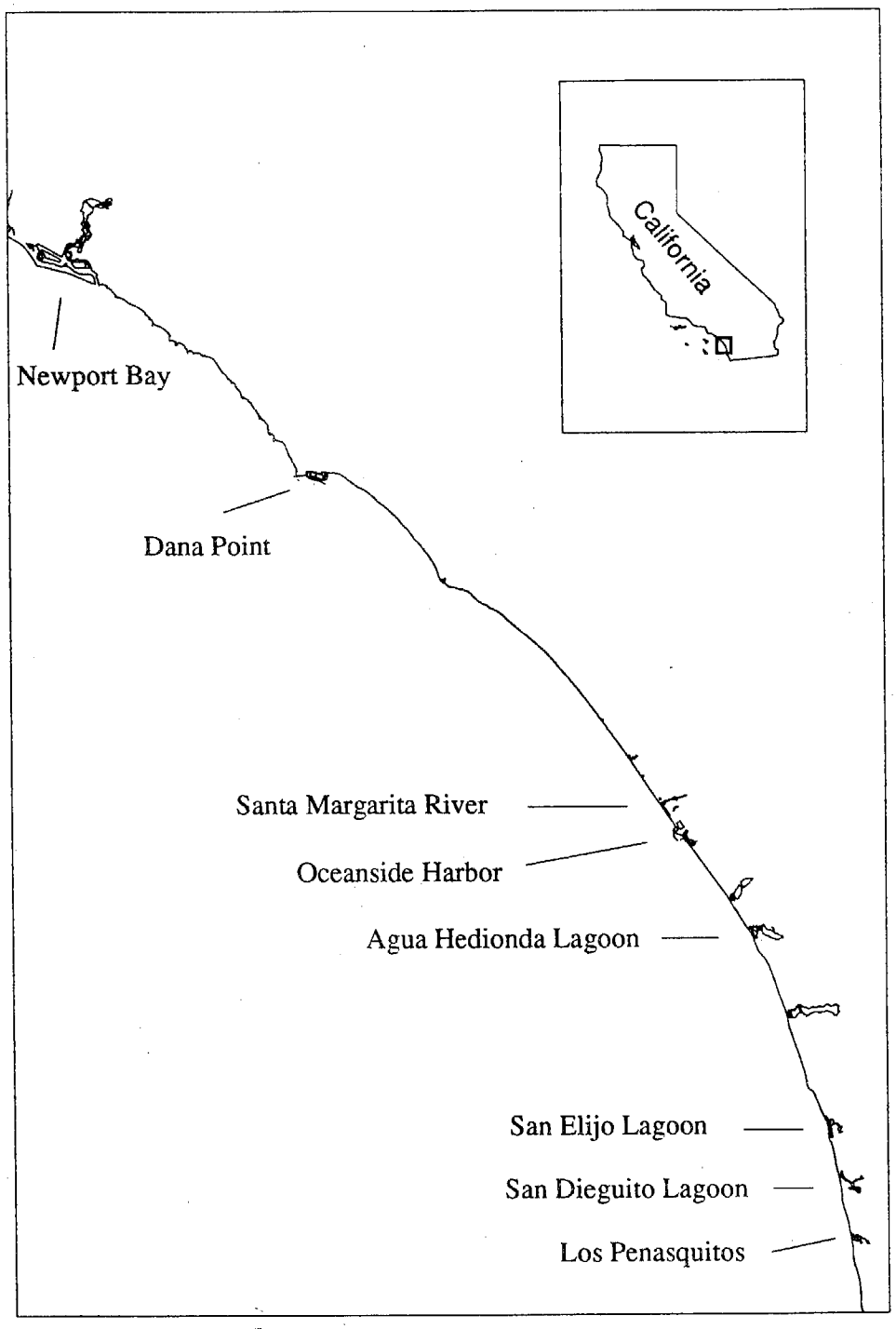


Figure 1. Southern California Bays and Estuaries EMAP study area.

Los Peñasquitos Lagoon

Los Peñasquitos Lagoon, the first significant estuary north of San Diego Bay, is managed by the California Department of Parks and Recreation as part of the Torrey Pines State Reserve. The lagoon comprises 630 acres and is the downstream estuary for a 98 square mile drainage which receives inputs from the cities of San Diego and Poway. The main tributaries are Los Peñasquitos and Carmel Valley creeks. The center of the lagoon is intersected by a railroad trestle and the Highway 1 Bridge, both of which have dramatically increased the sedimentation rate in the estuary. Sewage effluent was discharged into the lagoon from 1962 to 1972 in quantities ranging from 500,000 to 1 million gallons per day. Accidental spills of millions of gallons of raw sewage were a common occurrence in the lagoon until the mid-1980's. Two sewage pump stations close to the lagoon (No. 64 and 65) pump sewage from outlying areas to the POTW operated by the City of San Diego. Sewage enters the lagoon when these pumps fail (personal communication, P. Michael, SDRWQCB). The City of San Diego has attempted to address this problem by recently completing repairs to their sewage system. An industrial park borders the eastern boundary of the lagoon at the intersection of Interstate 5 and Highway 805 (personal communication, P. Michael, SDRWQCB).

San Dieguito Lagoon

San Dieguito Lagoon is one of six coastal lagoons in San Diego County. The lagoon is comprised of 300 acres adjacent to the City of Del Mar. It has the largest drainage of all the lagoons in this study (350 square miles); the San Dieguito River is the main tributary. The lagoon is bounded by several developments including the Del Mar Fairgrounds, the old Del Mar airport, a large shopping center, and moderate agriculture activity. Tidal flow in the lagoon is restricted because the lagoon is intersected by Highway 1 and Interstate 5. As a result, sedimentation in the lagoon is a problem. Approximately 200,000 to 300,000 gallons per day of sewage effluent was discharged into treatment ponds in the western area of the lagoon from 1940 to 1974.

San Elijo Lagoon

San Elijo Lagoon comprises 530 acres of shallow-water brackish wetland which receives inputs from a 77 square mile watershed including runoff from the cities of Escondido, Encinitas, and Solana Beach. The western boundary of the lagoon is intersected by Highway 1 and a railroad bridge. The lagoon received wastewater from the city of Escondido until as late as 1973. As with the other lagoons studied in this project, sedimentation is a major problem in San Elijo Lagoon due to lack of tidal influence, sediment inputs from Escondido and La Orilla creeks, and upland erosion

from urban stormwater. As is the case in Los Peñasquitos and San Dieguito Lagoons, lack of tidal flow combined with heavy sedimentation leads to anoxic conditions in certain parts of San Elijo Lagoon.

Agua Hedionda

Located near the City of Carlsbad, Agua Hedionda is composed of 400 acres which receive inputs from 29 square miles of watershed including the cities of Carlsbad, Vista, and Oceanside. Agua Hedionda is the main tributary stream. The watershed of Agua Hedionda is largely in agricultural use or undergoing development. The lagoon was completely dredged in 1954 to provide a deep basin and source of cooling water for the Encinitas Power Plant operated by San Diego Gas and Electric. Although the lagoon is subject to sedimentation, construction of jetties at the mouth of Agua Hedionda ensures year-round tidal flow and consequently, anoxic conditions are less of a problem in this lagoon.

Santa Margarita River and Estuary

The Santa Margarita River and Estuary is located on Camp Pendleton Marine Base and is comprised of 268 acres which receive inputs from a 740 square mile watershed draining Camp Pendleton Marine Base, and San Diego and Riverside County lands. The Santa Margarita River is considered to be the least disturbed river on the Southern California coast. Until 1970, the Marines used the salt flats of the estuary for tank exercises. At the same time, wastewater was discharged directly into the estuary, although discharge was stopped in the early 1970's. The estuary is now managed as a natural preserve by the Marines. Some agriculture occurs adjacent to the estuary.

Oceanside Harbor

Oceanside Harbor was constructed in the 1940's and was operated by the Marines until transferring the harbor to the City of Oceanside. The harbor consists of 210 acres adjacent to Camp Pendleton Marine Base and the City of Oceanside. The closest major tributary which potentially influences water quality in the harbor is the San Luis Rey River, which is approximately 1.5 miles south of the harbor mouth. This river drains a watershed of approximately 565 square miles. There is only minor agriculture activity around Oceanside Harbor. The south harbor is used primarily for small craft activities and contains one boatyard and some fueling stations. A number of storm drains discharge into the south harbor. Copper sulfate was applied in significant quantities directly to the harbor waters until the mid 1980's for algae control (personal

communication, P. Michael, SDRWQCB). Other possible sources of contaminants include light industrial activities, and urban residential runoff.

Dana Point Harbor

Dana Point Harbor was constructed in the early 1970's with the construction of jetties and subsequent dredging just north of Doheny State Beach. The harbor consists of 215 acres. San Juan Creek is the major tributary in the area; this creek runs into the ocean at Doheny State Beach. Sewage effluent was discharged near the harbor mouth until the late 1970's when the existing discharge pipe was extended off-shore. The harbor is used primarily for small craft activities and contains one boatyard and some fueling stations. There is only minor agricultural activity in this area. Other possible sources of contaminants include light industrial activities, and urban residential runoff.

Newport Bay

Adjacent to the cities of Newport Beach, and Corona Del Mar, Newport Bay is one of the largest small craft harbors in Southern California. Containing approximately 10,000 small craft, the Bay is split into upper and lower bays. Upper Newport Bay is owned and managed by the State Department of Fish and Game as a State Ecological Reserve. Lower Newport Bay is heavily developed with housing, hotels and restaurants, marinas, and light marine industry such as boatyards and fuel docks. The Newport Bay watershed encompasses 154 square miles. San Diego Creek is the largest tributary. Included among several smaller tributaries draining into the system are the Santa Ana-Delhi Channel and Big Canyon Wash.

Pollution problems in Newport Bay include pesticides/herbicides entering the system from urban runoff and agriculture runoff into the tributary creeks. High levels of certain trace metals have been detected in San Diego Creek and at certain locations in the bay. Toxicants associated with sedimentation from urban erosion and tributary creeks have also been identified (Santa Ana Regional Water Quality Control Board). Other toxicant sources include boatyard and fueling operations, small craft discharges and stormwater runoff.

METHODS

Sampling Design

Two sampling designs were used to meet the combined goals of the SWRCB, EMAP, and NOAA. A directed point sampling design was required to address SWRCB's objective of identifying specific toxic hot spots. A stratified random sampling design was required to address EMAP's and NOAA's goal of evaluating the spatial extent of pollution. This study consists of a data set of 43 samples collected during two sampling legs in September, 1994. Of the 43 total samples, 13 were collected from directed point sampled stations and 30 were collected from randomly sampled stations.

Prior to sample collection, a reconnaissance survey of all of the proposed water bodies was completed to identify and map appropriate sampling areas. During this survey rough maps were constructed indicating areas with the appropriate sediment characteristics (depositional sediment with greater than 30% fines, subtidal habitats with primarily marine or estuarine salinities). Information from these maps was transferred to topographic maps of the areas to be sampled.

For random sample location, the bays and estuaries were divided into three strata based on order of magnitude of size of area represented. Within these three areas, a total of 30 random samples were collected. Newport Bay was Stratum 1, Agua Hedionda, Dana Point Harbor, and Oceanside Harbor were in Stratum 2, and Los Peñasquitos Lagoon, San Elijo Lagoon, San Dieguito Lagoon, and Santa Margarita River were in Stratum 3. Stratum 1 had 12 sampling stations, and Stratums 2 and 3 had 9 sampling stations. Subdivision into these three strata ensured equitable areal representation of the varying size water bodies.

The following method was used to locate the sampling stations. A grid of hexagons was laid down over topographic maps of the areas demarcating the suitable sampling areas. Each hexagon was used to locate a single random point. The points within each stratum were counted, and a selection probability for each stratum was computed by dividing the desired number of points in the stratum by the total number of points in the stratum. A subsample of points from the set of random hexagon points determined the sample stations. Before taking the subsample, the points were randomized in a manner to ensure that the resulting stations were spread spatially over each bay. Total area sampled, calculated as the sum of all three sampling strata, was 5.01 km^2.

When directed point sampling design was required, the following process was used. Areas of interest were identified through the reconnaissance information, and by regional and state water board staff. These included areas presumed to be contaminated either from historical information or because of proximity to point source or non-point source discharges. Station locations (latitude & longitude) were predetermined by agreement with the SWRCB, EMAP, 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

Maps of the study area showing random and directed sampling stations are provided in **Figure 2 a-c.**

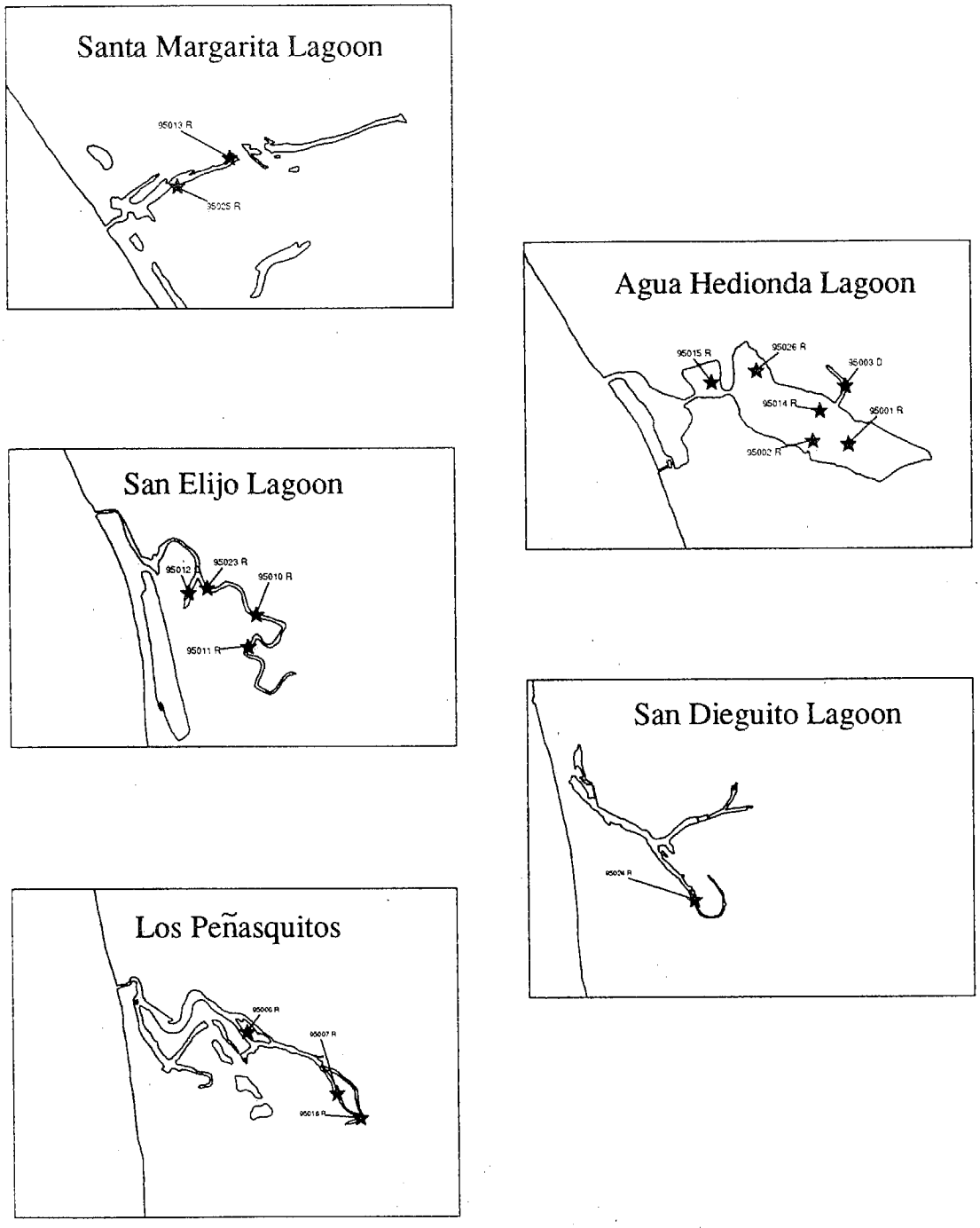


Figure 2a. Sampling locations in small estuaries and lagoons for southern California EMAP study. D = samples chosen using Directed point sampling design; R = samples chosen using stratified Random sampling design.

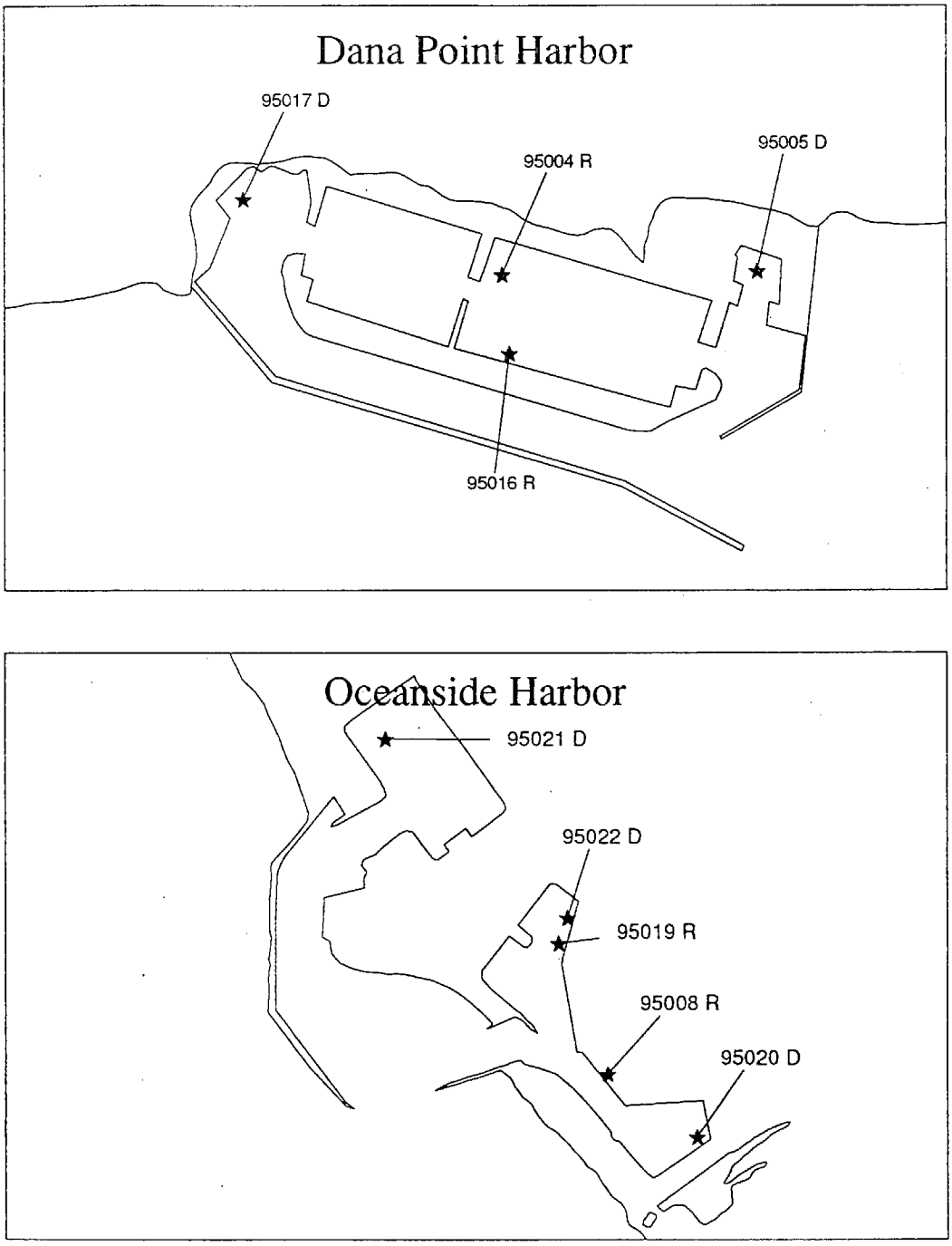


Figure 2b. Sampling locations in Oceanside and Dana Point Harbors for southern California EMAP study. D = samples chosen using Directed point sampling design; R = samples chosen using stratified Random sampling design.

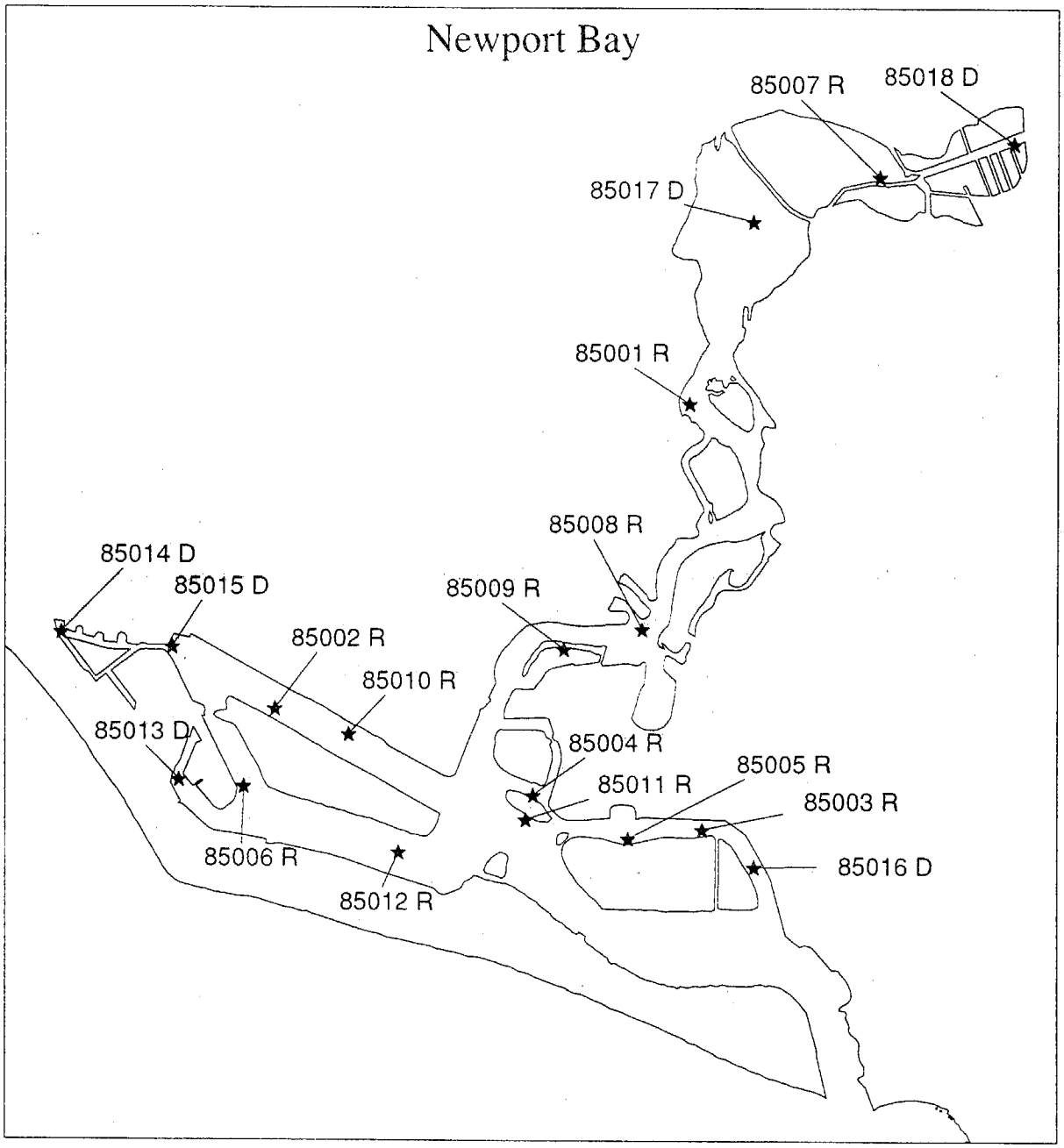


Figure 2c. Sampling locations in Newport Bay for southern California EMAP study. D = samples chosen using Directed point sampling design; R = samples chosen using stratified Random sampling design.

Sample Collection and Processing

Summary of Methods

This section describes specific techniques used for collecting and processing samples. Because collection of sediments influences the results of all subsequent laboratory and data analyses, it is 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 the accepted procedures of EMAP (Weisberg 1990), NS&T (NOAA 1991), and ASTM (1992) to ensure comparability in sample collection among crews and across geographic areas.

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 polyethylene gloves. All sample collection equipment (excluding the sediment sampler) 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 sampler was cleaned prior to entering the field, and between sampling stations, using the following steps: a vigorous Micro detergent wash and scrub, a sea-water rinse, a 10% HCl rinse, and a methanol rinse. The sediment sampler 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) 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, three Type II Milli-Q water rinses, and air dried.

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, 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 receiver, 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:

1. Sampler was not over-filled (*i.e.*, the sediment surface was not pressed against the top of the sampler).
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 (approx. >30% fines), not sandy or gravelly.
8. Sample did not include excessive shell, organic or man-made debris.

If a sample did not meet all the above criteria, it was rejected, dumped into the bay, and the sampler was re-deployed until a sufficient amount of material was obtained.

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 centimeters. 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=3) were obtained at predetermined sites from separate deployments of the Van Veen sampler. The three replicates were positioned according to the BPTCP sampling protocol (*e.g.*, located by previously assigned lat/long coordinates). 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 carefully 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 polycarbonate sample containers for chemistry and toxicity and benthic cores were packed in ice chests 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^{o}C) while sample containers for sediment chemistry (metals, organics, TOC and grain size) were stored in a freezer (-20^{o}C).

Procedures for the Extraction of Pore Water

All procedures for the extraction of pore water were performed using trace metal and trace organic clean techniques. Operations were performed in a positive pressure clean room with filtered air to prevent airborne contamination.

All sample containers or sampling equipment in contact with sediment or porewater received a scrub and 2 day soak in MICRO® detergent, followed by triple fresh and deionized water rinses. Equipment was then immersed in 10% HCl for 3 days, triple rinsed in MILLI-Q® Type II water, air dried, and triple rinsed with petroleum ether.

Samples were stored on ice at 4°C prior to centrifugation. Pre-cleaned Teflon scoops were used to transfer sediment from sample containers to centrifuge jars. High speed one-liter polycarbonate centrifuge jars were used for extraction of pore water. Samples were spun at 2500 G for 30 minutes at 4°C in a Beckman J-6B refrigerated centrifuge.

Porewater was transferred from each centrifuge jar into final sample containers (250 pre-cleaned borosilicate glass jars) using pre-cleaned polyethylene siphons. While decanting, care was used to avoid floating debris, fauna, shell fragments or other solid material. After transfer into final sample containers, porewater was immediately refrigerated at 4°C. Because of the number of samples processed, pore water extraction took 24 to 48 hours to complete. Testing was initiated within 24 hours of extraction of the final samples.

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), DFG 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 accompanies every sample so that each person releasing or receiving a subsample signs and dates 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 (after Standard Methods, 1992). 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).

Analytes and Detection Limits

Table 1 - Trace Metal Detection Limits in Sediments (ug/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

A 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. Vessels were 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 Teflon vessel and solution were recorded, and solution was poured into 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 occurred 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 analyzed and list method detection limits for sediments on a dry weight basis (after Standard Methods, 1992).

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
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 samples 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 L 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 l 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 undergoes additional cleanup using size-exclusion high performance liquid chromatography (HPLC/SEC). The collected PAH fraction was exchanged into hexane and concentrated to 250 L 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 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, regent 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 completely dry (approx. 48 hrs). Visual inspection of the dried sample before homogenization ensured 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 commercial ball jar 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 (Control Equipment Co., No. 440 Elemental Analyzer) to determine carbon and nitrogen concentrations. The manufacturer's suggested procedures were followed. The methods are comparable to the validation study of USEPA method MARPCPN I. Two to three aliquots 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 were 0.2 ug/mg, carbon and 0.01 ug/mg nitrogen dry weight.

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

Quality control was assessed 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 is 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 an unknown and comparing known theoretical percentages with resultant analyzed percentages. Acceptable limits of standard unknowns is less than $\pm$ 2%. Sample variance was assessed by duplicate or triplicate sample analysis, variance (standard deviation/mean) was always less than 7%.

Grain Size Analysis of Sediments

Sample Splitting and Preparation

This procedure uses wet and dry sieve techniques to determine particle size of sediment samples. Methods follow those of Folk (1974). 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 0.01 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 disappeared. 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 pre-weighed 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 pretared 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.

P450 Reporter Gene System Assay (RGS)

Subsamples (20 g) of the 30 randomly sampled sediment samples, which had been frozen, were shipped to Columbia Analytical Services (CAS), in Kelso Washington for extraction by EPA method 3540 to produce 2 mL samples in dichloromethane. These were then shipped to the Columbia Analytical Services laboratory in Carlsbad, California for application to a unique cell line which produces the luminescent enzyme, luciferase, as a function of the concentrations and potency of planar organic compounds present in the extract. The RGS assay responds to the presence of high molecular weight PAHs, coplanar PCBs, dioxins and furans, which attach to the Ah-receptor and induce the CYP1A1 site on the chromosome. Detailed descriptions of the procedure may be found in Standard Methods 8070 (APHA 1996) and ASTM E 1853-97 (ASTM 1997). Three replicate wells, each containing 2 mL of medium and about 1 million cells, were inoculated with 10 µL of each sediment extract. After 16 hours of exposure, cells are rinsed, then lysed, and the cells with medium were transferred to a microcentrifuge tube and spun for 10 seconds at 6,000 rpm. Fifty µL samples of the supernatant were transferred to a 96-well luminometer plate, and after addition of the luciferin substrate the relative light units (RLU) for each sample, a solvent blank and the standard reference inducer were recorded. The mean RLUs of the solvent were set equal to unity, and all other values were divided by this mean to produce fold induction values. Since 1 µg of benzo(a)pyrene/ mL has been shown to be equivalent to a 60 fold induction, the mean fold induction values of samples were converted to B(a)P equivalents by first multiplying by a factor (200) to determine the total inducing compounds in the 2 mL extracts, and then dividing by the dry weight of the sample and the factor 60. Over 300 samples of

sediment from California, Texas, Florida, and South Carolina have been tested for NOAA by the RGS procedure and expressed in B(a)P equivalents per g of sediment, allowing direct comparisons between stations and between regions of the country.

Toxicity Testing

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, pore water samples were stored in the dark, at 4°C, until required for testing. Samples were equilibrated to test temperature (15°C) 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 the 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‰). 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. Total ammonia concentrations were measured using an ammonium ion specific electrode (Orion model 95-12) following methods described in Phillips *et al.* (in press), and sulfide concentrations were measured on a spectrophotometer using the colorimetric methylene blue method (adapted from Fonselius, 1985).

Sediment Samples

Bedded sediment samples were held at 4°C until required for testing. All *Rhepoxynius abronius* and *Ampelisca abdita* solid phase sediment tests were initiated within 14 days of the sample collection date except where noted. 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 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 are given in ASTM 1995. 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 (33±2‰) 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. Tests were conducted at ambient seawater salinity (33±2‰). A 96-h positive control reference test was conducted concurrently with each pore water test using a dilution series of copper chloride as a reference toxicant.

After a 96-h exposure, 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 in ASTM 1995. 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. Embryos demonstrating retarded development 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 are 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. Test containers were polyethylene-capped, sea-water leached, 20ml glass scintillation vials containing 5 mls of pore water. All pore water samples from the first sampling leg 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. All pore water samples from the second sampling leg were tested with 100% pore water only due to logistical constraints. 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. Tests were conducted at ambient seawater salinity (33±2 ppt). A positive control reference test (1 hour sperm exposure) 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:

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

Amphipod Tests

Solid-phase sediment sample toxicity was assessed using the 10-day amphipod survival toxicity test protocol for *Rhepoxynius abronius* (ASTM 1993). A subset of samples was tested with the 10 day survival protocol using the amphipod *Ampelsica abdita* (ASTM 1993). All *Rhepoxynius* 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‰ per day) to 28‰ sea water (T =15°C). Once acclimated to 28‰, 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‰ 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‰ 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 Nitex 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‰ was compared to all cadmium concentrations.

Amphipod survival for each replicate was calculated as:

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

Methods for testing the amphipod *Ampelisca abdita* were identical to those described for *Rhepoxynius* except that different suppliers and therefore, different home sediment controls were used. *Rhepoxynius* were obtained from Northwest Aquatic Sciences; the home sediment for this test was from Yaquina Bay, OR. *Ampelisca* were obtained from East Coast Amphipods; the home sediment for this test was from Wickford, RI. *Ampelisca* were tested with 25 of the 30 randomly-collected samples. *Ampelisca* were tested on this subset for comparison with *Rhepoxynius*, the primary species used in the Bay Protection Toxic Cleanup program.

Toxicity Test Objectives and Data Analysis

There were three primary objectives for the toxicity testing portion of this study: 1) Investigate the spatial extent of toxicity in the Southern California Bays and Estuaries 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 relative sensitivity of each toxicity test protocol. In addition to comparing the relative sensitivity of the different protocols, interlaboratory comparisons of the *Ampelisca* test and sea urchin development test were conducted using 6 samples .

Statistical Analysis Of Toxicity Test Data

The different objectives required different sampling designs and different statistical approaches. The first objective, determination of the spatial extent of toxicity, was accomplished through a process hereafter referred to as the t-test-control approach, which involved 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 utilizing 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, we employed an alternative statistical method hereafter referred to as 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 bays and estuaries studied. 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 for the purposes of the State and Regional Water Quality Board's objective of identifying and prioritizing toxic hot spots. This method is also described in detail below.

t-test-control approach to determining spatial extent of toxicity in the Southern California Coastal Region

The Southern California bays and estuaries sampled in this study included 8 non-connecting water bodies: Los Peñasquitos Lagoon, San Dieguito Lagoon, Agua Hedionda, San Elijo Lagoon, Santa Margarita River, Oceanside Harbor, Dana Point Harbor, and Newport Harbor. 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 these bays were considered too few to accurately represent toxicity in a frequency distribution. Consequently, data from all 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); this method has been used in the EPA Environmental Monitoring Assessment Program (EMAP) and in similar NOAA studies nationwide (e.g. Long et al. 1994). Using the t-test-control approach, samples were defined as toxic if the following two criteria were met: 1) there was a significant difference ($p < 0.05$) 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 among 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. We used a "separate variance" t-test that adjusted 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 $90 \pm 2\%$ *Rhepoxynius* survival would be significantly different from a control with survival of $96 \pm 2\%$, 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 less significant. 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. Based on this observation, the 80% criterion has been used previously (Schimmel *et al.*, 1991). Samples identifed as toxic according to these criteria were used to estimate the percent of total area toxic within the Southern California bays and estuaries.

Using Cumulative Distribution Frequencies (CDFs) to characterize spatial extent

Cumulative Distribution Frequencies (CDFs) were determined using known areas of each sampling strata normalized to the number of samples per strata. By combining the area represented by each sample with their toxicity designations in a cumulative manner, the CDF's indicated the percentage of total area sampled that was toxic. Sample toxicity was determined from comparisons with laboratory controls as described above; 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. CDF's were generated from toxicity tests using *Rhepoxynius* (solid phase) and *Strongylocentrotus* fertilization and larval development in pore water; these were based on 30 random samples. A CDF was also generated from the *Ampelisca abdita* (solid phase) toxicity test based on a smaller subset of 15 random samples. CDF's were used to determine the percentage of area toxic for each toxicity test protocol. A 95% Confidence Interval was calculated for each areal toxicity determination based on EMAP methods.

The reference envelope approach to distinguish the most toxic samples

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 is an essential step in prioritizing efforts to improve sediment and water quality through regulation and remediation programs. An efficient use of funds requires that efforts be focused on localized areas that are significantly more toxic than optimal ambient conditions that presumably exist in the greater portion of the Southern California bays, estuaries, and coastal lagoons. In this study, we have employed a "reference envelope" statistical approach (Smith, 1995) to identify samples that exhibit significantly greater toxicity than expected in the area 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 area studied.

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 "t-test-control approach" described above), the reference envelope approach compares 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, includes 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 served 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, the bioassay results for the test locations were compared with the bioassay results from the population of reference locations.

If it is assumed that the bioassay results from the population of reference locations were normally distributed, then we could get an idea of the probability that the test sediment was from the underlying reference station distribution. 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 approximately 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 was not known because the number of samples from the underlying distribution were limited. Therefore, the location of the first percentile could only be estimated. If this value was estimated a large number of times using different random samples from the reference distribution, a non-central t distribution of estimates would be obtained, with the distribution mode at the actual first percentile (**Figure 3**). This figure shows that for this distribution of estimates, about one half of the time the estimate from the sample will be above the actual first percentile. Ideally, it would be preferable to identify an estimated toxicity value that would cover the actual first percentile for a large percentage of the estimates (say 95% of the time). This value can be obtained from the left tail of the distribution of estimates where 5% of the estimates are less than the chosen value. We define p as the percentile of interest, and alpha as the acceptable error probability associated with an estimate of the pth percentile. Thus, in this example, p=1 and alpha = .05.

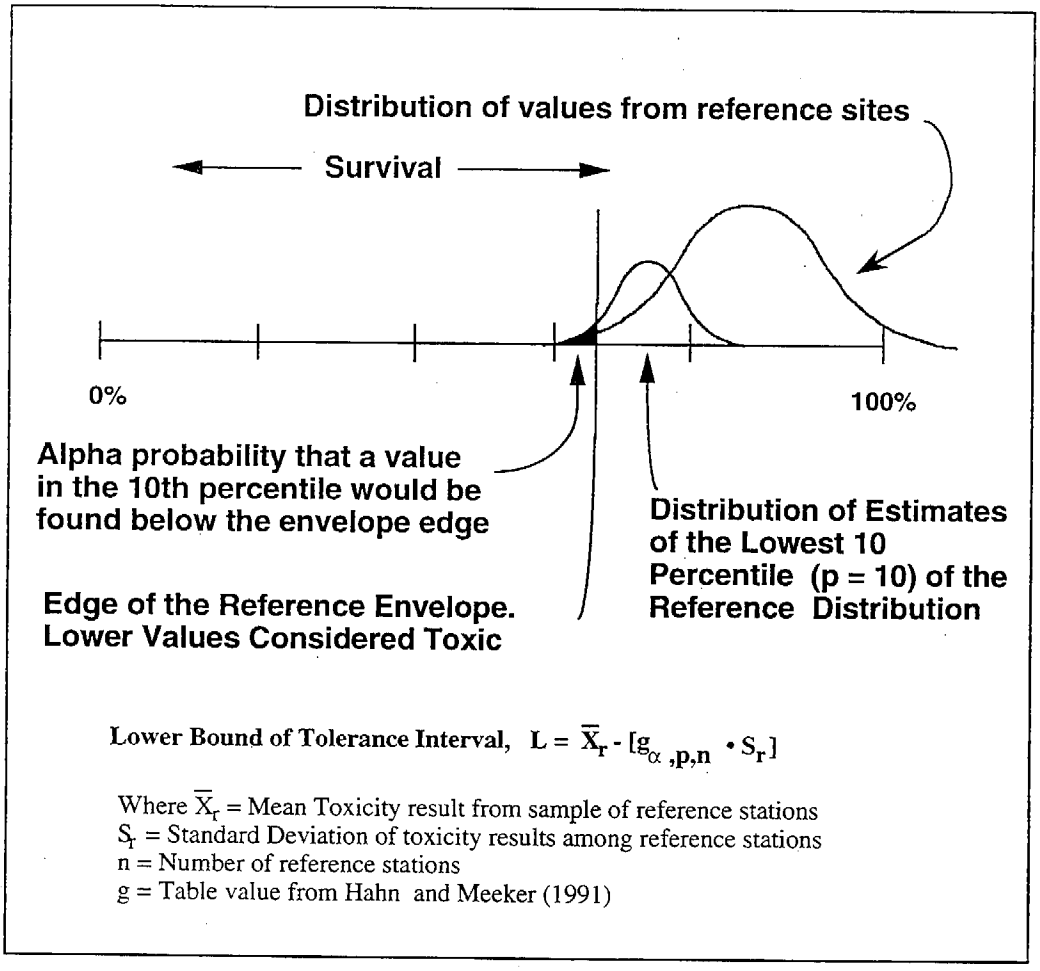


Figure 3. Schematic illustration of the method for determining the lower tolerance interval bound (edge of the reference envelope) to determine sample toxicity relative to a percentile of the reference site distribution.

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

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

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. L is called the "edge of the reference envelope" because it represents a cutoff toxicity level we will use 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 we chose p values equal to 1 and 10%, to distinguish the most toxic samples, that is, the samples that we are 95% certain are the most toxic 1 and 10% relative to the reference conditions defined below.

Reference station selection for use in developing reference envelope

Reference stations were selected to represent optimal ambient conditions available in the Southern California bays and estuaries sampled, 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 selected, a total of 43 samples were analyzed for toxicity, chemistry and benthic ecology in this study. After screening these 43 samples, six stations were selected as reference stations. Five stations were selected as baseline or reference stations from the results of P450 RGS analyses, as these produced low values of 1.7 to 2.5 µg of benzo(a)pyrene equivalents per g dry weight. 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.

Interlaboratory Comparisons of Toxicity Test Protocols

Interlaboratory comparisons were conducted to document test reproducibility and comparability of the results to other EMAP data collected in the Southern California Bight Pilot Project. These comparisons were conducted by staff from the Marine Pollution Studies Laboratory (MPSL) and Southern California Coastal Water Research Project (SCCWRP). Six sediment samples were collected on September 26, 1994, by personnel from Moss Landing Marine Laboratories using methods described above. Samples were homogenized, split into separate containers, and shipped on ice via overnight courier to SCCWRP, or by car in ice chests to MPSL so that both laboratories received solid-phase samples on the same day.

Two toxicity test protocols were compared between the two labs: the 10 day solid-phase survival test using the amphipod *Ampelisca abdita* and the 96-h development test in pore water using sea urchin embryos (*Strongylocentrotus purpuratus*). For the MPSL samples, pore water was extracted on September 27 and urchin development toxicity tests were initiated on September 28. The amphipod test was initiated on September 30. However, the first *Ampelisca* test conducted at MPSL failed due to poor home sediment control performance (control survival = 72%). Amphipods were then obtained from a second supplier (East Coast Amphipods) and this test was repeated on October 17. The results from the second test are presented. For the SCCWRP tests, pore water was extracted on September 30, and sea urchin and amphipod tests were initiated on September 30.

The interstitial water tests varied in two other respects. First, urchin development tests at MPSL were terminated after 96-h vs 72-h at SCCWRP. Interstitial water pH of SCCWRP samples was adjusted to approximately 8.0 using sodium hydroxide; pH of MPSL interstitial waters was not adjusted. Pore water extraction methods, test temperatures and salinities were similar between laboratories.

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 labeled internally and externally with project, date collected, site/station information, and IDORG. 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.

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 (Stephenson et al. 1994). This document describes procedures within the program which ensure data quality and integrity. 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

Distribution of Chemical Contaminants

Chemical Specific Screening Values

There have been several recent studies associating contaminant concentrations with biological responses which provide guidance for evaluating whether measured contaminant concentrations most likely contributed to observed biological effects (MacDonald 1996, Long et al. 1995). Reported guideline values are based on individual chemical concentrations; therefore their application may be confounded in sediments where biological effects may be attributed to synergistic or antagonistic effects of low concentrations of multiple compounds, unmeasured or unidentified compounds, or physical factors not accounted for.

The National Status and Trends Program has evaluated chemical and toxicological evidence from a

number or laboratory, field, and modeling studies to establish ranges of chemical concentrations which are rarely, sometimes, or usually associated with toxicity . Evaluation of available data (Long et al. 1995) has resulted in the identification of three concentration ranges for selected chemical compounds:

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

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

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

Two different methods were used to determine these chemical ranges. One method developed by NOAA (Long et al. 1995) used chemical data which were associated with toxic response. These data were used to determine the lower 10th percentile of ranked data where chemical concentration was associated with an effect (Effects Range- Low, or ERL). Chemical concentrations below the ERL are not expected to have an effect. 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 occasionally expected to occur when chemical concentrations fall between the ERL and ERM.

The screening concentrations described by MacDonald (1996) also identify three ranges of chemical concentrations associated with toxic biological response but use an alternate method. The ranges are identified as PEL (Probable Effects Level), and TEL (Threshold Effects Level). TELs 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. The ERL, ERM, TEL, and PEL values are provided in **Table 4**.

Although different data sets and percentiles were used in these two approaches to derive chemical screening concentrations, they are in close agreement, usually within a factor of 2. Values reported for both methods are given in Table 1. Neither of these methods is advocated over the other in this report. Both are used in the following analysis to establish a weight-of-evidence in order to help explain the observed effects.

It should be noted that the degree of confidence that MacDonald (1996) and Long et al. (1995) had in their respective numerical guidelines varied considerably among the different chemical substances. For example, both had little confidence in the values for nickel, mercury, DDTs, dieldrin, and endrin. DDT compounds were among those exceeding the PEL and ERM values most often at the 43 stations sampled in this study. MacDonald (1994) has recently revised guidelines for DDT and it's metabolites to derive Sediment Effect Concentrations (SECs) for these compounds.

Primary Chemicals of Concern

A summary of chemical compounds which exceeded the TEL/PEL values at the 43 sample stations are presented in **Figure 4**. Three pesticides occurred in relatively high concentrations, with chlordanes and DDT congeners exceeding PEL values in over 30% of the samples. Dieldrin exceeded the PEL in 3 of the samples. Copper, mercury and zinc were the only metals which exceeded the highest screening value (PEL) and the number of samples with exceedances were relatively few; a high proportion of samples exceeded the TELs for copper and zinc. High concentrations of total PCBs and low and high molecular weight PAHs were conspicuously absent in most of the samples.

Table 4. Sediment Quality Guidelines developed by the State of Florida, and NOAA.

	State of Florida (1)		NOAA		
SUBSTANCE	TEL	PEL	ERM (2)	ERL (3)	ERM (3)
Organics (ug/kg- dry weight)					
Total PCBs	21.550	188.79	380	22.70	180.0
PAHs					
Acenaphthene	6.710	88.90	650	16.00	500.0
Acenaphthylene	5.870	127.89		44.00	640.0
Anthracene	46.850	245.00	960	85.30	1100.0
Fluorene	21.170	144.35	640	19.00	540.0
2-methyl naphthalene	20.210	201.28	670	70.00	670.0
Naphthalene	34.570	390.64	2100	160.00	2100.0
Phenanthrene	86.680	543.53	1380	240.00	1500.0
Total LMW-PAHs	311.700	1442.00		552.00	3160.0
Benz(a)anthracene	74.830	692.53	1600	261.00	1600.0
Benzo(a)pyrene	88.810	763.22	2500	430.00	1600.0
Chrysene	107.710	845.98	2800	384.00	2800.0
Dibenzo(a,h)anthracene	6.220	134.61	260	63.40	260.0
Fluoranthene	112.820	1493.54	3600	600.00	5100.0
Pyrene	152.660	1397.60	2200	665.00	2600.0
Total HMW-PAHs	655.340	6676.14		1700.00	9600.0
Total PAHs	1684.060	16770.54	35000	4022.00	44792.0
Pesticides					
p,p'-DDE	2.070	374.17	15	2.20	27.0
p,p'-DDT	1.190	4.77			
Total DDT	3.890	51.70	350	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	85	8.20	70.0
Antimony				2.00	2.5
Cadmium	0.676	4.21	9	1.20	9.6
Chromium	52.300	160.40	145	81.00	370.0
Copper	18.700	108.20	390	34.00	270.0
Lead	30.240	112.18	110	46.70	218.0
Mercury	0.130	0.70	1.3	0.15	0.7
Nickel	15.900	42.80		20.90	51.6
Silver	0.733	1.77	2.5	1.00	3.7
Zinc	124.000	271.00	280	150.00	410.0

(1)-D.D. MacDonald, 1996; (2)-Long and Morgan, 1990; (3)-Long et al., 1995.

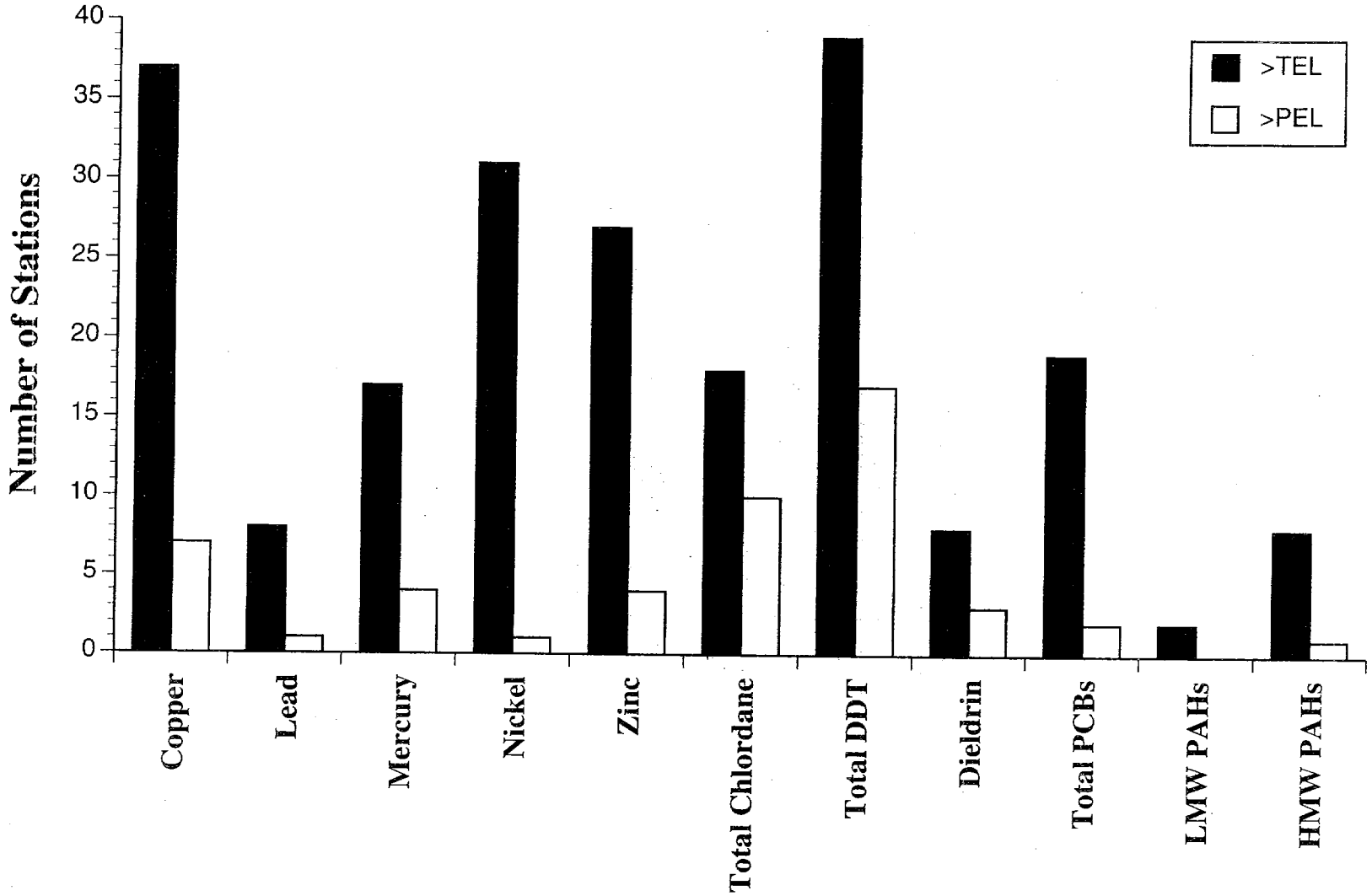


Figure 4. Number of stations, out of 43, exceeding either the TEL or PEL sediment quality guidelines (see text for details).

Total chlordane is the summation of the major constituents of technical grade chlordane and its metabolites (in this case CCHLOR, TCHLOR, OCDAN; **Appendix B**), and comprise a group of nonsystemic stomach and contact insecticides which until the mid 1970's had been used extensively in home and agricultural applications. Although the use of this compound was discontinued in this country due to it's widespread occurrence, biomagnification through the foodchain, and persistence in non-target systems, chlordane continues to occur in aquatic ecosystems. Due to their limited water solubility, chlordane compounds tend to bind to organic carbon and settle out of the water column, accumulating in sediments (Wilcok et al., 1993). High concentrations of chlordane were measured at 10 of the 43 stations sampled (23%). Almost all of the samples with chlordane concentrations exceeding the ERM (Long and Morgan 1990) or PEL (MacDonald 1994) came from Newport Bay (**Fig. 5a**) with highest concentrations occurring at the Arches Storm Drain (Station 85015; 7.5x the PEL) and Newport Island (85014; 5x the PEL). Of the 18 stations sampled in Newport Bay 50% had concentrations of chlordane which exceeded the PEL. One station from Dana Point Harbor had chlordane concentrations exceeding the PEL (**Figure 5b**).

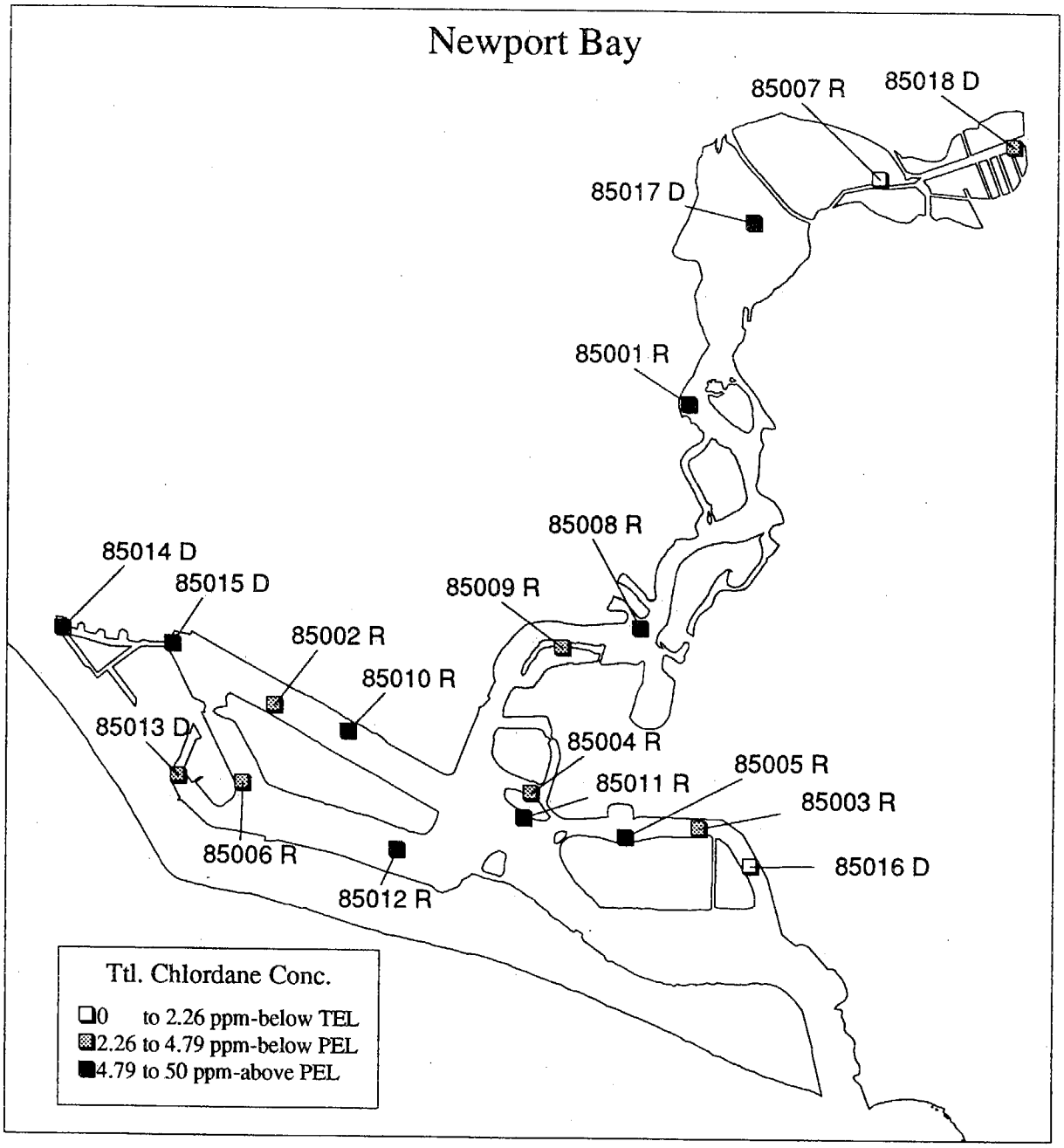
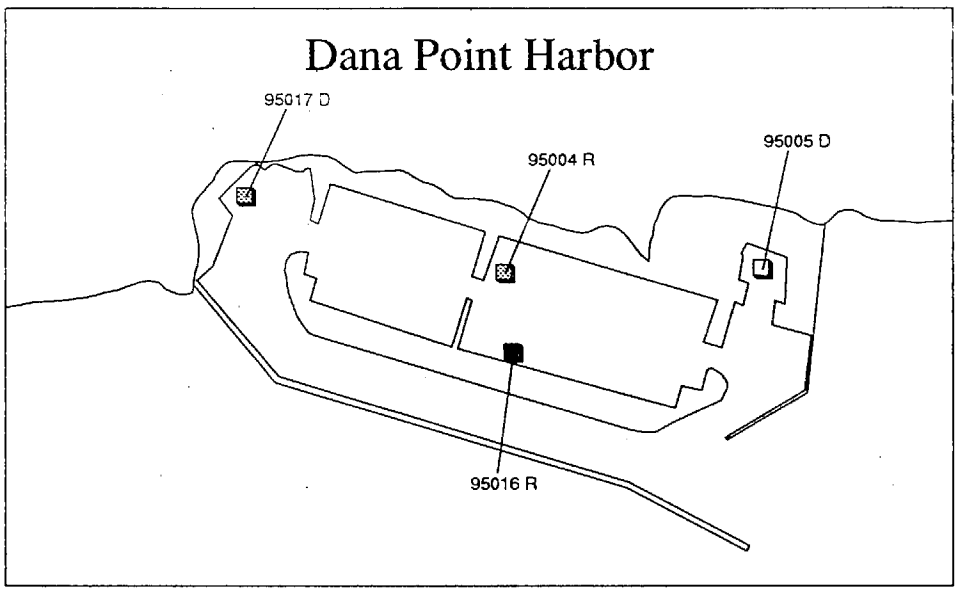


Figure 5a. Distribution of samples in Newport Bay exceeding the PEL for chlordane.



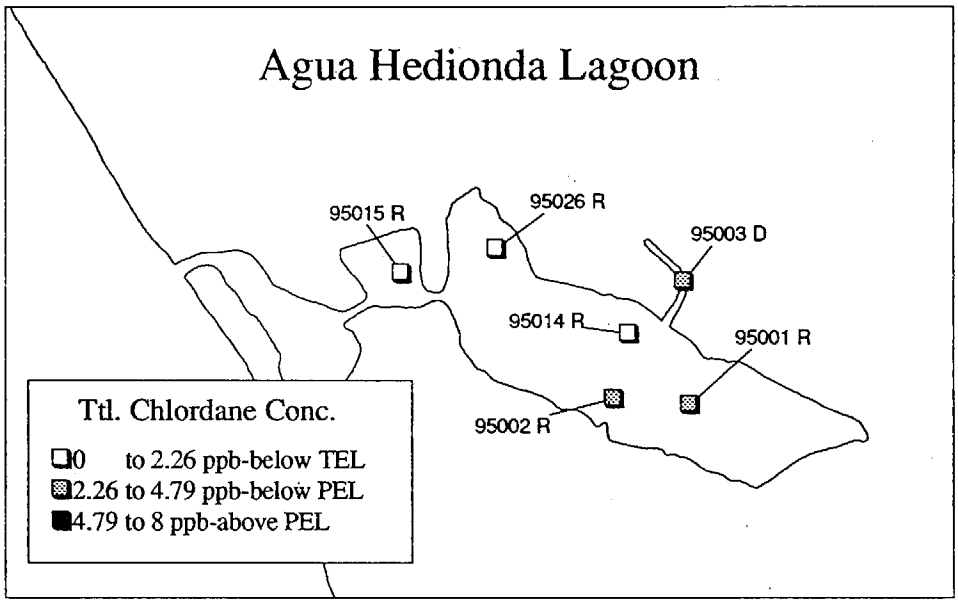


Figure 5b-c. Distribution of samples in Dana Point Harbor and Agua Hedionda Lagoon exceeding the PEL for chlordane.

DDT and its metabolites are a class of relatively water insoluble organo-chlorine compounds which also tend to bind to organic particulates and thus accumulate in the sediments. Concentrations of these compounds have generally declined in aquatic ecosystems since they were banned for most insecticide applications in 1972, although concentrations of some DDT metabolites have increased. Like chlordane and dieldrin, it is persistent in sediments and may be of significant environmental concern at higher concentrations (Hoke et al., 1994, Swartz et al., 1994). Elevated concentrations of total DDT were found at 17 of the 43 stations sampled (40%). As with chlordane, the majority of the stations with total DDT exceeding the ERMs or PELs were located within Newport Bay. Of the 18 Newport Bay stations, 13 (72%) had total DDT concentrations exceeding the PEL (MacDonald 1994; **Figure 6a**). The highest concentrations occurred at Arches Drain (85015; 2x the PEL) and Newport Bay Station No. 85012 (2x the PEL). In addition, 4 of the 6 (67%) Agua Hedionda stations and 1 station each in San Elijo Lagoon and the Santa Margarita River had total DDT concentrations exceeding the PEL (**Figure 6b**). One of the DDT metabolites (p'p DDE) also occurred at high concentrations at these stations. This compound exceeded the ERM (Long et al. 1995) value in 21 of the 43 stations sampled (49%) with highest concentrations occurring in Newport Bay (**Figure 7a**) and Agua Hedionda (**Figure 7b**). Over 80% of the Newport Bay stations exceeded the ERM for p'p DDE.

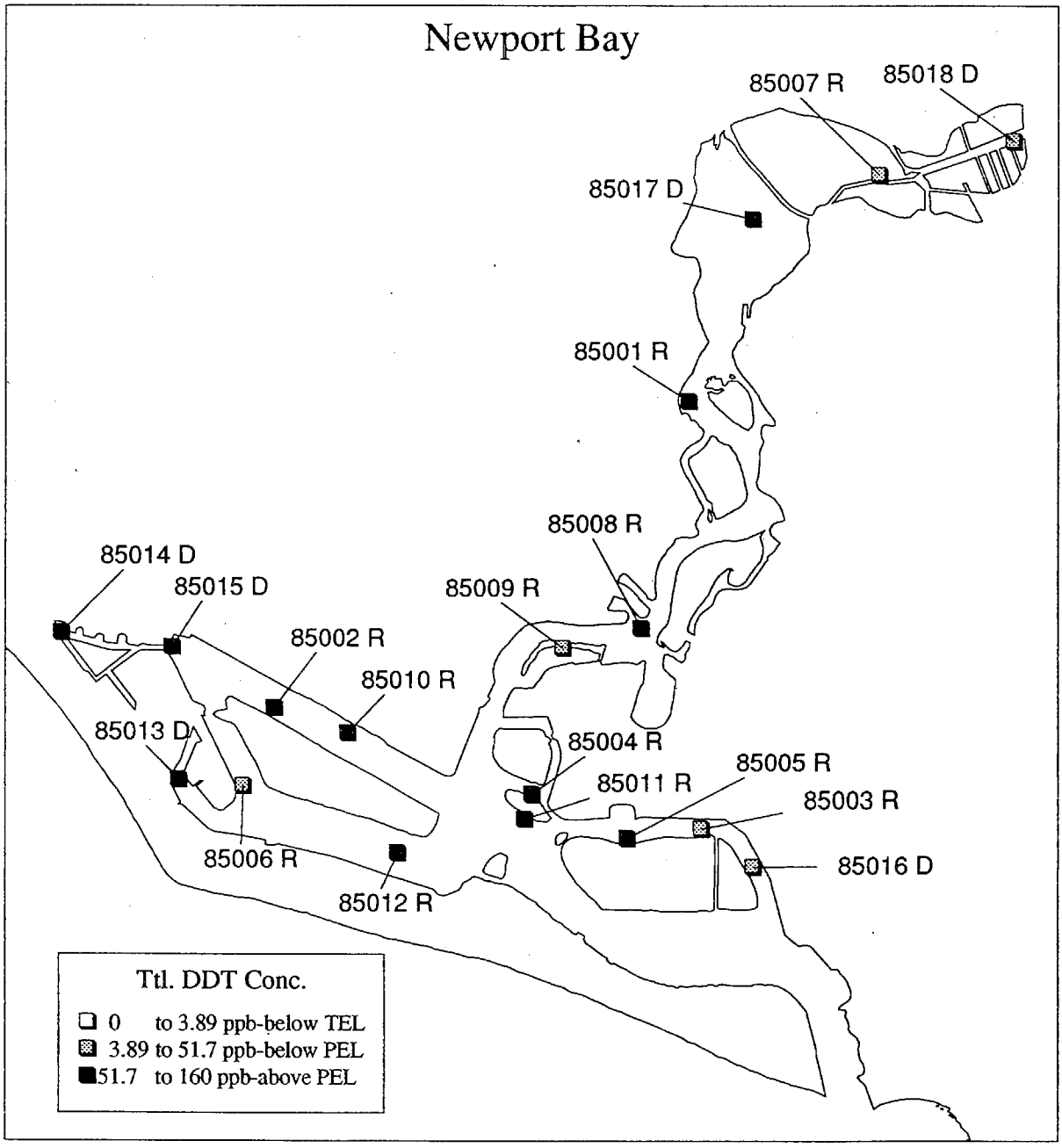


Figure 6a. Distribution of samples in Newport Bay exceeding PEL for Total DDT.

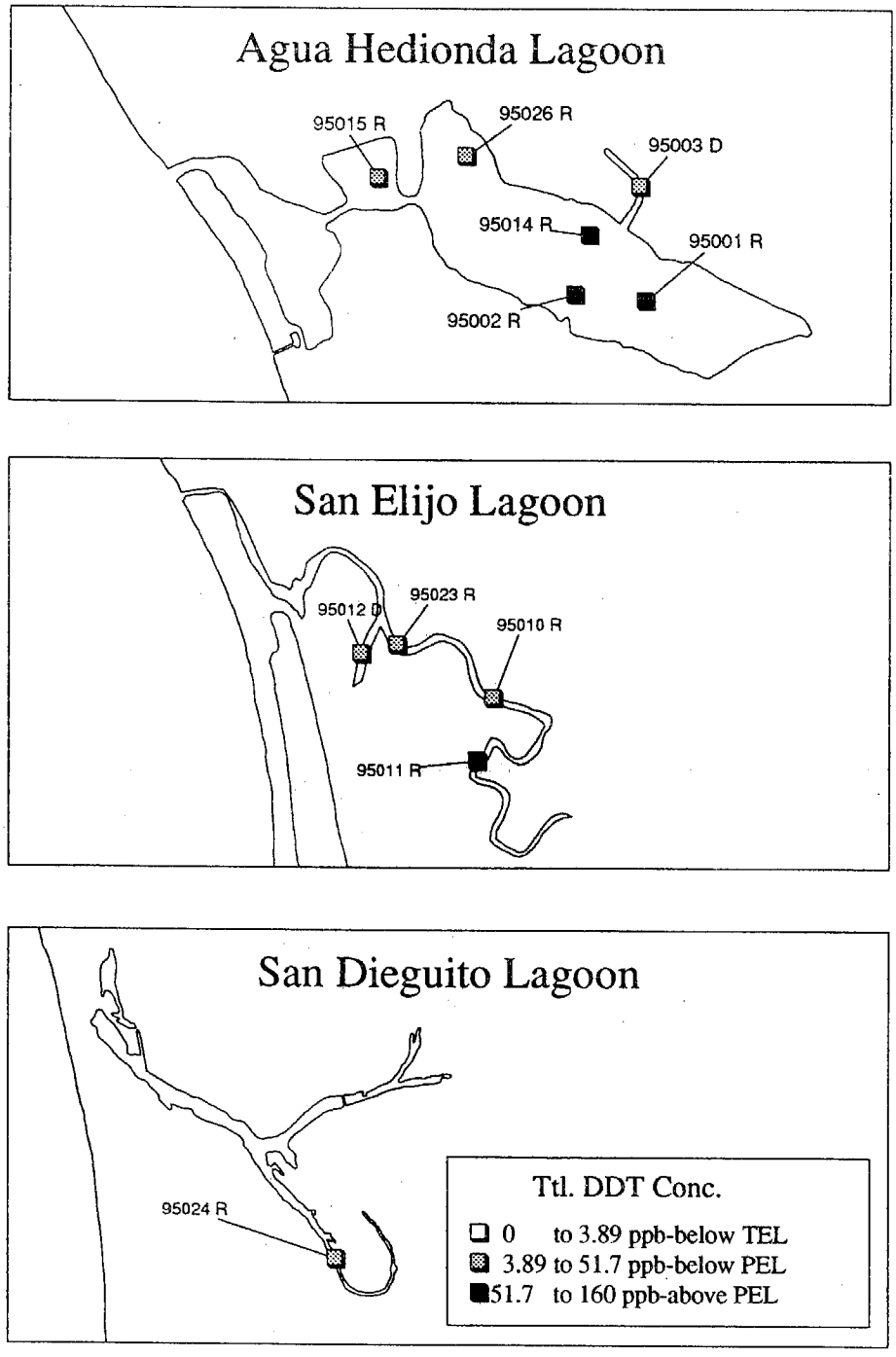


Figure 6b-d. Distribution of samples in Agua Hedionda, San Elijo, and San Dieguito Lagoons exceeding the PEL for Total DDT.

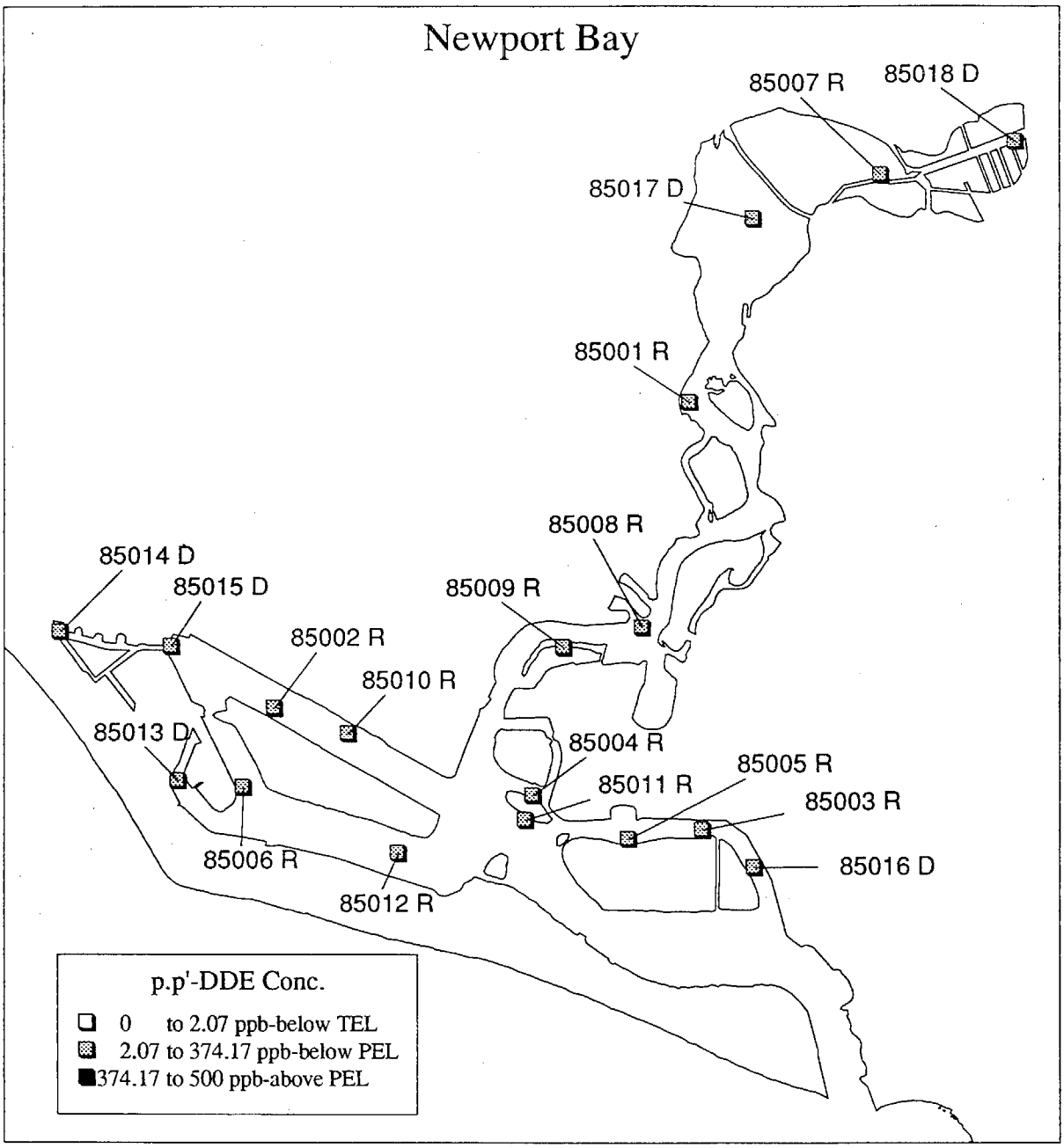


Figure 7a. Distribution of samples in Newport Bay exceeding PEL for p,p'-DDE.

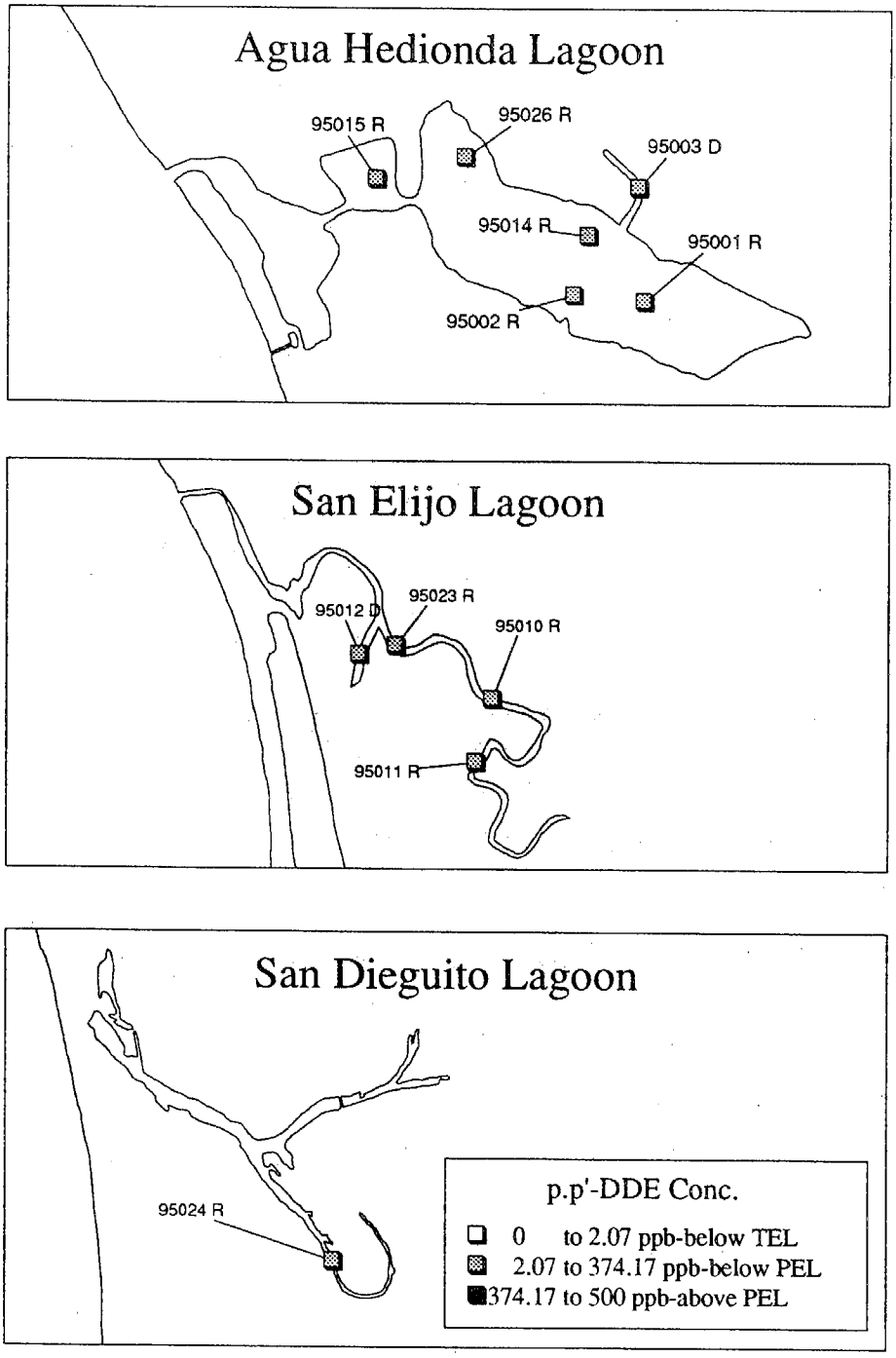


Figure 7b-d. Distribution of samples in Agua Hedionda, San Elijo, and San Dieguito Lagoons exceeding the PEL for p,p'-DDE.

Of the remaining pesticides detected at these stations, only dieldrin occurred at concentrations exceeding the screening criteria. High concentrations of dieldrin occurred at Rhine Channel in Newport Bay (1.1x the PEL; MacDonald 1994) and at San Elijo and San Dieguito Lagoons (2x and 3x the PEL, respectively).

Polycyclic Aromatic Hydrocarbons (PAHs) are base-neutral organic compounds which are components of crude and refined petroleum products and a product of incomplete combustion of hydrocarbons. These compounds are common components of contaminated sediments and are toxic to infaunal invertebrates (Eisler 1987; Neff 1979; Neff and Anderson 1981), in particular amphipods (Swartz et al. 1995). Due to their similar modes of toxicity, individual PAHs are combined into low and high molecular weight groups. The majority of the stations sampled had PAH concentrations considerably less than the screening values (**Figure 4**). Elevated concentrations of high molecular weight PAHs occurred at the Arches Storm Drain in Newport Bay (Station number 85015; **Figure 8**) where only dibenzo(a,h) anthracene exceeded the PEL (MacDonald 1996). Five other PAHs detected at this station (benzo(a)pyrene, chrysene, fluoranthrene, phenanthrene, and pyrene) had elevated concentrations (65 to 85% of the PEL value; **Figure 8**). The only other station with elevated concentrations of PAHs was Rhine Channel in Newport Bay (Station No. 85013) where dibenzo(a,h)anthracene was 65% of the PEL value.

Concentrations of total PCBs were elevated at two Newport Bay stations: Rhine Channel (Station No. 85013 = 2x the ERM for total PCBs), and Newport Island (Station No. 85014 = 1x the ERM for total PCBs).

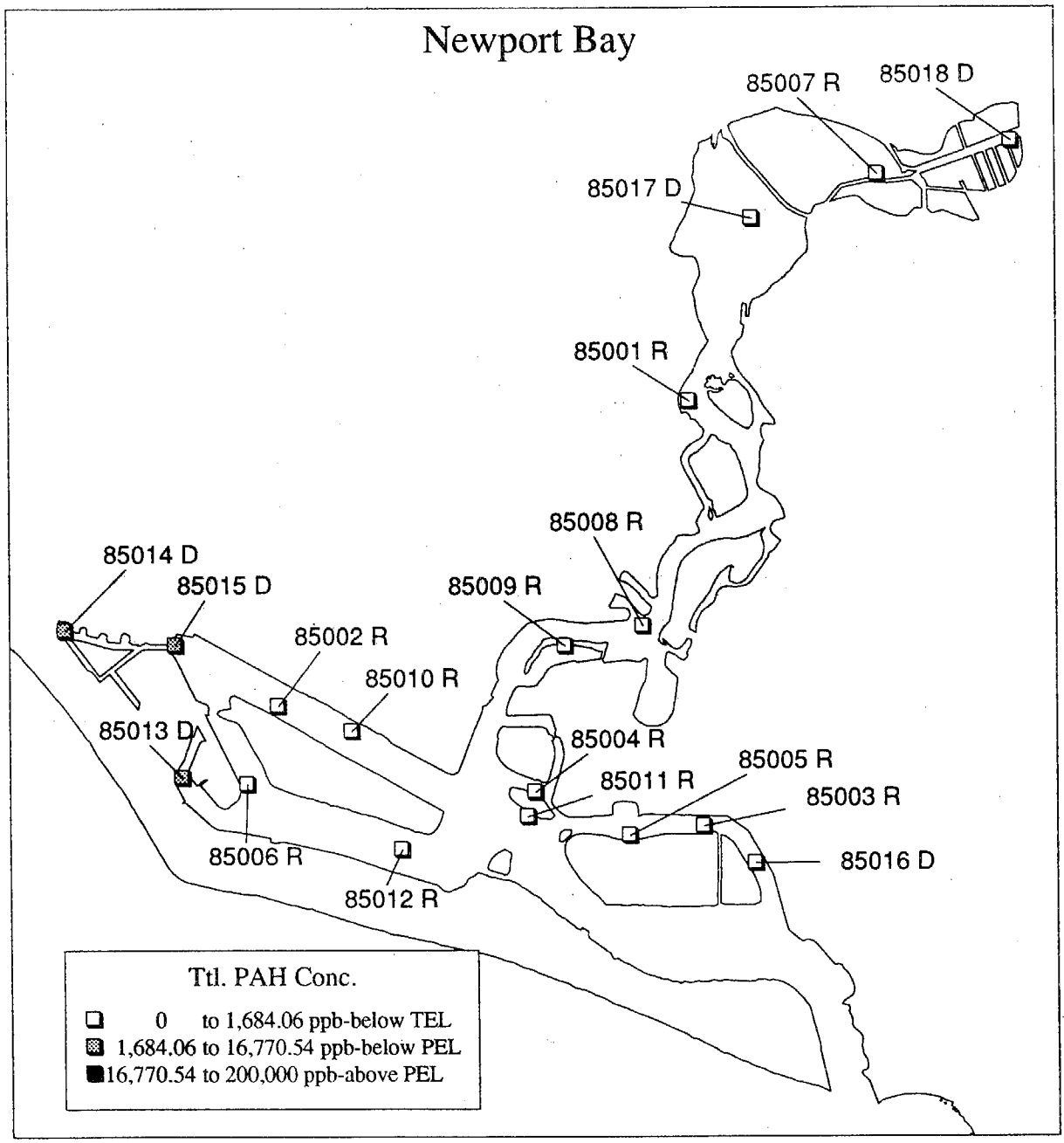


Figure 8. Distribution of samples in Newport Bay exceeding the PEL for Total PAHs.

Two metals, copper and mercury, occurred at concentrations exceeding the sediment screening guidelines. Copper exceeded the PEL (MacDonald 1996) at two stations in Newport Bay: Rhine Channel (4.7x the PEL) and Newport Island (2.2x the PEL; **Figure 9a**). In addition, several other stations in Newport Bay had concentrations almost equal to the PEL. Three stations in Dana Point Harbor and three in Oceanside Harbor had copper concentrations exceeding the PEL; the copper concentration at Station Number 95016 in Dana Point Harbor was 3.8x the PEL (**Figure 9b**). Mercury concentrations exceeded the PEL (MacDonald 1996) at 4 stations in Newport Bay. The highest mecury concentrations were measured at Rhine Channel (12.5x the PEL), Station Number 85006 (2.6x the PEL) and Station Number 85014 (2.9x the PEL; **Figure 10**).

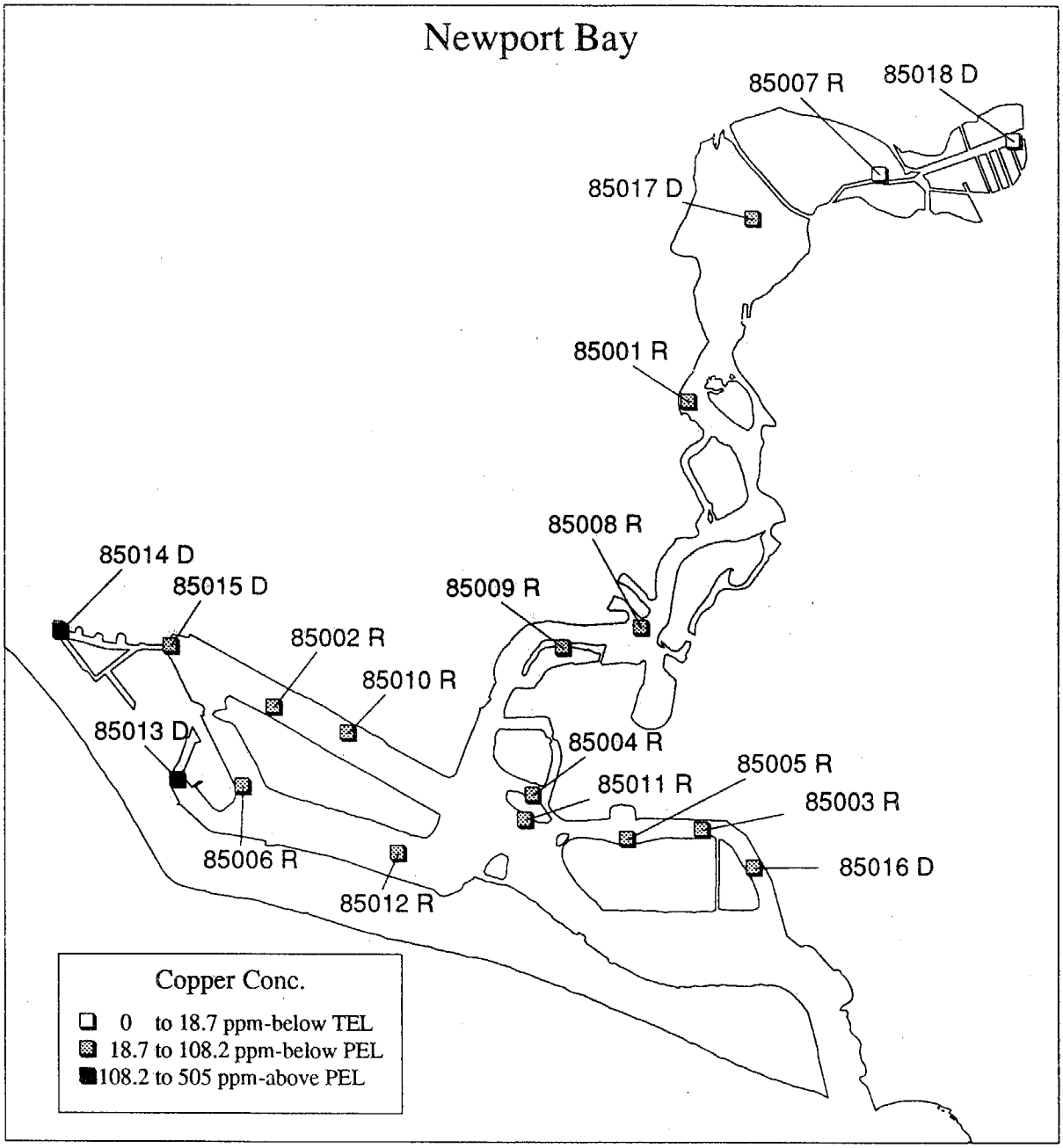


Figure 9a. Distribution of samples in Newport Bay exceeding the PEL for copper.

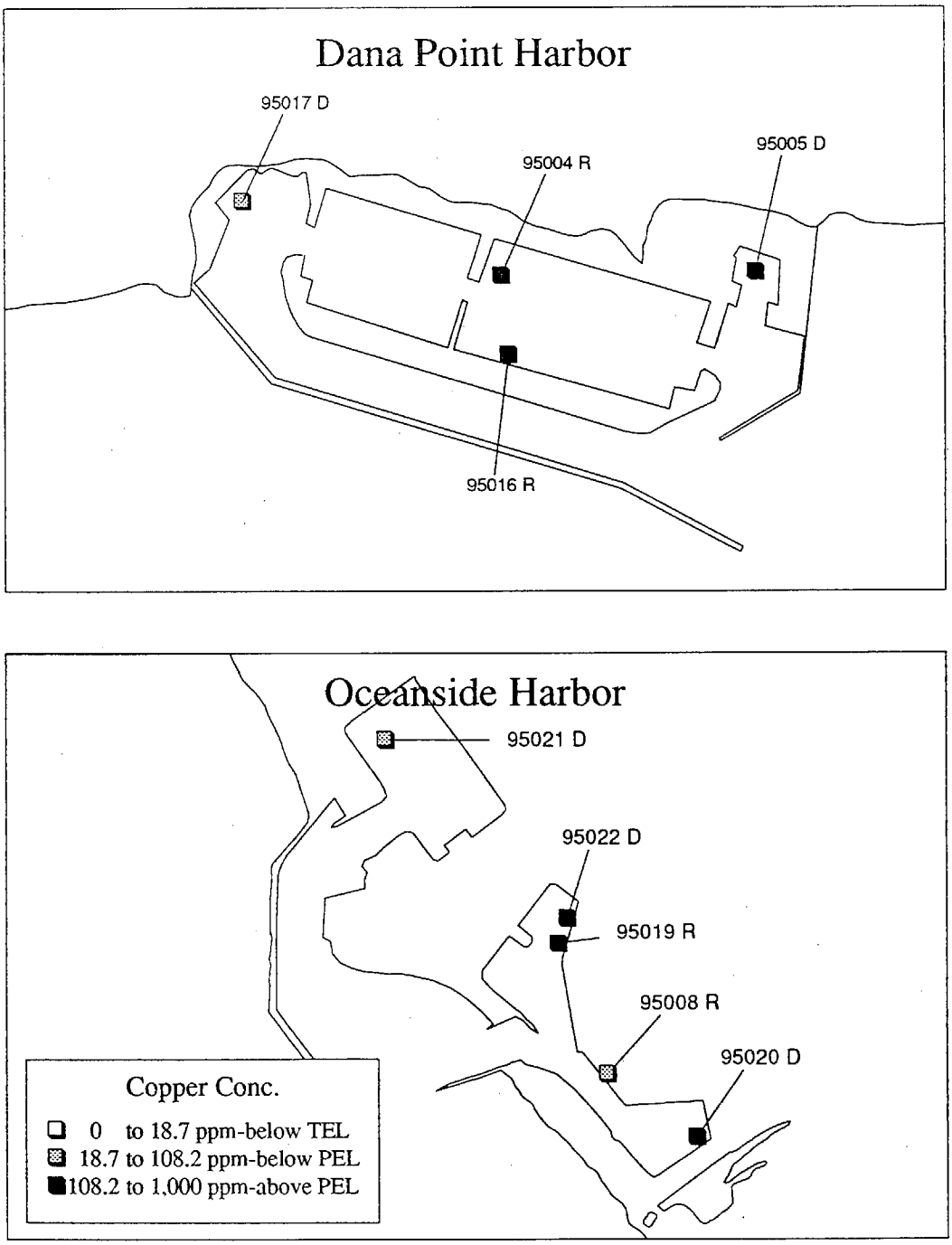


Figure 9b-c. Distribution of samples in Dana Point and Oceanside Harbors exceeding the PEL for copper.

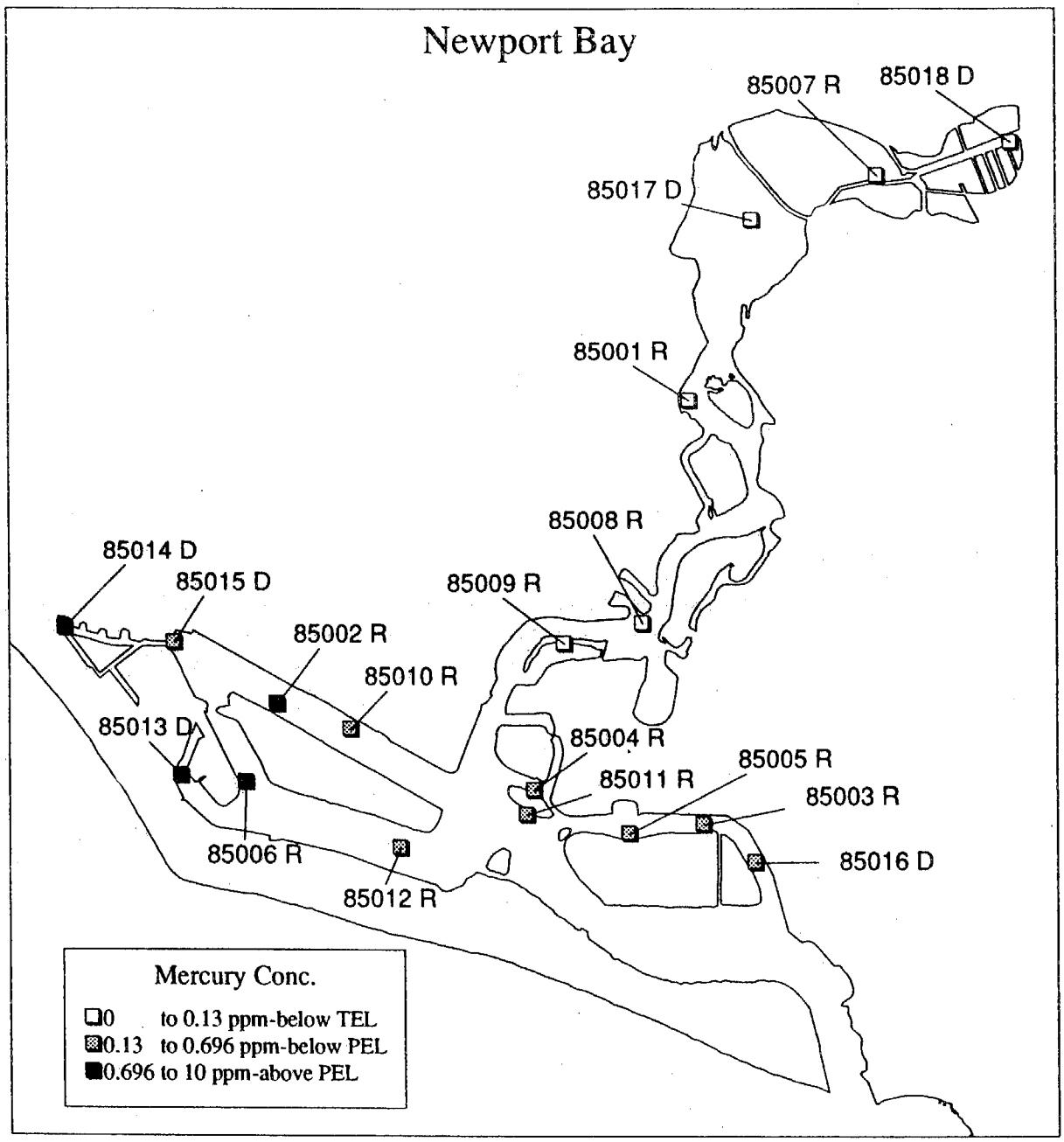


Figure 10. Distribution of sediment samples in Newport Bay exceeding the PEL for mercury.

ERM and PEL Quotients

The effects-based numerical guidelines listed above may also be used to assess the relative degree of contamination at these stations. In order to compare contamination using these guidelines, ERM quotients (ERMQ) and PEL quotients (PELQ) were calculated for all of the compounds for which these values exist. These are summations of chemical concentrations of the chemicals listed in **Tables 1-3**, divided by their respective ERM or PEL value. In cases where concentrations of measured chemicals were below the analytical method detection limit (MDL), a value of one-half the MDL was used for summations. ERM and TEL quotients are reported as average quotient values. The average ERM quotient was calculated by summing ERM quotient values for the following chemicals: Antimony, Arsenic, Cadmium, Chromium, Copper, Lead, Mercury, Silver, Zinc, Total DDT, Total Chlordane, Dieldrin, Endrin, Total PCBs, LMW PAHs, and HMW PAHs. This sum was then divided by the total number of analyte quotients (16) to give an average ERM quotient value. The average PEL quotient was calculated by summing PEL quotient values for the following chemicals: Arsenic, Cadmium, Chromium, Copper, Lead, Mercury, Silver, Zinc, Total DDT, Total Chlordane, Dieldrin, Lindane, Total PCBs, LMW PAHs, and HMW PAHs. This sum was then divided by the total number of analyte quotients (15) to give an average PEL quotient value. This is a simple approach to addressing chemical contamination in situations where there are multiple compounds present, and is intended for use in conjunction with the standard chemical-specific method discussed earlier. Although synergistic effects are possible with the different contaminants, this is not implied by the quotient summations. Quotients are presented as a method for comparing relative degree of contamination at these stations to aid management efforts (**Table 5**).

Many of the stations sampled in this study are from coastal bays and estuaries which are removed from industrial and commercial activities associated with pollution. Therefore, a majority of the stations reflect low contaminant concentrations. Three of the stations in Newport Bay (Rhine Channel, Newport Island, and Arches Storm Drain) were the most heavily contaminated of the 43 stations in this study and had PELQs and ERMQs considerably higher than the other stations (**Table 5**).

It should be noted that although these stations had relatively high quotient values relative to the other stations, these values were driven, in some cases by compounds for which the authors of the guideline values had less confidence. For example, at Rhine Channel the high quotients for PELs and ERMs are largely driven by mercury (12x the PEL). The high quotients were driven mainly by total chlordane at Newport Island (Station No. 85014) and Arches Storm Drain (Station No.

85015; 5x and 7x the PEL, respectively) . Benthic community degradation and toxicity test results for these and the other stations are discussed in more detail in the following sections.

Table 5. Average ERM quotients (ERMQ) and PEL quotients (PELQ) for 43 Southern California EMAP stations.

Station No.	Station Name	Sampling Design	ERMQ	PELQ
85013.0	NEWPORT BAY (RHINE CHANNEL)	Directed	1.270	1.684
85014.0	NEWPORT BAY (NEWPORT ISLAND)	Directed	0.733	1.039
85015.0	NEWPORT BAY (ARCHES S. DRAINS)	Directed	0.668	0.972
95016.0	DANA POINT HARBOR (396)	Random	0.322	0.579
85006.0	NEWPORT BAY (1009)	Random	0.318	0.426
85017.0	NEWPORT BAY (UNIT II BASIN)	Directed	0.256	0.373
85005.0	NEWPORT BAY (949)	Random	0.244	0.359
85002.0	NEWPORT BAY (616)	Random	0.239	0.340
85010.0	NEWPORT BAY (819)	Random	0.216	0.329
85012.0	NEWPORT BAY (1064)	Random	0.212	0.316
95024.0	SAN DIEGUITO LAGOON (306)	Random	0.174	0.307
95023.0	SAN ELIJO LAGOON (18)	Random	0.181	0.304
85011.0	NEWPORT BAY (905)	Random	0.200	0.295
95004.0	DANA POINT HARBOR (386)	Random	0.166	0.294
85004.0	NEWPORT BAY (877)	Random	0.198	0.290
95005.0	DANA POINT HARBOR(COMM. BASIN)	Directed	0.178	0.285
95022.0	OCEANSIDE HARBOR(STORM DRAINS)	Directed	0.183	0.284
85001.0	NEWPORT BAY (523)	Random	0.180	0.283
95017.0	DANA POINT HARBOR(STORM DRAIN)	Directed	0.169	0.280
85008.0	NEWPORT BAY (670)	Random	0.175	0.267
95019.0	OCEANSIDE HARBOR (90)	Random	0.158	0.262
95020.0	OCEANSIDE HARBOR (COMM. BASIN)	Directed	0.157	0.262
85016.0	NEWPORT BAY (YACHTMANS COVE)	Directed	0.163	0.247
95021.0	OCEANSIDE HARBOR (PENDLETON)	Directed	0.153	0.234
95003.0	AGUA HEDIONDA LAGOON (FINGER)	Directed	0.144	0.216
95008.0	OCEANSIDE HARBOR (110)	Random	0.128	0.214
85003.0	NEWPORT BAY (791)	Random	0.147	0.212
85009.0	NEWPORT BAY (705)	Random	0.131	0.209
95001.0	AGUA HEDIONDA LAGOON (190)	Random	0.126	0.187
95002.0	AGUA HEDIONDA LAGOON (234)	Random	0.123	0.185
95013.0	SANTA MARGARITA RIVER (33)	Random	0.116	0.180
95014.0	AGUA HEDIONDA LAGOON (179)	Random	0.107	0.161
95011.0	SAN ELIJO LAGOON (269)	Random	0.103	0.153
85018.0	NEWPORT BAY (UNIT I BASIN)	Directed	0.093	0.152
95010.0	SAN ELIJO LAGOON (24)	Random	0.088	0.147
95006.0	LOS PENASQUITOS (319)	Random	0.093	0.126
95025.0	SANTA MARGARITA RIVER (48)	Random	0.077	0.123
95026.0	AGUA HEDIONDA LAGOON (144)	Random	0.076	0.117
95007.0	LOS PENASQUITOS (331)	Random	0.080	0.105
95015.0	AGUA HEDIONDA LAGOON (212)	Random	0.066	0.103
95012.0	SAN ELIJO LAGOON (WASTE SITE)	Directed	0.065	0.100
85007.0	NEWPORT BAY (431)	Random	0.070	0.100
95018.0	LOS PENASQUITOS (336)	Random	0.077	0.097

P450 RGS Biomarker Results

Application of 10 µL of extracts from the 30 randomly collected sediment samples to the RGS assay, with human liver cancer cells, produced fold induction values of from 5 to 67 times the solvent blank (fold induction). Utilizing the volumes of the solvent extract and the amount applied, the dry weight of the sample and the factor of 60 fold induction for 1 µg of benzo(a)pyrene, data were converted to a range of µg of B(a)P equivalents/g of sediment. These values ranged from 1.7 to 22.8 µg of B(a)P equivalents/g. **Figure 11 and Table 6** show the distribution of these data, where 7 of the highest values were from sediments collected in Newport Bay, and the other sample in the top 8 was from Dana Point Harbor. The five samples with the lowest levels of CYP1A1 inducing compounds (reference) were two from Agua Hedionda Lagoon, two from Los Penasquitos Lagoon, and one from the Santa Margarita River. It should be noted that the sample locations were not provided to the researchers until after the data were reported, so testing was indeed blind. The relationship of these RGS findings to chemical analyses and biological responses will be discussed in later sections of this report.

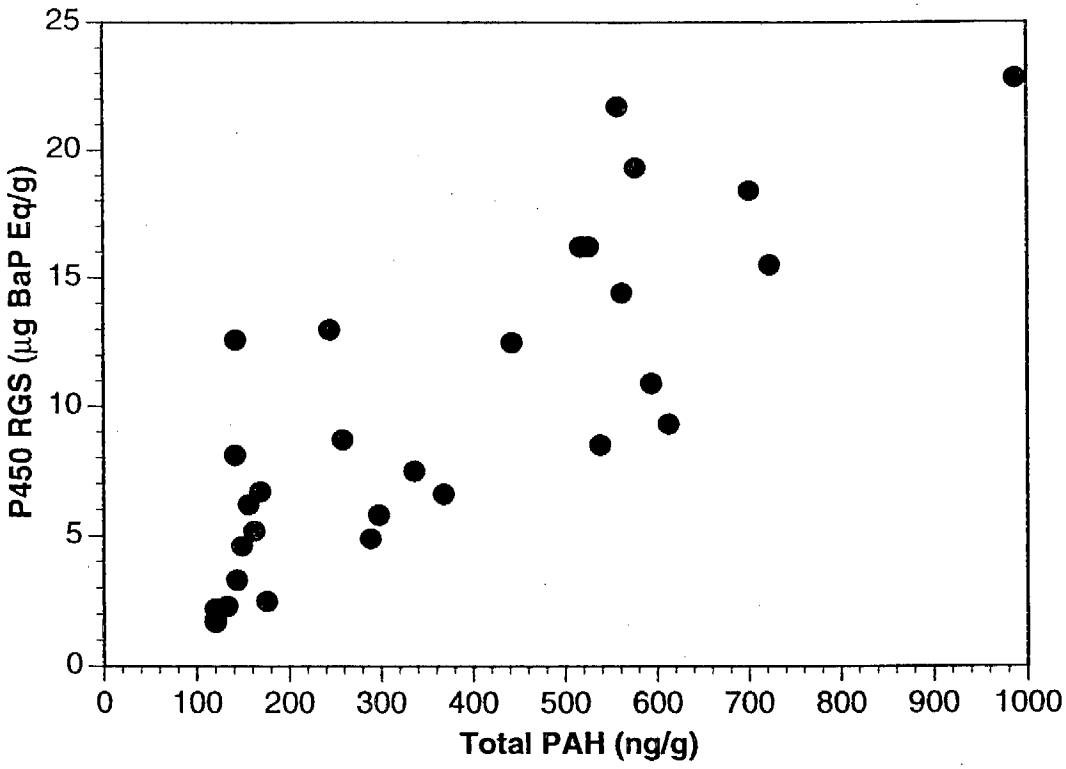


Figure 11. Relationship between Total PAH concentrations and response of P450 RGS assay to sediment extracts from 30 EMAP samples from Southern California bays and estuaries

Table 6. Response of P450 Reporter Gene System (RGS) screening assay at 30 Southern California EMAP stations. Bulk sediment concentrations of high molecular weight and total PAHs at these stations are also given.

Station Number	Station Name	IDORG	HMW PAH (ng/g)	TTL PAH (ng/g)	BaP eq (ug/g)
95025.0	SANTA MARGARITA RIVER (48)	1436	37.50	120.00	1.7
95018.0	LOS PENASQUITOS (336)	1417	37.50	120.00	1.8
95007.0	LOS PENASQUITOS (331)	1386	37.50	120.00	2.2
95015.0	AGUA HEDIONDA LAGOON (212)	1414	60.11	132.61	2.3
95001.0	AGUA HEDIONDA LAGOON (190)	1380	104.45	175.45	2.5
85007.0	NEWPORT BAY (431)	1418	76.80	143.07	3.3
95013.0	SANTA MARGARITA RIVER (33)	1397	81.19	148.69	4.6
85009.0	NEWPORT BAY (705)	1420	206.70	288.52	4.9
95002.0	AGUA HEDIONDA LAGOON (234)	1381	89.39	162.09	5.2
95010.0	SAN ELIJO LAGOON (24)	1394	218.27	297.36	5.8
95026.0	AGUA HEDIONDA LAGOON (144)	1412	89.96	155.94	6.2
95011.0	SAN ELIJO LAGOON (269)	1395	288.32	368.12	6.6
95014.0	AGUA HEDIONDA LAGOON (179)	1413	98.88	168.58	6.7
95019.0	OCEANSIDE HARBOR (90)	1430	242.06	336.33	7.5
95024.0	SAN DIEGUITO LAGOON (306)	1435	68.90	141.40	8.1
85006.0	NEWPORT BAY (1009)	1392	467.10	538.20	8.5
95008.0	OCEANSIDE HARBOR (110)	1393	181.48	258.25	8.7
85010.0	NEWPORT BAY (819)	1421	532.90	612.65	9.3
85008.0	NEWPORT BAY (670)	1419	520.90	593.75	10.9
95004.0	DANA POINT HARBOR (386)	1383	341.40	442.40	12.5
95006.0	LOS PENASQUITOS (319)	1385	74.68	142.18	12.6
95023.0	SAN ELIJO LAGOON (18)	1434	169.27	244.07	13.0
85012.0	NEWPORT BAY (1064)	1423	490.20	561.50	14.4
95016.0	DANA POINT HARBOR (396)	1415	654.10	722.50	15.5
85001.0	NEWPORT BAY (523)	1387	453.30	525.50	16.2
85004.0	NEWPORT BAY (877)	1390	407.60	516.70	16.2
85011.0	NEWPORT BAY (905)	1422	620.60	700.40	18.4
85003.0	NEWPORT BAY (791)	1389	459.90	576.50	19.3
85002.0	NEWPORT BAY (616)	1388	434.90	557.30	21.7
85005.0	NEWPORT BAY (949)	1391	888.60	987.69	22.8

Spatial Extent of Chemical Contamination

The spatial extent of chemical contamination was determined based on a Cumulative Distribution Function (CDF) using the PEL/TEL sediment quality guidelines proposed by MacDonald (1996). CDF's were calculated for the 30 random samples analyzed for substances which have PEL/TEL values. If DDT is excluded from the calculation, 89% of the randomly sampled study area had at least one exceedance of a TEL guideline. If samples having exceedances of the TEL for total DDT are included, the percentage of the randomly sampled study area having $\geq$ 1TEL exceedance increased to 94% (**Table 7**). If DDT is excluded from the calculation, 52% of the randomly sampled study area had at least one exceedance of a PEL guideline. If samples having exceedances of the PEL for total DDT are included, the percentage of the randomly sampled study area having $\geq$ 1 PEL exceedance increased to 67% (**Table 7**). As indicated in **Table 8**, a large percentage of the study area exceeded the TELs for a variety of metals, particularly copper, nickel, and zinc. In addition, organic substances such as chlordanes, DDT, and PCBs exceeded the TEL guidelines in much of the study area.

Table 7. Spatial extent of chemical contamination in Southern California bays and estuaries. Total area sampled = 5.01 km sq.

Degree of Contamination	N*	Percent Area Contaminated‡
Samples exceeding ≥ 1 TEL, excluding Total DDT TEL	27	88.9%
Samples exceeding ≥1 PEL, excluding Total DDT PEL	12	51.6%
Samples exceeding ≥ 1 TEL, including Total DDT TEL	28	94.1%
Samples exceeding ≥ 1 PEL, including Total DDT PEL	18	67.1%

* Number of contaminated stations out of 30 random samples. ‡ Percent Area Contaminated based on Cummulative Distribution Function of contamination at "n" random stations.

Table 8. Percent of area exceeding contaminant thresholds in Southern California bays and estuaries.‡

Chemical Analyte	TEL (% Area)	PEL (% Area)
Arsenic	0	0
Cadmium	36.7	0
Chromium	71	0
Copper	86.4	6.1
Lead	18.8	0
Mercury	43.3	5.2
Nickel	73.8	0
Silver	0.1	0
Zinc	71.8	0
LMW PAH	0	0
HMW PAH	0.1	0
Total DDT	90.5	52.1
Total Chlordane	53.8	34.5
Dieldrin	11.5	0
Total PCBs	42.8	0

‡ Percent Area Contaminated based on Cummulative Distribution Function of contamination at "n" random stations.

Toxicity Results

Distribution and Spatial Extent of Toxicity

A total of 43 sediment samples were tested for toxicity to amphipods (*Rhepoxynius abronius)* and sea urchins (*Strongylocentrotus purpuratus*) in this study. A subset of 30 samples was tested with the amphipod *Ampelisca abdita*.

All toxicity test data were evaluated for acceptability using the Quality Assurance guidelines presented in the BPTCP Quality Assurance Project Plan (BPTCP QAPP 1994). Most of the data reported here met test acceptability standards for each test protocol. Departures from acceptability standards are recorded in the Quality Assurance report which accompanies this data report. Almost all of these were departures in water quality parameters such as pH and dissolved oxygen exceedances, and in most cases were considered to be of minimal concern. Concentrations of dissolved oxygen in two pore water samples (Idorg # 1418, and 1419) were below the acceptability criteria and in both samples percent normal sea urchin development was zero. Low DO is often associated with organic enrichment resulting in high Biological Oxygen Demand (BOD), or in some cases specific contaminants resulting in high Chemical Oxygen Demand (COD). Conclusions regarding sea urchin toxicity associated with contamination at these stations should be considered preliminary due to the low D.O. in these samples.

Sediment holding time was 20 days in the 30 samples tested with *Ampelisca* because the initial test failed due to low control survival; the holding time specified in the BPTCP QAPP is two weeks. This test was repeated using amphipods from an alternative supplier (East Coast Amphipods) and home sediment controls in this test exceeded the 90% survival criterion (Home sediment from Wickford, RI) . See the Quality Assurance Report (**Appendix G**) for a discussion of possible effects of extended sediment holding time.

The results of all toxicity tests conducted are presented in tables in **Appendix C**. These tables show mean toxicity responses (e.g. percent survival of *Rhepoxynius and Ampelisca;* percent fertilization or normal development of larval sea urchins) of three to five replicates of each sample tested. Associated ammonia and hydrogen sulfide concentrations are also included.

Distribution of Toxicity

Estimations of the distribution and spatial extent of toxicity were based on a two-tiered approach for determining toxicity (ie., t-test and < 80% 0f the control value). Samples which met these criteria were considered to be highly toxic. The distributions of results for the four toxicity test protocols are presented in **Tables 9-12**. Toxicity for each protocol is presented in descending order from most to least toxic. These tables show toxicity data from samples collected using both sampling designs. The experimental design used for each particular sample is indicated by an "R" for randomly selected samples and by a "D" for samples selected using the directed design. The following discussion of the distribution and spatial extent of toxicity considers all samples collected using only the stratified random design described previously. A comparison of results based on the two sampling designs is discussed in a later section. There were no significant correlations between results of any of the toxicity tests.

Table 9. Toxicity of Southern California sediments to *Rhepoxynius abronius;* sediment toxicity ranked in descending order

Station No.	Idorg No.	Mean Proportion Survival	sd	Sampling Design‡	Toxicity
95006	1385	0.23	0.08	R	**
95018	1417	0.28	0.14	R	**
85001	1387	0.29	0.15	R	**
95007	1386	0.42	0.12	R	**
95002	1381	0.50	0.22	R	**
85014	1425	0.56	0.15	D	**
85008	1419	0.57	0.14	R	**
85002	1388	0.58	0.16	R	**
85012	1423	0.59	0.16	R	**
85013	1424	0.60	0.21	D	**
85005	1391	0.63	0.19	R	**
95012	1396	0.63	0.34	D	**
95024	1435	0.64	0.16	R	**
95004	1383	0.67	0.20	R	**
95022	1433	0.68	0.14	D	**
85004	1390	0.70	0.10	R	**
95011	1395	0.70	0.21	R	**
85003	1389	0.72	0.10	R	**
95005	1384	0.73	0.06	D	**
95013	1397	0.73	0.07	R	**
85010	1421	0.74	0.14	R	**
95014	1413	0.76	0.07	R	**
95023	1434	0.78	0.07	R	**
85006	1392	0.79	0.10	R	*
95008	1393	0.79	0.14	R	*
95010	1394	0.80	0.29	R	ns
85011	1422	0.80	0.17	R	*
95020	1431	0.80	0.05	R	*
85017	1428	0.81	0.04	D	*
95019	1430	0.82	0.09	R	*
95001	1380	0.85	0.15	R	ns
85016	1427	0.85	0.08	D	*
95016	1415	0.86	0.07	R	*
95017	1416	0.87	0.03	D	*
95021	1432	0.87	0.10	D	ns
95025	1436	0.88	0.06	R	*
85018	1429	0.89	0.11	D	ns
85015	1426	0.93	0.06	D	ns

Table 9 (cont.) Toxicity of Southern California sediments to *Rhepoxynius abronius;* sediment toxicity ranked in descending order.

95003	1382	0.93	0.06	D	ns
85007	1418	0.93	0.06	R	*
85009	1420	0.93	0.06	R	*
95015	1414	0.95	0.05	R	ns
95026	1412	0.95	0.07	D	ns
	home 1	1.00	0.00		
	home 2	0.95	0.05		

** indicates highly significant toxicity using separate variance t test and survival < 80 % of home sediment control value, * indicates significant toxicity using t-test only, ns = not significant using t-test.
‡ R indicates random sampling design; D indicates directed sampling design
home 1 & 2 = Yaquina Bay home sediment tested during legs 1 and 2.

Table 10. Toxicity of Southern California sediments to *Ampelisca abdita;* sediments ranked in descending order.

Station No.	Idorg	Mean surv	s.d	Sampling Design‡	Toxicity
85008	1419	0.00	0.00	R	**
85013	1424	0.04	0.05	D	**
85014	1425	0.26	0.20	D	**
85012	1423	0.67	0.39	R	*
85010	1421	0.76	0.13	R	*
85015	1426	0.77	0.16	D	ns
95019	1430	0.78	0.24	R	ns
95020	1431	0.81	0.20	R	ns
95021	1432	0.81	0.16	D	ns
95025	1436	0.81	0.17	R	ns
95022	1433	0.83	0.23	D	ns
95018	1417	0.84	0.15	R	ns
95015	1414	0.86	0.09	R	ns
85018	1429	0.86	0.13	D	ns
95023	1434	0.87	0.11	R	ns
85007	1418	0.87	0.13	R	ns
85009	1420	0.87	0.10	R	ns
85016	1427	0.89	0.11	D	ns
95014	1413	0.89	0.13	R	ns
95026	1412	0.91	0.15	D	ns
95016	1415	0.93	0.08	R	ns
85017	1428	0.93	0.06	D	ns
95024	1435	0.94	0.05	R	ns
85011	1422	0.95	0.05	R	ns
95017	1416	0.96	0.04	D	ns
	home	0.92	0.13		

** indicates highly significant toxicity using separate variance t test and survival < 80 % of home sediment control value, * indicates significant toxicity using t-test only, ns = not significant using t-test.
‡ R indicates random sampling design; D indicates directed sampling design.
home = Chesapeake Bay home sediment.

Table 11. Toxicity of pore water to sea urchin embryo development. Stations ranked by toxicity in descending order.

Percent Pore Water	Station No.	Idorg	% Norm. Develop.	sd	Sample Design‡	Toxicity
100	85001.0	1387	0.00	0.00	R	**
100	85002.0	1388	0.00	0.00	R	**
100	85003.0	1389	0.00	0.00	R	**
100	85004.0	1390	0.00	0.00	R	**
100	85005.0	1391	0.00	0.00	R	**
100	85006.0	1392	0.00	0.00	R	**
100	95005.0	1384	0.00	0.00	D	**
100	95008.0	1393	0.00	0.00	R	**
100	95010.0	1394	0.00	0.00	R	**
100	95011.0	1395	0.00	0.00	R	**
100	95012.0	1396	0.00	0.00	D	**
100	85007.0	1418	0.00	0.00	R	**
100	85008.0	1419	0.00	0.00	R	**
100	85009.0	1420	0.00	0.00	R	**
100	85010.0	1421	0.00	0.00	R	**
100	85011.0	1422	0.00	0.00	R	**
100	85013.0	1424	0.00	0.00	D	**
100	85014.0	1425	0.00	0.00	D	**
100	85017.0	1428	0.00	0.00	D	**
100	85018.0	1429	0.00	0.00	D	**
100	95015.0	1414	0.00	0.00	R	**
100	95018.0	1417	0.00	0.00	R	**
100	95023.0	1434	0.00	0.00	R	**
100	95025.0	1436	0.00	0.00	R	**
100	85015.0	1426	0.00	0.01	D	**
100	95003.0	1382	0.02	0.03	D	**
100	85012.0	1423	0.02	0.03	R	**
100	95002.0	1381	0.06	0.06	R	**
100	95024.0	1435	0.17	0.11	R	**
100	95004.0	1383	0.25	0.19	R	**
100	95026.0	1412	0.26	0.29	D	**
100	95021.0	1432	0.36	0.31	D	**
100	95006.0	1385	0.42	0.39	R	ns
100	95001.0	1380	0.43	0.11	R	**
100	95014.0	1413	0.56	0.14	R	**
100	95017.0	1416	0.67	0.24	D	ns
100	95016.0	1415	0.75	0.08	R	**

Table 11 (cont.). Toxicity of pore water to sea urchin embryo development. Stations ranked by toxicity in descending order.

Percent Pore Water	Station No.	Idorg	% Norm. Develop.	sd	Sample Design‡	Toxicity
100	85016.0	1427	0.81	0.08	D	*
100	95020.0	1431	0.81	0.22	D	ns
100	95019.0	1430	0.91	0.03	R	*
100	95013.0	1397	0.92	0.01	R	ns
100	95007.0	1386	0.92	0.04	R	ns
100	95022.0	1433	0.98	0.01	D	ns
		DC	0.92	0.02		
		BC	0.95	0.03		
		DC	0.98	0.01		
		BC	0.76	0.02		
50	85001.0	1387	0.00	0.00	R	**
50	85002.0	1388	0.00	0.00	R	**
50	85003.0	1389	0.00	0.00	R	**
50	85004.0	1390	0.00	0.00	R	**
50	85005.0	1391	0.00	0.00	R	**
50	85006.0	1392	0.00	0.00	R	**
50	95002.0	1381	0.00	0.00	R	**
50	95004.0	1383	0.00	0.00	R	**
50	95005.0	1384	0.00	0.00	D	**
50	95008.0	1393	0.00	0.00	R	**
50	85007.0	1418	0.00	0.00	R	**
50	85008.0	1419	0.00	0.00	R	**
50	85010.0	1421	0.00	0.00	R	**
50	85011.0	1422	0.00	0.00	R	**
50	85014.0	1425	0.00	0.00	D	**
50	85018.0	1429	0.00	0.00	D	**
50	95015.0	1414	0.00	0.00	R	**
50	95025.0	1436	0.00	0.00	R	**
50	95023.0	1434	0.00	0.01	R	**
50	95010.0	1394	0.01	0.01	R	**
50	85009.0	1420	0.01	0.01	R	**
50	85017.0	1428	0.01	0.02	D	**
50	95001.0	1380	0.02	0.03	R	**
50	95026.0	1412	0.31	0.40	D	**
50	95012.0	1396	0.36	0.25	D	**

Table 11 (cont.). Toxicity of pore water to sea urchin embryo development. Stations ranked by toxicity in descending order.

Percent Pore Water	Station No.	Idorg	% Norm. Develop.	sd	Sample Design‡	Toxicity
50	95011.0	1395	0.39	0.04	R	**
50	85012.0	1423	0.43	0.16	R	**
50	95013.0	1397	0.62	0.54	R	ns
50	85013.0	1424	0.70	0.09	D	*
50	95003.0	1382	0.76	0.05	D	*
50	95018.0	1417	0.84	0.04	R	*
50	85015.0	1426	0.87	0.10	D	ns
50	95024.0	1435	0.90	0.08	R	ns
50	95006.0	1385	0.92	0.01	R	ns
50	95021.0	1432	0.93	0.01	D	*
50	95007.0	1386	0.93	0.08	R	ns
50	95014.0	1413	0.95	0.01	R	*
50	95017.0	1416	0.96	0.01	D	ns
50	95016.0	1415	0.96	0.02	R	ns
50	95019.0	1430	0.96	0.03	R	ns
50	95020.0	1431	0.96	0.01	D	ns
50	95022.0	1433	0.97	0.02	R	ns
50	85016.0	1427	0.97	0.01	D	ns
50		DC	0.98	0.01		
50		BC	0.95	0.06		
50		DC	0.92	0.02		
50		BC	0.86	0.07		
25	85001.0	1387	0.00	0.00	R	**
25	85007.0	1418	0.00	0.00	R	**
25	85008.0	1419	0.00	0.00	R	**
25	95015.0	1414	0.00	0.00	R	**
25	85003.0	1389	0.02	0.03	R	**
25	85018.0	1429	0.02	0.00	D	**
25	85011.0	1422	0.03	0.04	R	**
25	85005.0	1391	0.22	0.37	R	**
25	85006.0	1392	0.23	0.21	R	**
25	85012.0	1423	0.23	0.04	R	**
25	95023.0	1434	0.29	0.05	R	**
25	85004.0	1390	0.34	0.31	R	ns
25	85010.0	1421	0.50	0.47	R	ns

Table 11 (cont.). Toxicity of pore water to sea urchin embryo development. Stations ranked by toxicity in descending order.

Percent Pore Water	Station No.	Idorg	% Norm. Develop.	sd	Sample Design‡	Toxicity
25	85009.0	1420	0.51	0.15	R	**
25	95002.0	1381	0.51	0.41	R	ns
25	95010.0	1394	0.56	0.04	R	**
25	85002.0	1388	0.58	0.48	R	ns
25	95005.0	1384	0.58	0.34	D	ns
25	85014.0	1425	0.62	0.21	D	**
25	95008.0	1393	0.70	0.23	R	ns
25	95025.0	1436	0.71	0.14	R	**
25	95003.0	1382	0.77	0.17	D	ns
25	95001.0	1380	0.78	0.27	R	ns
25	85017.0	1428	0.80	0.06	D	*
25	95013.0	1397	0.81	0.19	R	ns
25	95011.0	1395	0.83	0.05	R	ns
25	85013.0	1424	0.86	0.15	D	ns
25	95004.0	1383	0.86	0.05	R	ns
25	95026.0	1412	0.87	0.09	D	*
25	95012.0	1396	0.91	0.01	D	ns
25	95014.0	1413	0.92	0.11	R	ns
25	95006.0	1385	0.93	0.02	R	ns
25	95017.0	1416	0.94	0.02	D	*
25	95007.0	1386	0.94	0.03	R	ns
25	95020.0	1431	0.95	0.02	R	*
25	95019.0	1430	0.95	0.02	R	ns
25	85015.0	1426	0.95	0.03	D	ns
25	95021.0	1432	0.95	0.03	D	ns
25	95016.0	1415	0.96	0.03	R	ns
25	95022.0	1433	0.97	0.02	D	ns
25	95018.0	1417	0.97	0.01	R	ns
25	85016.0	1427	0.97	0.00	D	ns
25	95024.0	1435	0.98	0.02	R	ns
25		DC	0.98	0.01		
25		BC	0.96	0.01		
25		DC	0.92	0.02		
25		BC	0.91	0.02		

** indicates highly significant toxicity using separate variance t test and survival < 80% of control. * indicates toxicity using t-test only, ns = not significant using t-test. ‡R=random sample; D= Directed sample.
DC = Dilution Water (Sea Water) Control; BC = Brine Control

Table 12. Toxicity of pore water to sea urchin fertilization test.
Stations ranked by toxicity in descending order.

% Pore Water	Station	Idorg	Prop fert	sd	Sample Design‡	Toxicity
100	95003.0	1382	0.00	0.00	D	**
100	95006.0	1385	0.00	0.00	R	**
100	95010.0	1394	0.00	0.00	R	**
100	95011.0	1395	0.00	0.00	R	**
100	95012.0	1396	0.00	0.00	D	**
100	85007.0	1418	0.00	0.00	R	**
100	85008.0	1419	0.00	0.00	R	**
100	85009.0	1420	0.00	0.00	R	**
100	95023.0	1434	0.00	0.00	R	**
100	95024.0	1435	0.00	0.00	R	**
100	95025.0	1436	0.00	0.00	R	**
100	95016.0	1415	0.01	0.01	R	**
100	85018.0	1429	0.29	0.15	D	**
100	95007.0	1386	0.32	0.11	R	**
100	85001.0	1387	0.47	0.12	R	**
100	95013.0	1397	0.51	0.04	R	**
100	95014.0	1413	0.61	0.08	R	**
100	95021.0	1432	0.61	0.10	D	**
100	95022.0	1433	0.65	0.05	D	**
100	95019.0	1430	0.66	0.04	R	**
100	95017.0	1416	0.67	0.07	D	**
100	95001.0	1380	0.68	0.10	R	**
100	85010.0	1421	0.72	0.05	R	**
100	95026.0	1412	0.74	0.11	D	*
100	95020.0	1431	0.78	0.03	R	ns
100	95005.0	1384	0.79	0.06	D	*
100	85012.0	1423	0.86	0.06	R	ns
100	85016.0	1427	0.86	0.04	D	ns
100	85003.0	1389	0.91	0.02	R	ns
100	85004.0	1390	0.92	0.02	R	ns
100	85015.0	1426	0.92	0.04	D	ns
100	85002.0	1388	0.93	0.03	R	ns
100	95002.0	1381	0.93	0.04	R	ns
100	85013.0	1424	0.93	0.05	D	ns
100	95004.0	1383	0.94	0.03	R	ns
100	85006.0	1392	0.94	0.00	R	ns
100	95008.0	1393	0.95	0.02	R	ns
100	95018.0	1417	0.95	0.01	R	ns
100	85011.0	1422	0.95	0.03	R	ns

Table 12 (cont.). Toxicity of pore water to sea urchin fertilization test.
Stations ranked by toxicity in descending order.

% Pore Water	Station	Idorg	Prop fert	sd	Sample Design‡	EMAP TOX.*
100	95015.0	1414	0.96	0.02	R	ns
100	85017.0	1428	0.96	0.01	D	ns
100	85005.0	1391	0.96	0.03	R	ns
100	85014.0	1425	0.96	0.02	D	ns
100	BC		0.97	0.02		
100	DC		0.91	0.08		
100	BC		0.77	0.02		
100	DC		0.92	0.02		

** indicates highly significant toxicity using separate variance t test and survival < 80% of control. * indicates toxicity using t-test only, ns = not significant using t-test. ‡R=random sample; D= Directed sample.
DC = Dilution Water (Sea Water) Control; BC = Brine Control

For the amphipod (*Rhepoxynius abronius*), 18 out of the 30 randomly selected samples were highly toxic (60%) and nearly half of the toxic sites were in Newport Bay. Three of the four most toxic sites were in Los Peñasquitos Lagoon with survival ranging between 23 and 42%. Survival at Newport Bay Station No. 85001 was also among the lowest recorded at 29%. The magnitude of toxic response to *Rhepoxynius* in the 14 remaining toxic samples indicated moderate toxicity relative to the range of toxic response previously reported for this species from samples tested nationwide (see Swartz 1994; Table 8). The distributions of samples toxic to *Rhepoxynius* are presented in **Figures 12-15**. For the amphipod *Ampelsica abdita,* 15 of the 25 samples tested with this species were selected using the random design. Only 1 of the 15 randomly collected samples tested with this species was significantly toxic; this station was in Newport Bay (**85008, Figures 16-19**).

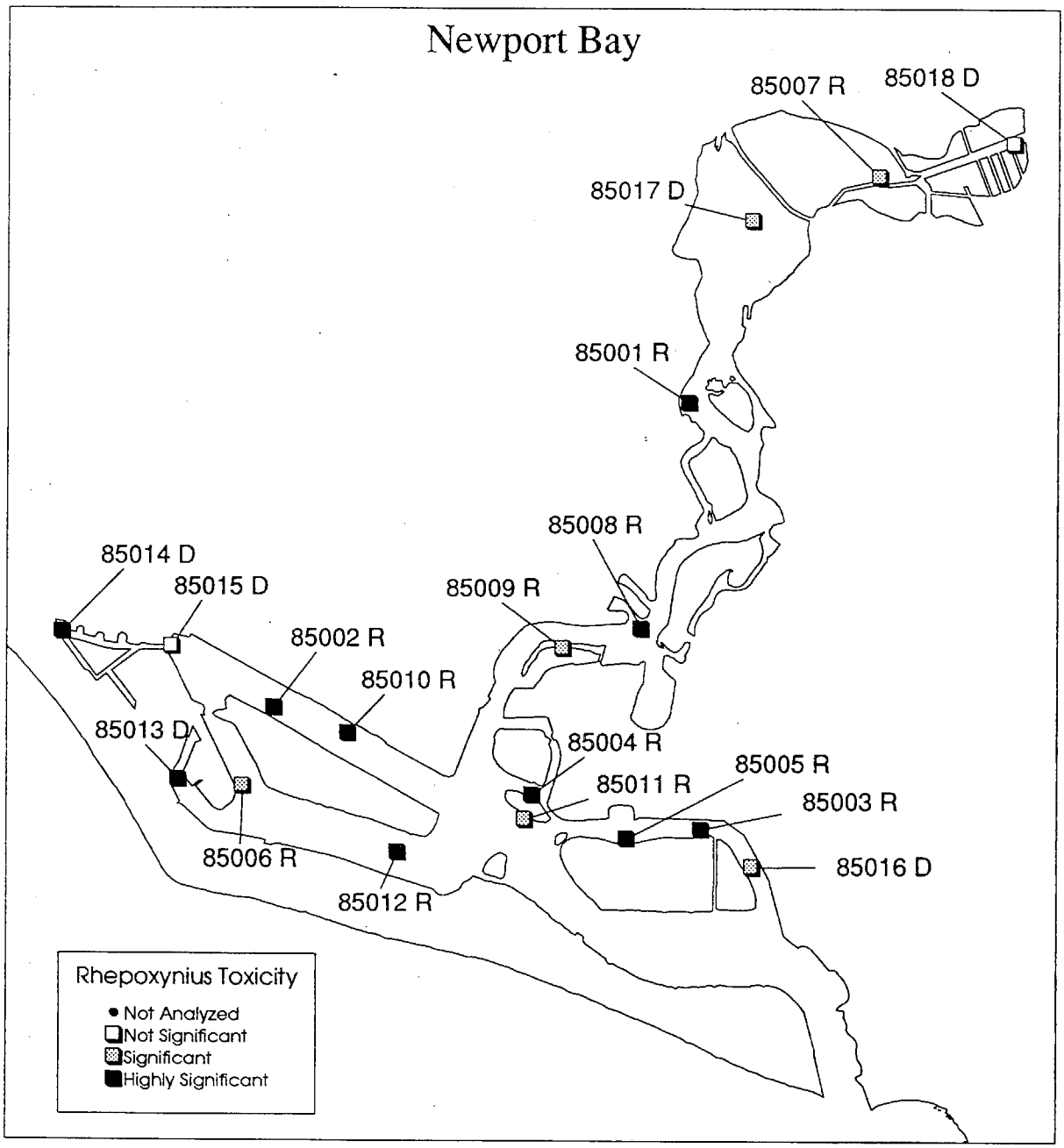


Figure 12. Distribution of sediment samples in Newport Bay significantly toxic to amphipods (*Rhepoxynius abronius*).

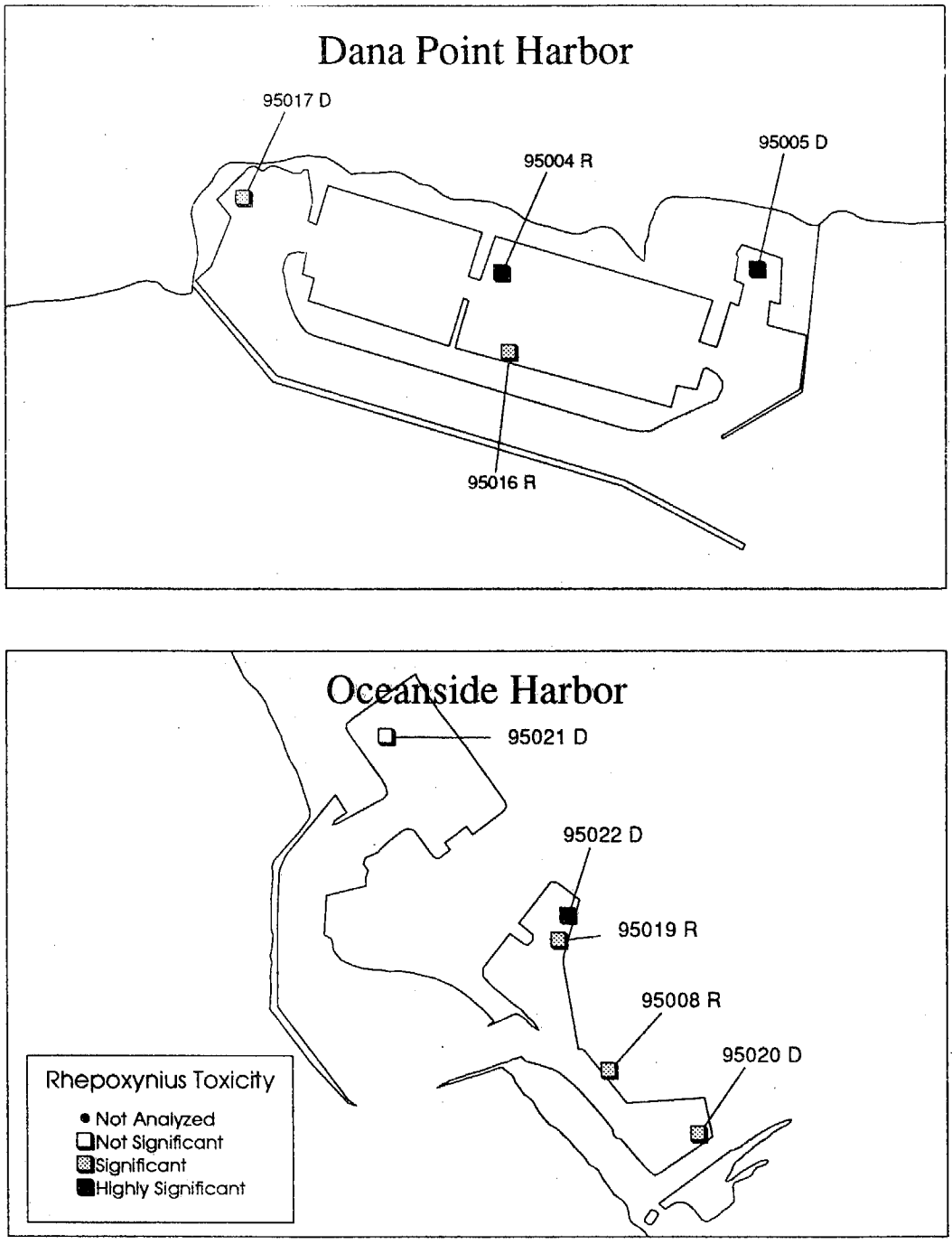


Figure 13. Distribution of sediment samples in Dana Point and Oceanside Harbors significantly toxic to amphipods (*Rhepoxynius abronius*).

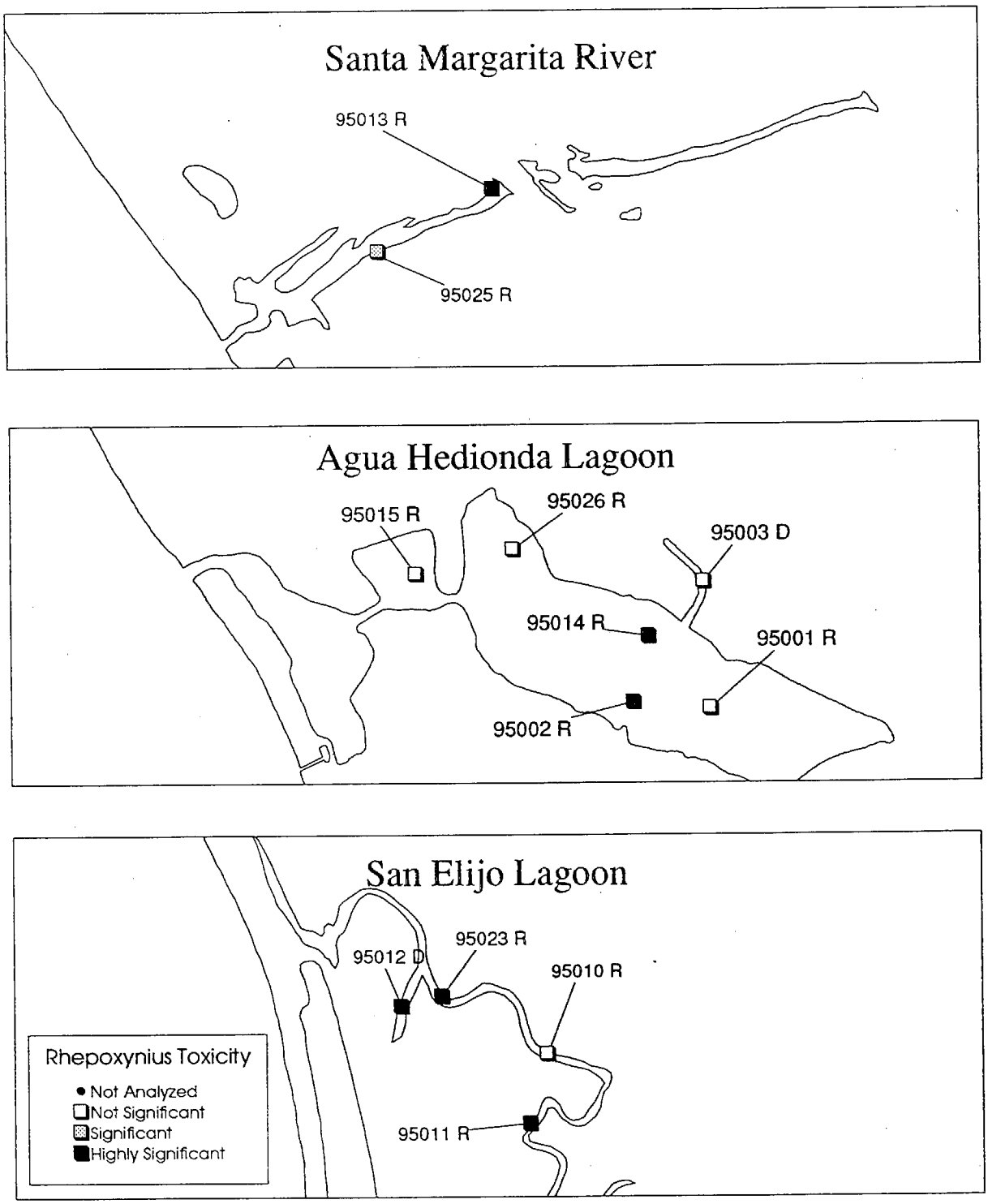


Figure 14. Distribution of sediment samples in Santa Margarita, Agua Hedionda, and San Elijo Lagoons significantly toxic to amphipods (*Rhepoxynius abronius*).

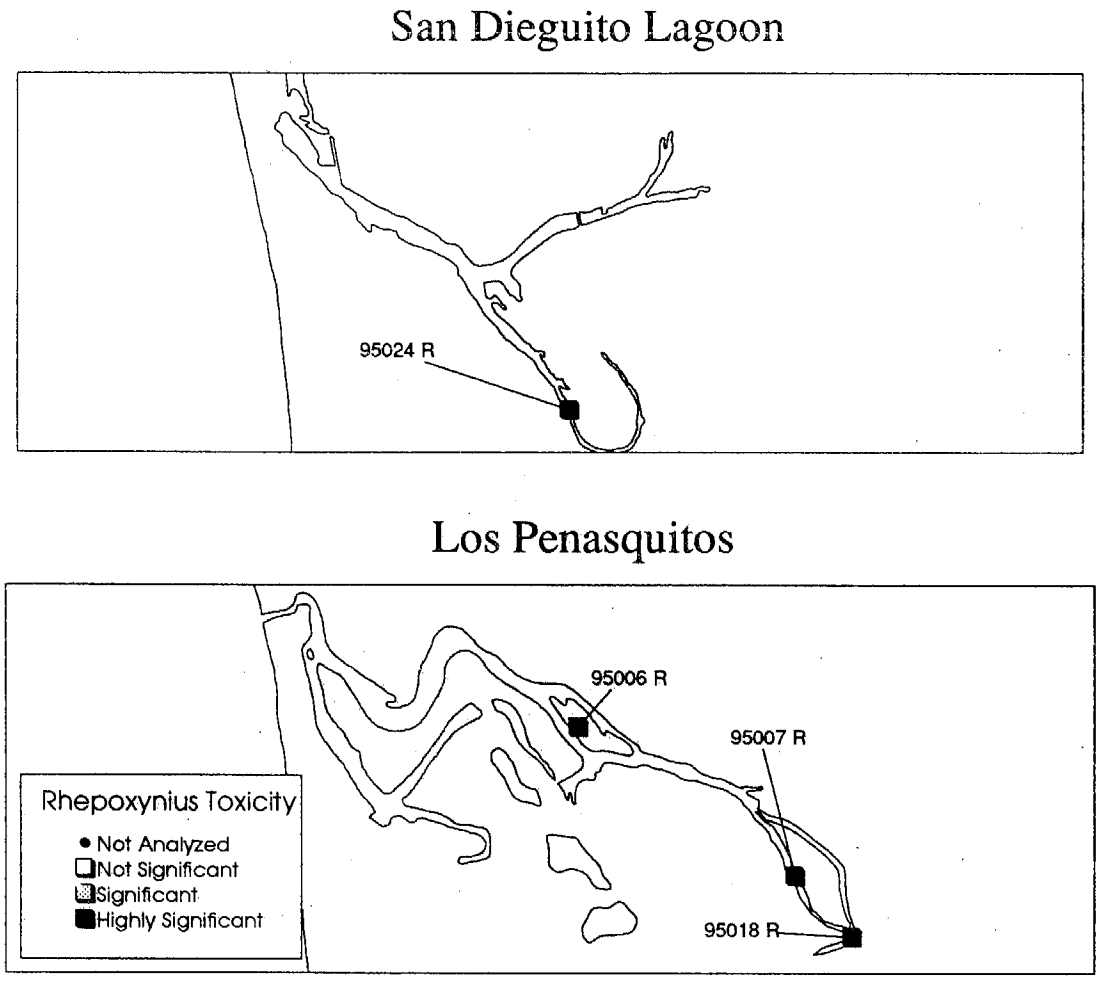


Figure 15. Distribution of sediment samples in San Dieguito and Los Peñasquitos Lagoons significantly toxic to amphipods (*Rhepoxynius abronius*).

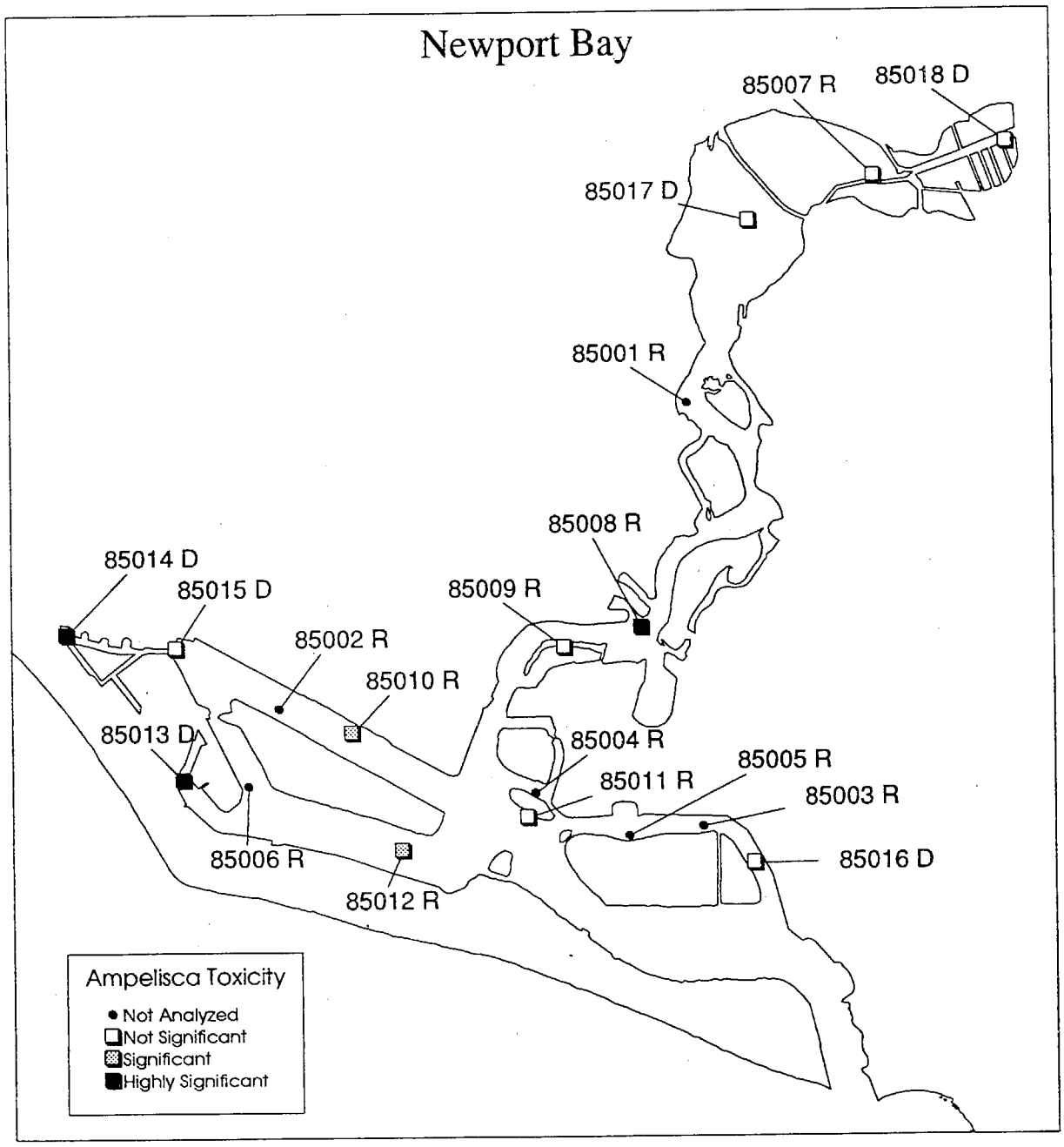


Figure 16. Distribution of sediment samples in Newport Bay significantly toxic to amphipods (*Ampelisca abdita*).

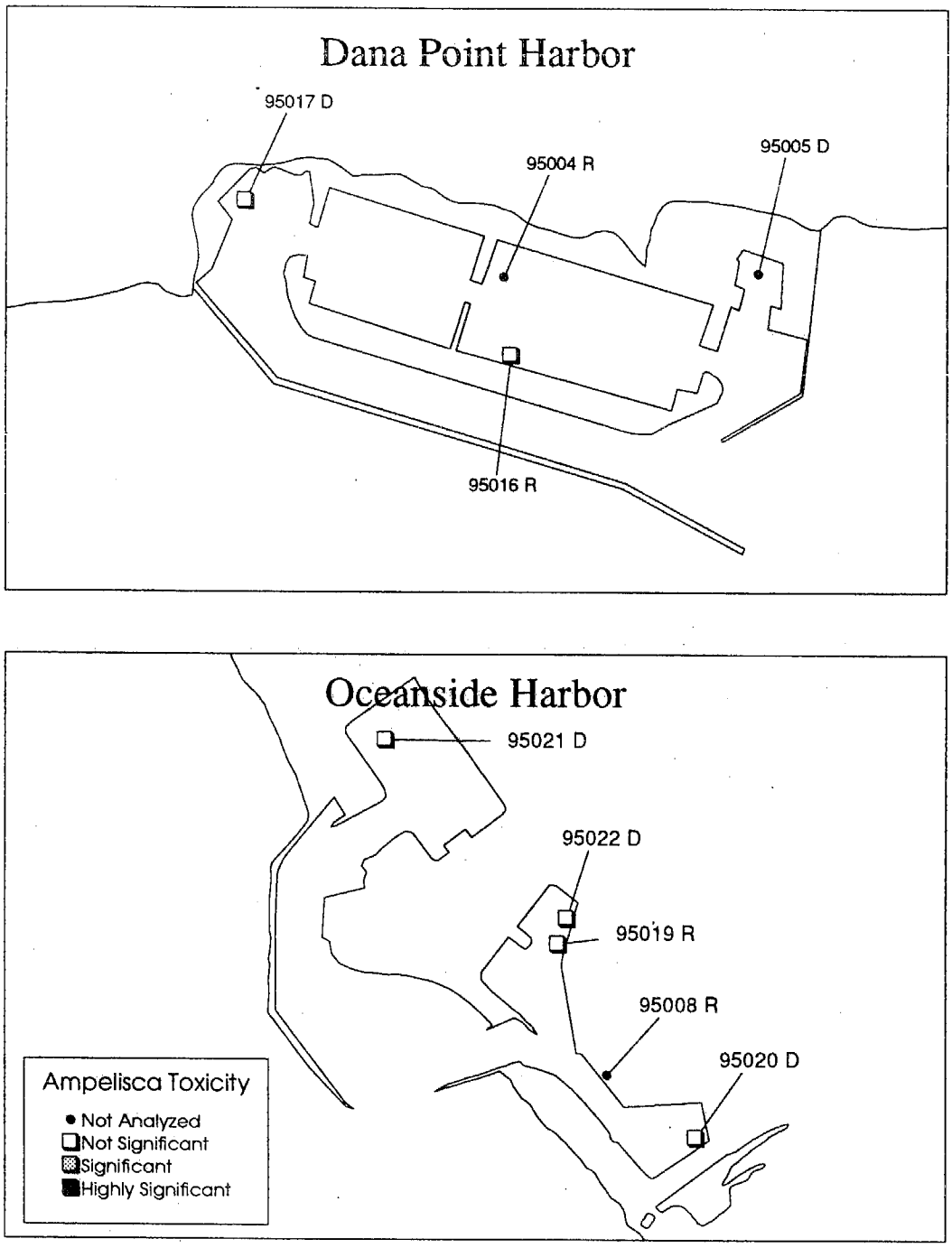


Figure 17. Distribution of sediment samples in Dana Point and Oceanside Harbors significantly toxic to amphipods (*Ampelisca abdita.*)

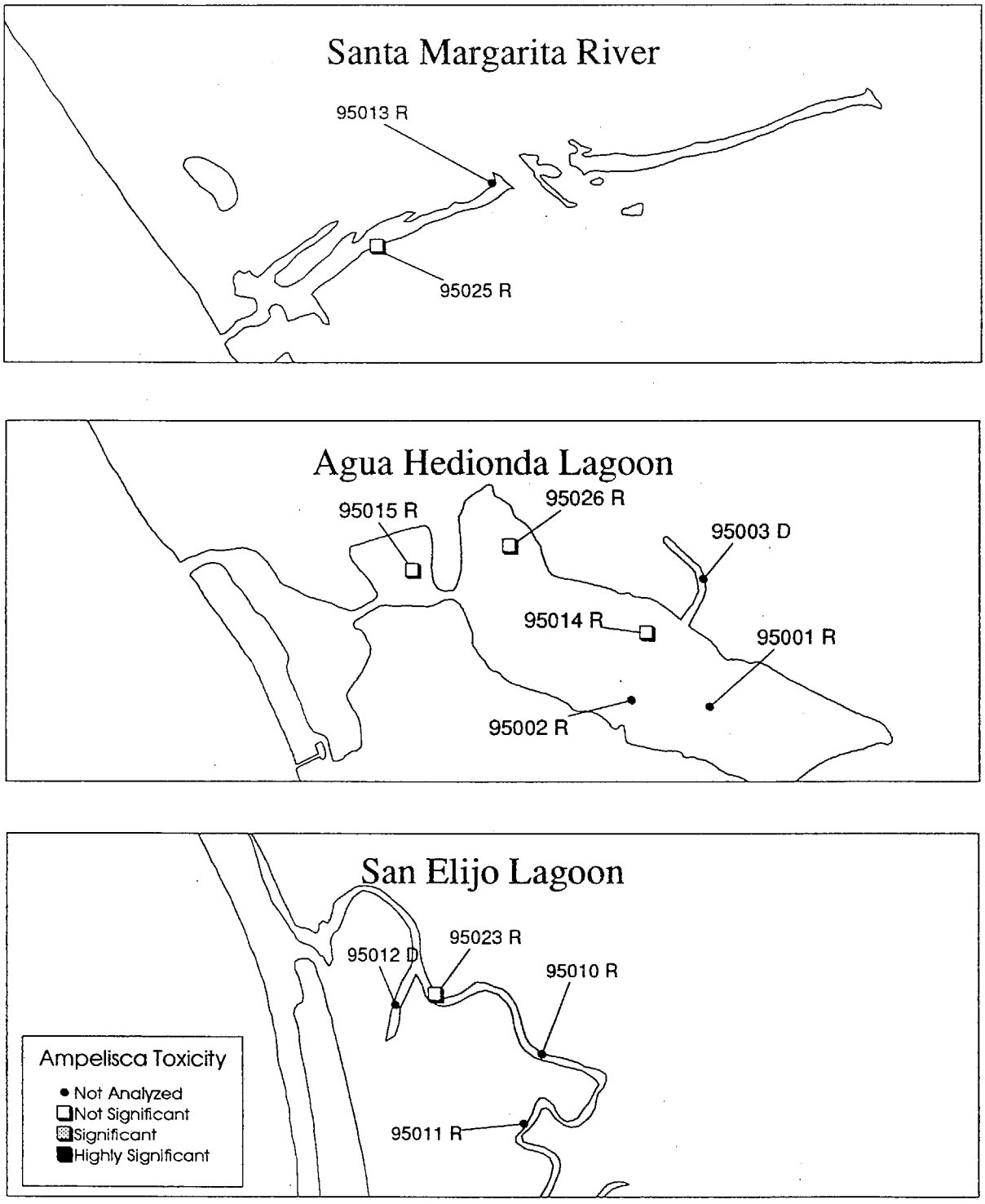


Figure 18. Distribution of sediment samples in Santa Margarita, Agua Hedionda, and San Elijo Lagoons significantly toxic to amphipods (*Ampelisca abdita.*)

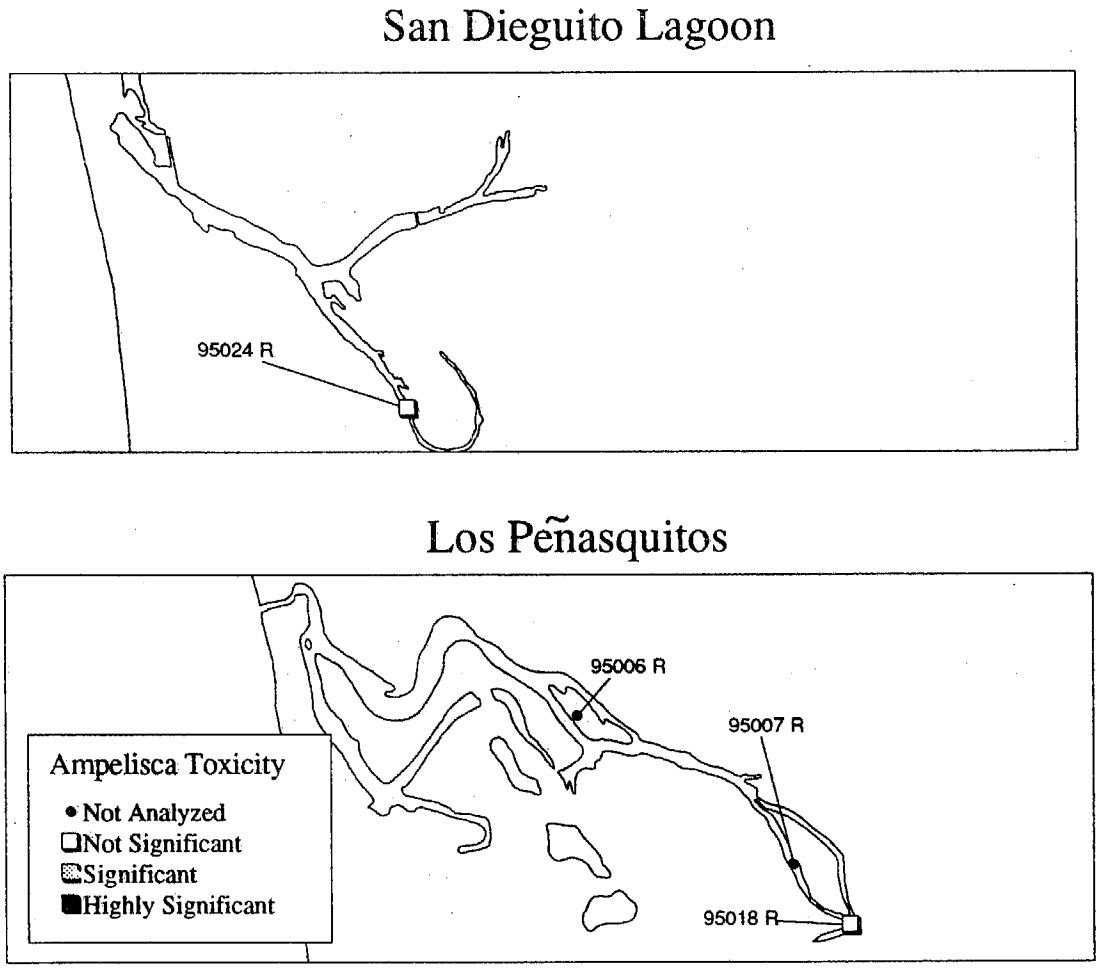


Figure 19. Distribution of sediment samples in San Dieguito and Los Peñasquitos Lagoons significantly toxic to amphipods (*Ampelisca abdita.*).

Considerably more toxicity was detected with the sea urchin development tests. Using 100% pore water, 26 of 30 randomly selected stations were highly toxic to sea urchin development (87% of the samples). Toxicity was reduced at lower dilutions of pore water. Using 50% pore water, 21 of the 30 random samples were highly toxic (70% of the samples); using 25% pore water the number of highly toxic samples was reduced to 13 (26% of the samples). The distribution of samples toxic to sea urchin development are presented in **Figures 20-23**.

The sea urchin fertilization test detected less toxicity than the sea urchin development test. Using 100% pore water (the only concentration tested), 17 of the 30 random stations were highly toxic to sea urchin sperm (57% of the samples). The distribution of samples toxic to sea urchin fertilization are presented in **Figures 24-27**.

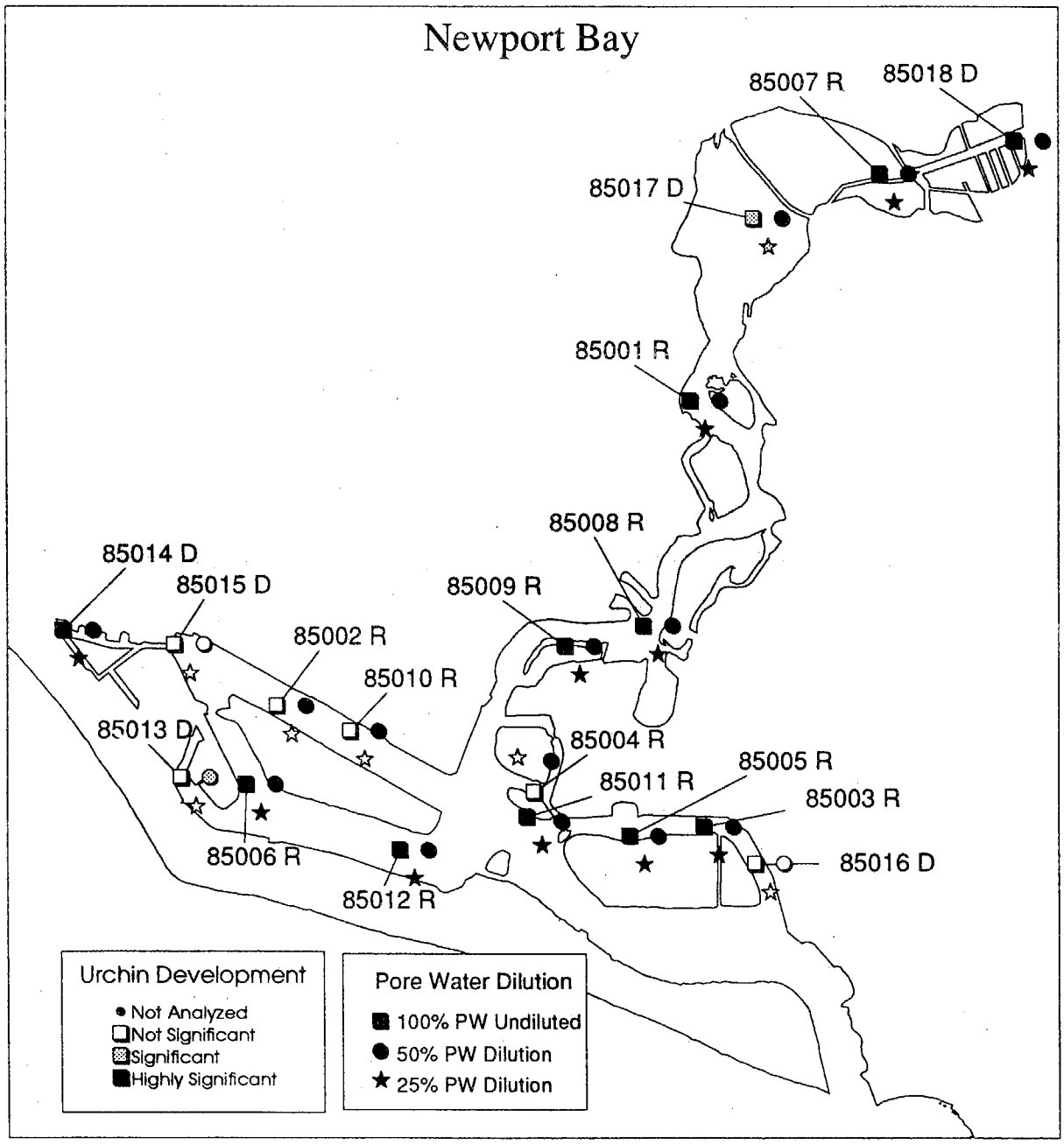


Figure 20. Distribution of sediment interstitial water samples in Newport Bay significantly toxic to sea urchin embryo development (*Strongylocentrotus purpuratus*).

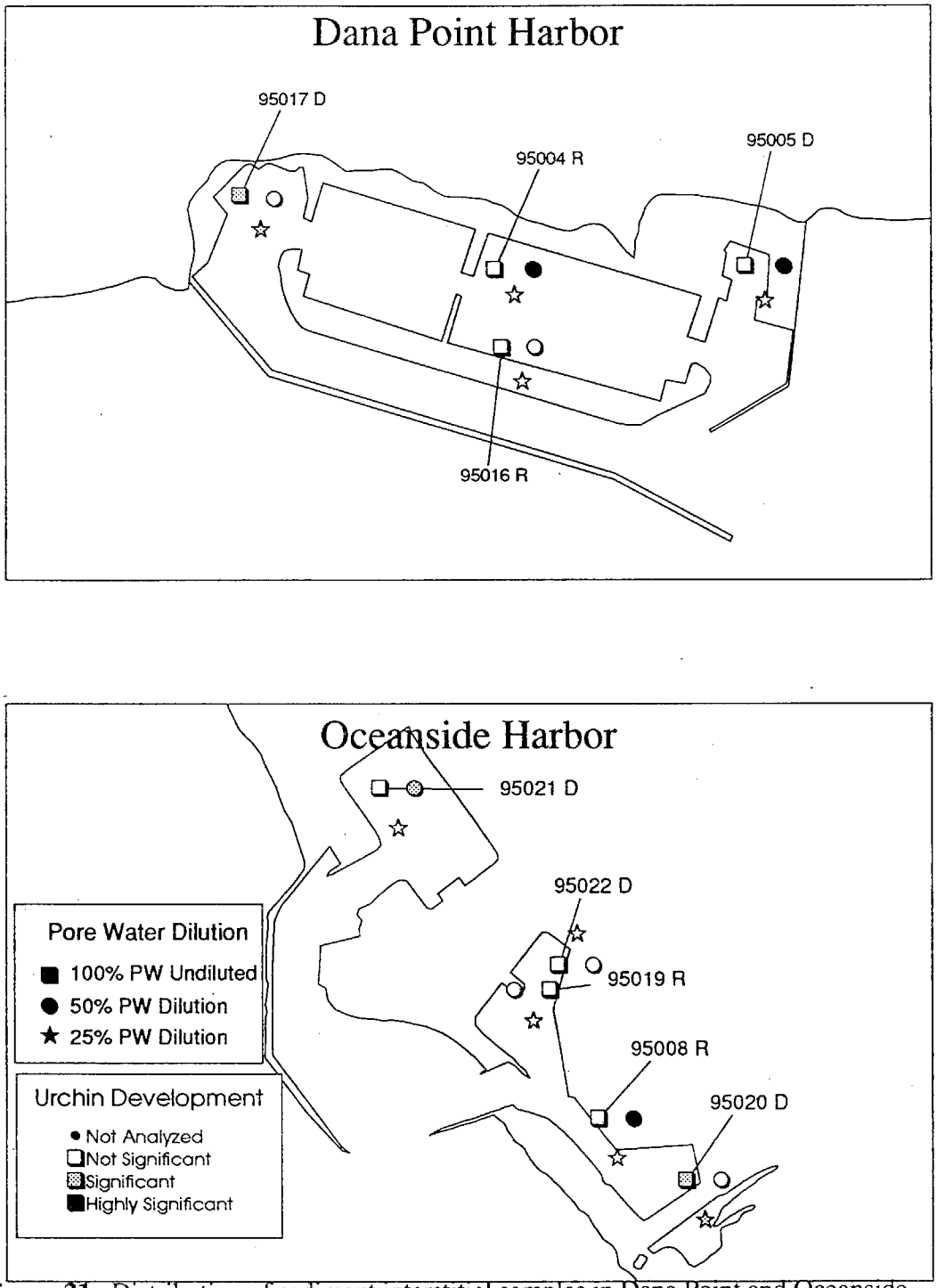


Figure 21. Distribution of sediment interstitial samples in Dana Point and Oceanside Harbors significantly toxic to sea urchin embryo development (*Strongylocentrotus purpuratus*).

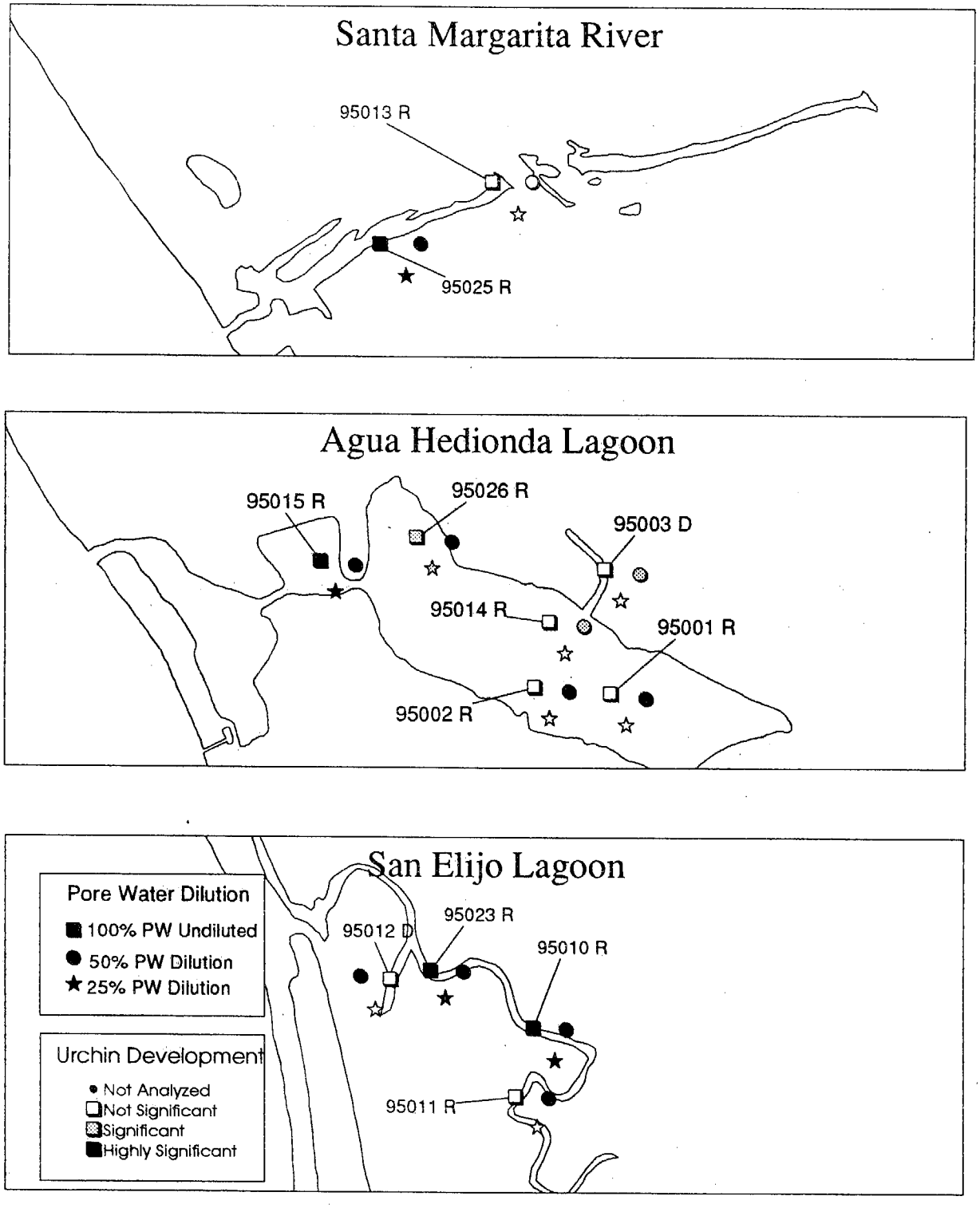
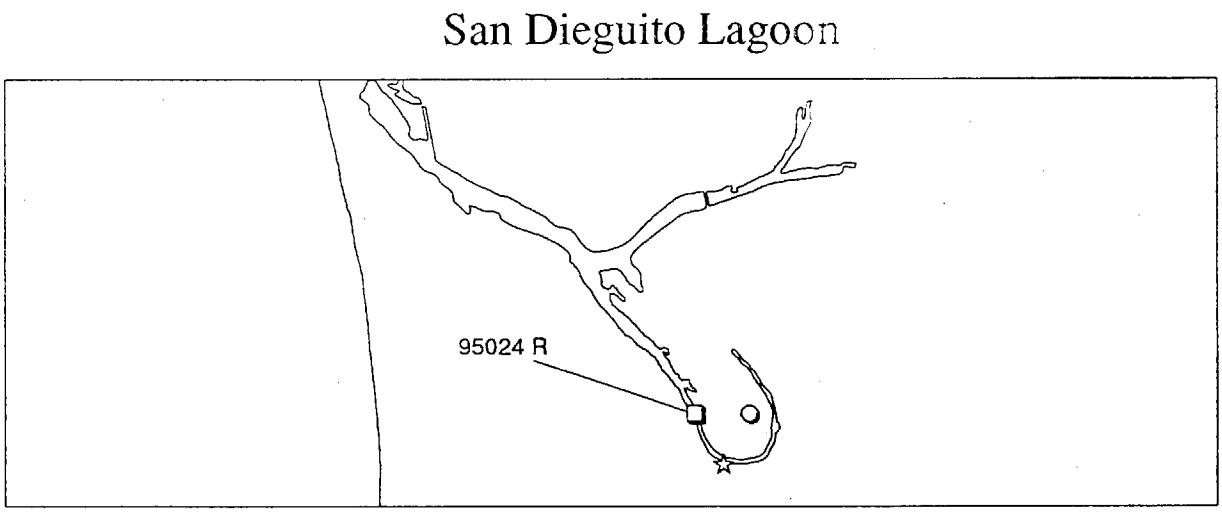


Figure 22. Distribution of sediment interstitial water samples in Santa Margarita, Agua Hedionda, and San Elijo Lagoons significantly toxic to sea urchin embryo development (*Strongylocentrotus purpuratus*).



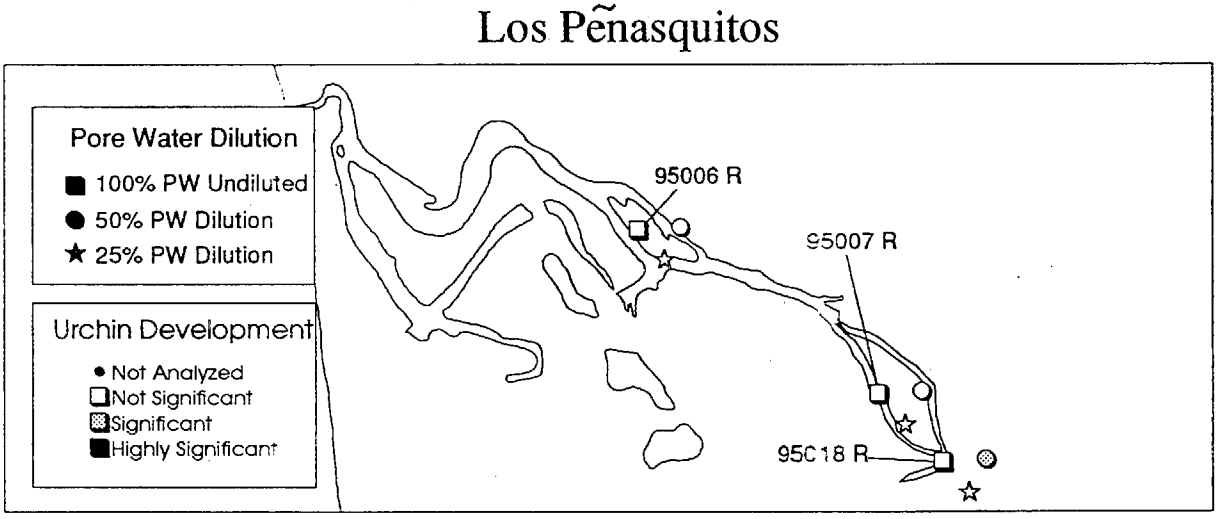


Figure 23. Distribution of sediment interstitial water samples in San Dieguito and Los Peñasquitos Lagoons significantly toxic to sea urchin embryo development (*Strongylocentrotus purpuratus*).

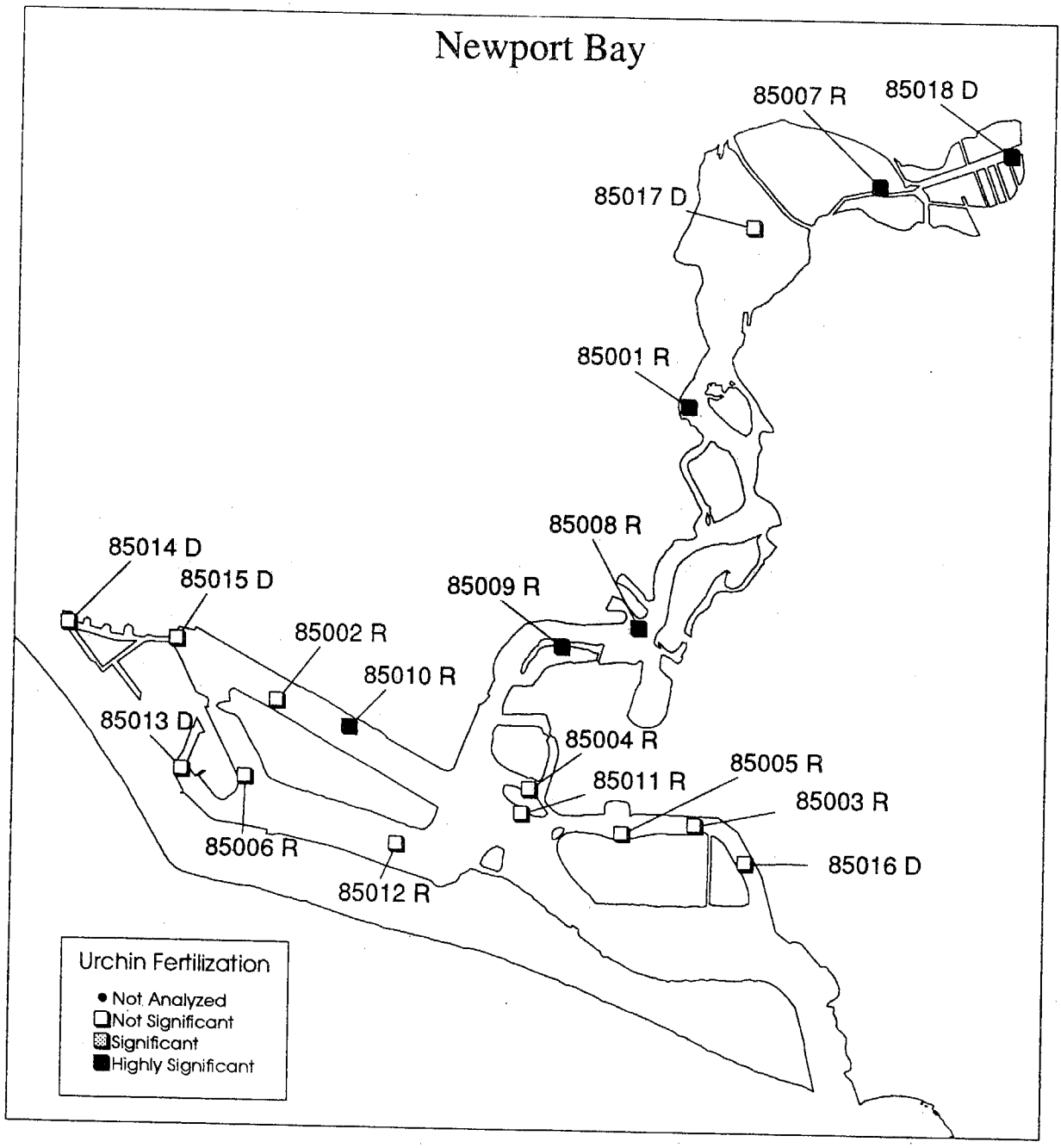


Figure 24. Distribution of sediment interstitial water samples in Newport Bay significantly toxic to sea urchin fertilization (*Strongylocentrotus purpuratus*).

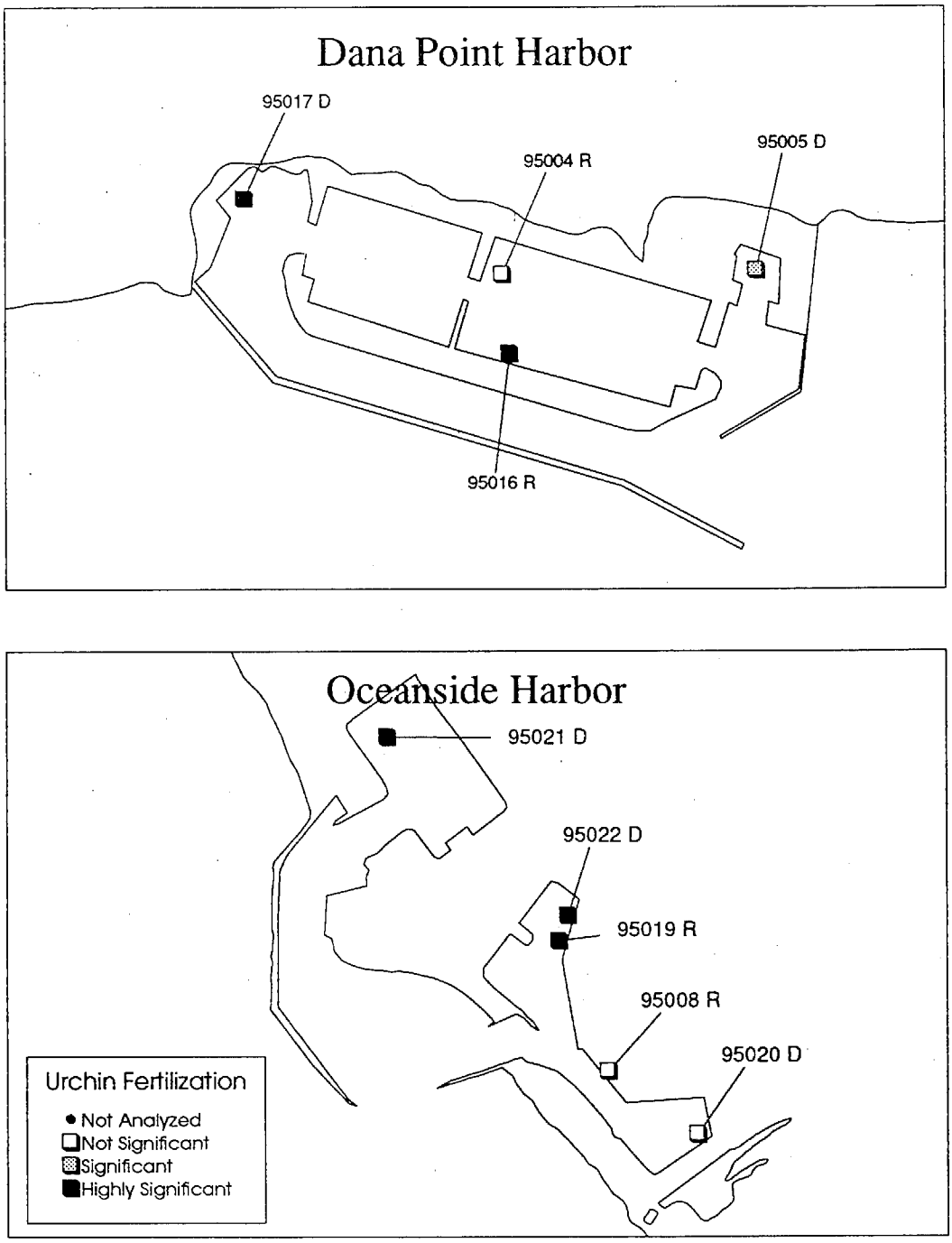


Figure 25. Distribution of sediment interstitial samples in Dana Point and Oceanside Harbors significantly toxic to sea urchin fertilization (*Strongylocentrotus purpuratus*).

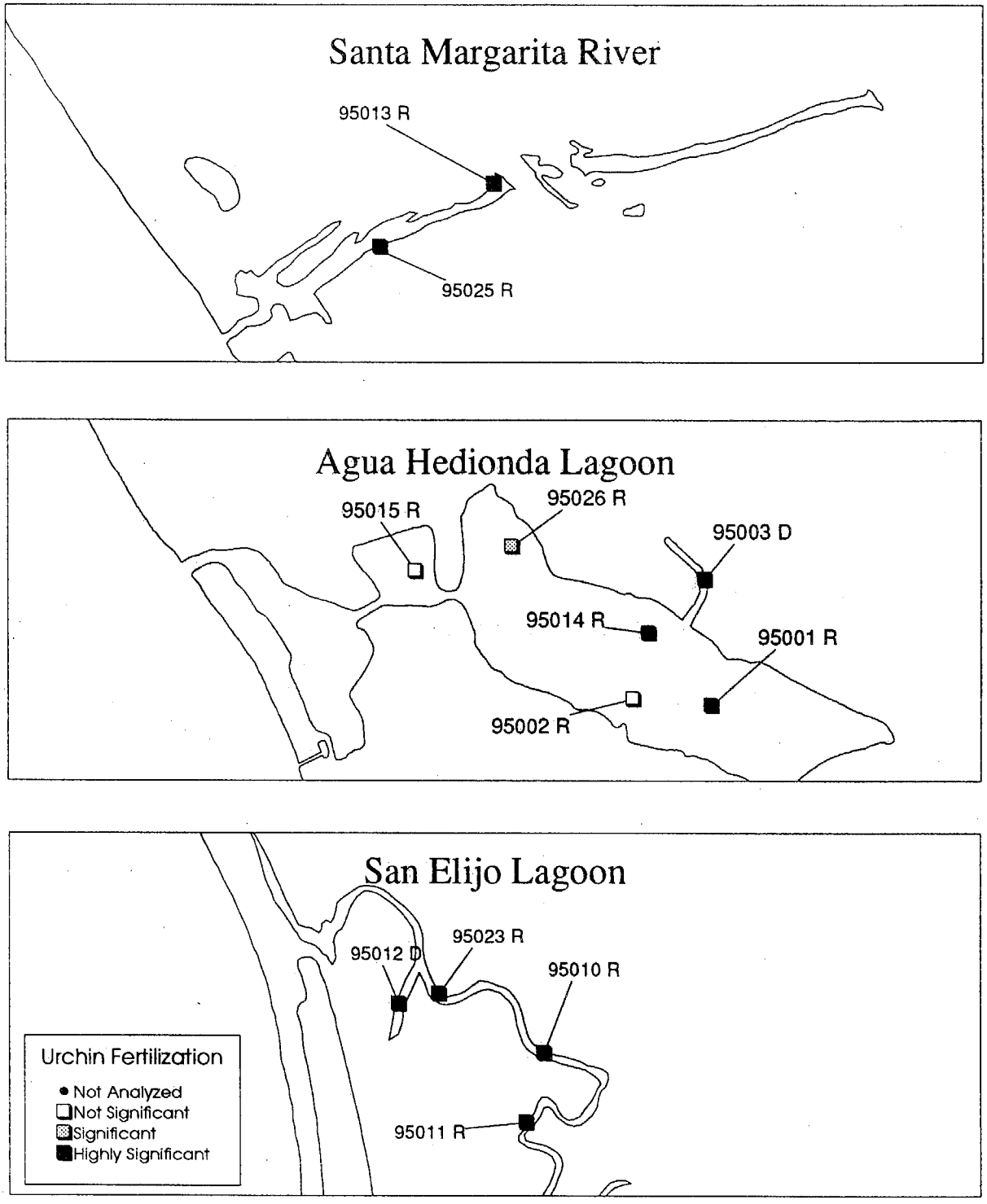


Figure 26. Distribution of sediment interstitial water samples in Santa Margarita, Agua Hedionda, and San Elijo Lagoons significantly toxic to sea urchin fertilization (*Strongylocentrotus purpuratus*).

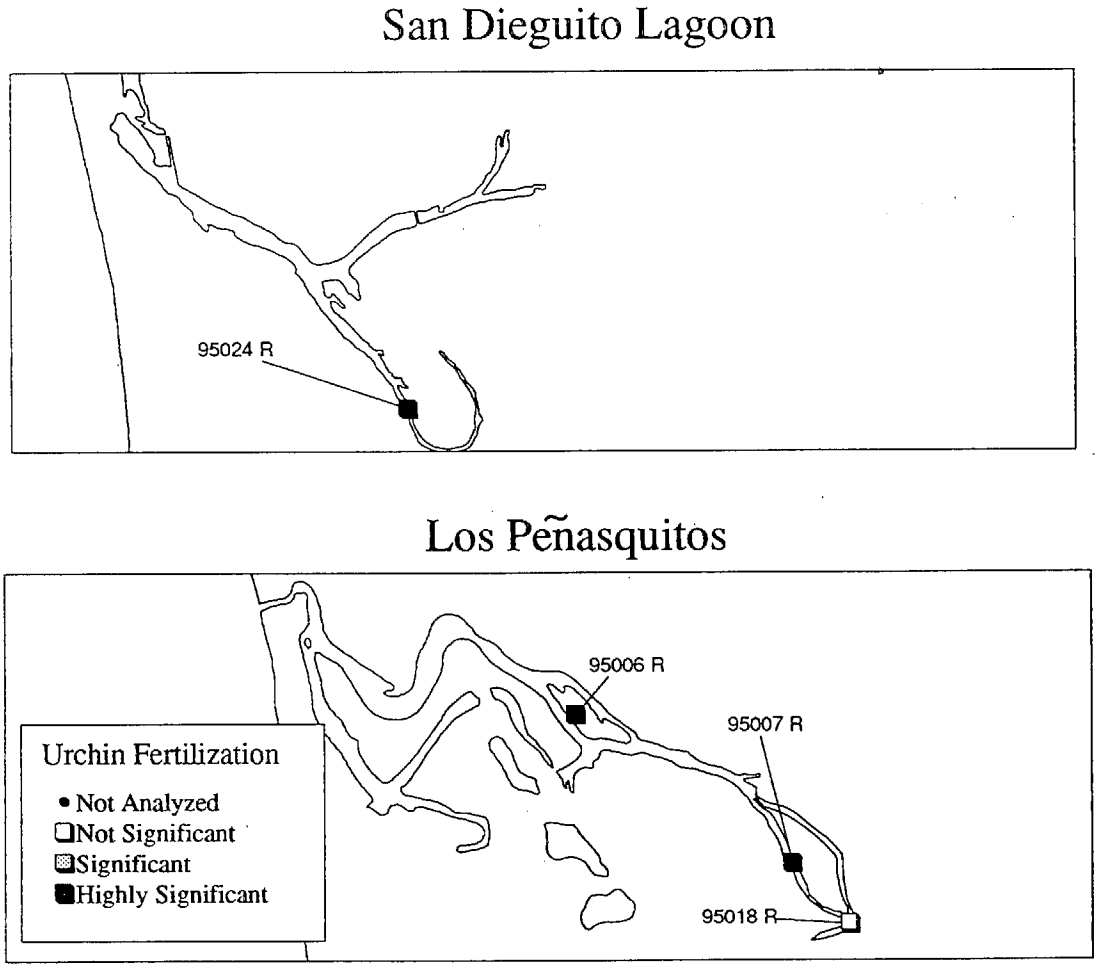


Figure 27. Distribution of sediment interstitial water samples in San Dieguito and Los Peñasquitos Lagoons significantly toxic to sea urchin fertilization (*Strongylocentrotus purpuratus*).

Spatial Extent of Toxicity

The spatial extent of toxicity was determined based on Cumulative Distribution Functions (CDFs) using the toxicity criteria of statistical significance using a t-test and response less than 80% of the control value. CDFs were calculated for the 30 random samples tested with each protocol (15 only for *Ampelisca*). The results show that 58% of the area sampled was significantly toxic to the amphipod *Rhepoxynius abronius* using these criteria for toxicity (**Table 13**). Results for the amphipod *Ampelsica abdita* showed that 11% of the area tested with this species was significantly toxic. Results using the sea urchin (*Strongylocentrotus*) development test showed considerably greater toxicity. At 100, 50, and 25% pore water concentrations, the percent area significantly toxic to sea urchin development was 91, 83, and 51%, respectively. The sea urchin fertilization protocol was less sensitive. Using 100% pore water, 43% of the area sampled was significantly toxic to sea urchin sperm (**Table 13**).

Table 13. Spatial extent of toxicity in Southern California bays and estuaries.
Total area sampled = 5.01 km sq.

Toxicity Test Protocol	N*	Percent Area Toxic‡	95% CI
Rhepoxynius abronius survival	30	57.9%	19.0%
Ampelisca abdita survival	15	10.7%	NC
Strongylocentrotus purpuratus development (100% PW)	30	90.5%	5.8%
Strongylocentrotus purpuratus development (50% PW)	30	83.3%	8.6%
Strongylocentrotus purpuratus development (25% PW)	30	51.3%	16.4%
Strongylocentrotus purpuratus fertilization (100% PW)	30	42.7%	19.7%

* Number of random samples.
‡ Percent Area Toxic based on Cumulative Distribution Function of toxicity at "n" random stations.
NC = not calculatable because only one random station was toxic.

Toxicity Relative to the Reference Envelope

After screening the chemistry and benthic community data for all 43 random and directed samples, 6 stations were selected as reference stations based on the criteria described previously (**Table 14a**). These were stations where benthic community structure was considered to be undisturbed (the criteria used are described in a later section) and where chemical contamination was considered to be minimal based on comparisons with the ERM and PEL guidelines.

At 5 of the 6 stations, DDT and its metabolites (particularly tDDT and p,p'-DDE) exceeded the ERM and/or PEL for these compounds. Long et al. (1995) had less confidence in the ERMs for total DDT and p,p'-DDE because they found the incidence of associated biological effects did not increase consistently with increasing concentrations of these compounds. This was due, in part, because the ERM values may have been overly influenced by relatively low equilibrium-partitioning values. These are based upon chronic marine water quality criteria intended to protect against bioaccumulation in marine fish and birds, not acute toxicity to benthic organisms. MacDonald (1994) used a variety of field and laboratory bioeffects data, including DDT-spiked sediment bioassay data using *Rhepoxynius,* to develop Sediment Effects Concentrations (SECs) for four groups of DDT (ΣDDT, ΣDDE, and ΣDDD and tDDT). These are expressed on a bulk sediment basis and normalized to TOC (MacDonald 1994, Table 16). Because these values include spiked sediment data with *Rhepoxynius*, as well as sea urchin fertilization data using DDT contaminated field sediment, we feel they are more applicable to acute sediment bioassay results. We evaluated concentrations of each of these DDT groups at the 43 EMAP stations sampled, including the 6 proposed reference stations. DDT concentrations at the 6 reference stations were all considerably lower than the SECs proposed by MacDonald (1994). Based on this and the low confidence these authors had in the ERM and PEL guidelines for DDT compounds, we consider chemical contamination at these stations to be sufficiently low to justify their inclusion in the reference population for the Southern California bays and estuaries.

It should be noted, however, that the 6 reference stations in **Table 14a** had a number of substances which exceeded the ERL/TEL guidelines. For example most exceeded the TEL guideline for copper, and zinc. In addition, several exceeded the TEL for total PCBs. Of these 6 reference stations, Agua Hedionda (Station No. 95015) was the least contaminated relative to the TEL guidelines; this station had TEL exceedances for Nickel, Chromium, and Total DDT only.

The stations selected as reference sediments for the P450 RGS assay were two samples from Agua Hedionda Lagoon, two from Los Penasquitos Lagoon, and one from the Santa Margarita River.

The range of B(a)P equivalents for these five stations was 1.7 to 2.5, and based on data from several previous sediment surveys (Anderson 1995a,b; 1996) these levels are well below response values that would be associated with any adverse biological effects from the PAHs or PCBs which induce this test system.

Using toxicity data for the 6 reference stations, a reference envelope toxicity threshold was calculated for each protocol using statistical methods described above. Because histogram plots indicated skewed distributions for all toxicity data, all data were arc-sine transformed prior to analysis to normalize the distributions. The results can be used to indicate the most toxic stations for each protocol (**Table 14b**). At the p value of 1%, the toxicity threshold for the amphipod *Rhepoxynius* was < 32.8% survival. Three stations were less than this threshold for *Rhepoxynius*; two from Los Peñasquitos Lagoon and one from Newport Bay (**Table 9**). At the p value of 1%, the toxicity threshold for the sea urchin fertilization test was 48.9% fertilized; 15 samples were less than this value (**Table 12**). Because of relatively high toxicity and considerable variability in response at the 6 reference stations, a reference envelope threshold could not be calculated for the sea urchin development data (**Table 14a and b**). There were an insufficient number of samples to calculate a reference envelope for the *Ampelisca* data.

Table 14a. Southern California bay and estuarine stations used to develop reference envelope.

Station No.	Station Name	IDORG No.	Benthic Index	Toxicity Results *Rhepoxynius* % Survival	*Ampelisca* % Survival	*Strongylocentrotus* % Fert.‡	*Strongylocentrotus* % Norm.Dev.*
85003	Newp. Bay 791	1389	0.80	72	nc	91	2
85004	Newp. Bay 877	1390	0.86	70	nc	92	34
85005	Newp. Bay 949	1391	0.70	63	nc	96	22
95015	Agua. Hed. Lag. 212	1414	0.81	95	86	96	0
85010	Newp. Bay 819	1421	0.80	74	76	72	50
85016	Newp. Bay Yacht.s Cove	1427	0.85	85	89	86	97
Mean			0.80	76.5	83.7	88.8	34.2
S.D.			0.06	11.5	6.8	9.0	36.2

nc = not conducted at this site; *25% pore water data; ‡100% pore water data

Table 14b. Reference envelope toxicity thresholds and number of toxic samples for each protocol at two values of "p" (see text for details).

	Rhepoxynius % Survival	*Ampelisca* % Survival	*Strongylocentrotus* % Fert.‡	*Strongylocentrotus* % Norm. Dev.*
Toxicity threshold at p = 1	32.8	not calc.°	48.9	not calc.°
No. of stations less than p = 1 threshold	3		15	-
Toxicity threshold at p = 10	51.5	not calc.°	66.8	not calc.
No. of stations less than p = 10 threshold	5		20	-

°Reliable toxicity thresholds could not be calculated for Ampelisca because of small sample size; reliable toxicity thresholds could not be calculated for sea urchin development because of high variability.

Using the less conservative p value of 10%, the toxicity threshold for Rhepoxynius was 51.5% survival (**Table 14b**). Five samples were less than this threshold (**Table 9**). Using the p value of 10%, the toxicity threshold for the sea urchin fertilization test was 66.8% fertilized; 20 samples were less than this value (**Table 12**).

The reference envelope toxicity thresholds determined for the Southern California bays and estuaries were lower than those developed for San Diego Bay and San Francisco Bay. Based on 11 reference site samples in San Diego Bay, the toxicity threshold for *Rhepoxynius* at a p value of 1% was 48% survival in San Diego Bay; at a p value of 10%, the toxicity threshold for *Rhepoxynius* was 63% survival (Fairey et al. 1996). Based on 33 reference site samples from San Francisco Bay, toxicity thresholds of 57% and 68% survival at p values of 1% and 10%, respectively, were determined for the amphipod *Eohaustorius estuarius* (SFRWQCB in review). Using sea urchin development data from these same 33 samples in San Francisco Bay, toxicity thresholds of 93% and 97% normal development were calculated for p values of 1% and 10%, respectively. The reference envelope toxicity thresholds for the different regions were clearly influenced by the number of stations included in the calculations, and variability in response of the test organisms.

Used in conjunction with comparisons to laboratory control values, the reference envelope approach has the potential to be a more appropriate method for assessing relative toxicity, particularly in moderately impacted areas, because it incorporates several sources of variability affecting test response. With the addition of more data from a variety of areas, resolution of reference from impacted conditions should improve. Several issues need to be addressed before this approach is implemented in a regulatory context. For example, it is not clear how many samples are necessary to accurately characterize the reference threshold for a given area. In addition, it is not certain whether reference conditions determined for one area can be applied to determining toxicity at other geographically isolated areas. Criteria such as level of chemical contamination, benthic community structure, and ammonia and hydrogen sulfide concentrations need to be further examined in the context of determining reference conditions. Finally, decision criteria regarding the appropriate p value for setting toxicity limits needs more consideration.

Correlations of the P450 RGS Assay with Chemical Contaminants

The RGS assay would be expected to respond to high molecular weight PAHs and the coplanar PCBs present at low concentrations (a few percent) in Aroclors. The findings demonstrated that

this screening test did identify sediments, which contained these contaminants. The RGS responses, in μg B(a)P equivalents / g, were highly correlated ($p = 0.001$) with the sum of high molecular weight PAHs, with total PAHs, Aroclor 1254, and Aroclor 1260. In addition, the RGS findings were also highly correlated ($p = 0.001$) with the ratios of these compounds to the PEL and the ERM values for low and high molecular weight PAHs and total PAHs.

Correlations of Toxicity with Chemical Contaminants

Statistical associations between solid phase and pore water toxicity and bulk phase chemical concentrations were determined using Spearman Rank Correlations to determine which chemicals may have co-varied with the measures of toxicity. Correlations between sediment chemistry and amphipod (*Rhepoxynius*) survival using all 43 sediment samples indicated weak negative correlations between survival and antimony and o'p DDE (**Table 15a**). Substances for which analyses were performed and not listed in **Table 15a** were not significantly correlated ($p > 0.05$). Because a majority of the contamination occurred in the more heavily urbanized marinas, the data for marinas was separated and correlations were conducted using the 27 samples from Newport, Dana Point, and Oceanside Harbors. For these samples significant correlations were detected for zinc, PCB52, un-ionized ammonia, and sediment grain size (**Table 15b**). None of the correlation coefficients improved when the data were analyzed using TOC-normalized bulk phase chemical concentrations.

Correlations between chemistry and amphipod (*Ampelisca abdita*) survival in the 25 samples tested with this species indicated more associations. Relatively weak correlations were determined for four metals (mercury, selenium, tin, and zinc), and several PCBs. Two PCBs (PCB44, and PCB1254) had stronger correlations with toxicity (**Table 16**). None of the correlation coefficients improved when the data were analyzed using TOC-normalized bulk phase chemical concentrations.

Table 15a. Spearman Rank Correlation Coefficients for selected toxicants significantly correlated with amphipod *Rhepoxynius abronius* survival. Data for all sample locations; n=43. * = sig. @ $p \leq 0.05$

Toxicant	Spearman rho
Antimony	-0.331 *
OP DDE	-0.312 *

Table 15b. Spearman Rank Correlation Coefficients for selected toxicants significantly correlated with amphipod *Rhepoxynius abronius* survival. Data for marinas: Newport Harbor, Dana Point Harbor, and Oceanside Harbor; n=27. * = sig. @ $p < 0.05$

Toxicant	Spearman rho
Zinc	-0.390 *
PCB52	-0.415 *
NH3	-0.410 *
Fines	-0.404 *

Table 16. Spearman Rank Correlation Coefficients for selected toxicants significantly correlated with amphipod *Ampelisca abdita* survival; n=25. * = sig. @ $p \leq 0.05$, ** = sig. @ $p \leq 0.01$

Toxicant	Spearman rho
Mercury	-0.436 *
Selenium	-0.465 *
Tin	-0.390 *
Zinc	-0.476 *
PCB28	-0.483 *
PCB44	-0.524 **
PCB66	-0.426 *
PCB101	-0.402 *
PCB105	-0.446 *
PCB118	-0.423 *
PCB128	-0.483 *
PCB138	-0.409 *
PCB153	-0.391 *
PCB195	-0.483 *
PCB206	-0.489 *
PCB209	-0.479 *
ARO 1254	-0.529 **
ARO 1260	-0.404 *
TTLPCB	-0.407 *

Toxicity to sea urchin development was significantly correlated with interstitial un-ionized ammonia concentration (**Figure 28**). Measurement of interstitial water ammonia indicated that 24 of the 43 sediment samples had un-ionized ammonia concentrations which exceeded the Lowest Observed Effect Concentration for sea urchin development (LOEC $\simeq$ 0.06 mg/l un-ionized ammonia; MPSL unpublished data). Ammonia was significantly correlated with abnormal sea urchin development (Spearman Rank rho = 0.560; sig @ alpha = 0.0001)). Correlations were conducted using the 25% pore water data to reduce the effect of ammonia toxicity in order to clarify analysis of the effects of other contaminants. At this concentration only 9 of the 43 samples had un-ionized ammonia concentrations which exceeded the NOEC ($\simeq$ 0.05 mg/l UNH_3). These correlations indicated that cadmium, silver, ammonia, two DDT metabolites and two chlordane compounds were significantly associated with abnormal larval development (**Table 17a**). When the 9 samples with high ammonia were eliminated from the analysis, cadmium, chlordane, three DDTs and PCB170 were found to be significantly correlated (**Table 17b**). There were only two significant correlations between reduced sea urchin fertilization and chemical contamination. Aluminum and un-ionized ammonia were weakly correlated with toxicity to sea urchin sperm (data not shown).

In addition to determinations of linear correlations between toxicity results and single chemical concentrations, the toxicity data were plotted against the ERM and PEL quotients discussed above to determine whether there was a threshold quotient value above which significant toxicity occurred. Three samples had PEL quotients above 1. Toxicity in these samples varied depending on the test used. All samples were significantly toxic to sea urchin development (in 100% pore water), none of the samples were significantly toxic to sea urchin fertilization, and 2 of these 3 samples were significantly toxic to amphipod survival (**Figure 29**). In a database compiled from studies performed nation wide, the incidence of highly significant toxicity in amphipod survival tests (*Rhepoxynius* and *Ampelisca*) was $\leq$ 33% in samples with average ERM quotients of $\leq$ 0.064, or average PEL quotients $\leq$ 0.25. The incidence of toxicity increased to $\geq$ 60% in samples with average ERM quotients of $\geq$ 1.0, or average PEL quotients $\geq$ 1.6 (E. Long, NOAA, unpublished data).

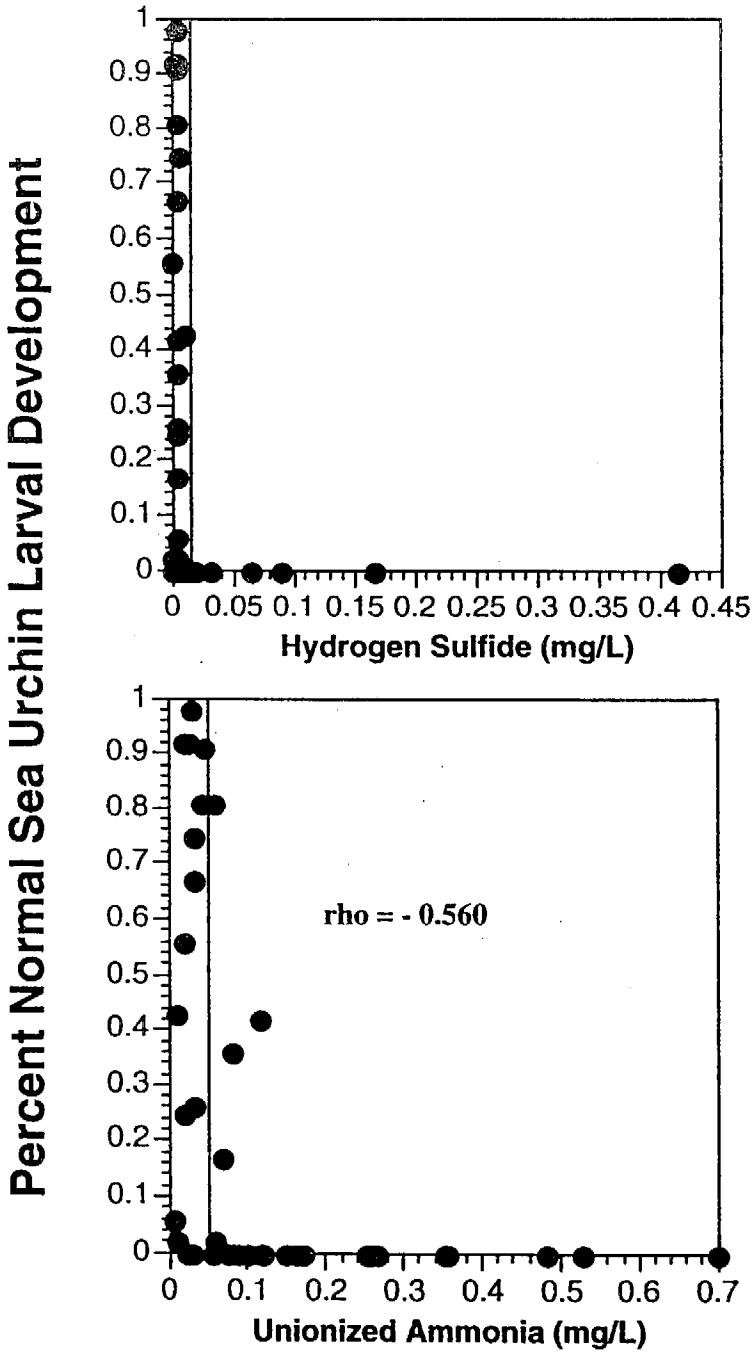


Figure 28. Relationship between sea urchin larvae development and interstitial water hydrogen sulfide and un-ionized ammonia concentrations in 43 EMAP samples. Vertical lines indicate Lowest Observed Effect Concentrations for H2S and NH3. rho = -.560 indicates significant negative correlation using Spearman Rank correlation.

Table 17a. Spearman Rank Correlation Coefficients for selected toxicants significantly correlated with sea urchin *Strongylocentrotus* embryo development in 25% pore water. Data for all samples; n=43. * sig @ 0.05; ** sig. @ 0.01

Toxicant	Spearman rho
Cadmium	-0.441 **
Silver	-0.424 **
Ammonia	-0.490 **
cis Chlordane	-0.354 *
pp DDE	-0.398 **
pp DDT	-0.486 **
t Nonachlor	-0.333 *

Table 17b. Spearman Rank Correlation Coefficients for selected toxicants significantly correlated with sea urchin *Strongylocentrotus* embryo development in 25% pore water. Data for samples with unionized ammonia less than 0.2 mg/l; n=34; * sig. @ 0.05, ** sig. @ 0.01.

Toxicant	Spearman rho
Cadmium	-0.401 *
cis Chlordane	-0.364 *
pp DDD	-0.365 *
pp DDE	-0.426 *
pp DDT	-0.454 **
PCB 170	-0.399 *

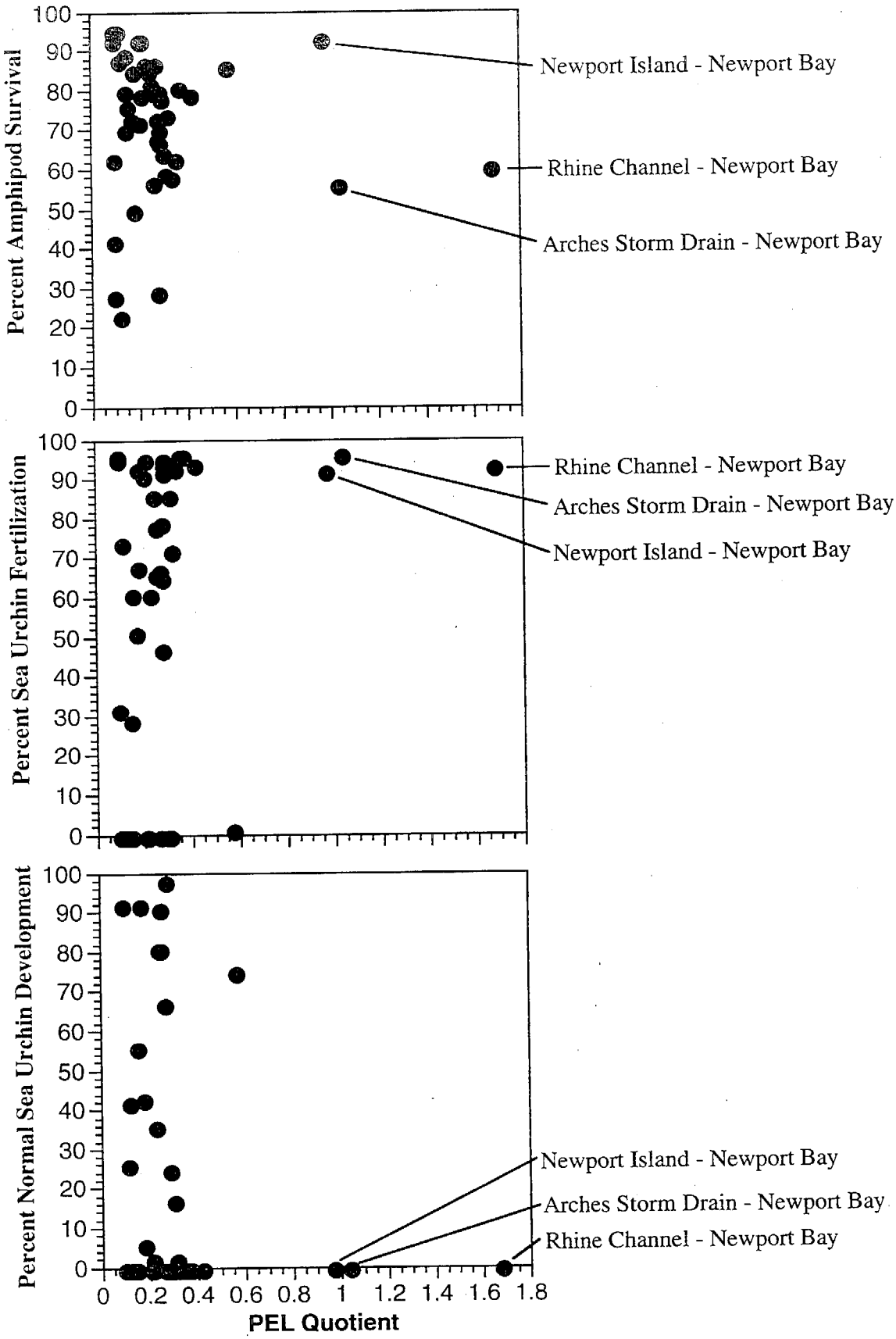


Figure 29. Toxicity response vs PEL quotient in 43 EMAP samples.

Given the relatively moderate level of contamination in these samples, the significance of these correlations is not clear. For example, SEM-AVS (simultaneously extracted metals-acid volatile sulfide) analysis was not conducted on these samples so it is impossible to determine whether molar concentrations of metals exceeded concentrations of AVS. Therefore it is difficult to determine whether associations between toxicity and metal concentrations are plausible.
The relatively large number of associations between chemistry and toxicity to the amphipod *Ampelisca* is suprising given the fact that so few samples were actually toxic to this species. In fact, 2 of the 3 samples toxic to *Ampelisca* (Station No. 85008 and Station No. 85013 in Newport Bay) had un-ionized ammonia concentrations which exceeded EPA's effect level for this species (**Appendix D**).

Based on known effect levels of un-ionized ammonia on sea urchin development, it is clear that ammonia played a major role in toxicity of the interstitial water to sea urchin embryos. Un-ionized ammonia is relatively non-toxic to sperm of the sea urchin *Strongylocentrotus purpuratus* (Bay et al. 1993) so the weak negative correlation between fertilization and un-ionized ammonia may be due to some covarying factor, such as hydrogen sulfide (**Figure 28**).

Comparison of the RGS Screening Test to Toxicity Tests

The RGS assay results from application of extracts of sediments to a human cell line exhibited a weak negative association with the percent survival of *Rhepoxynius abronius*. Much better correlations ($p = 0.001$) were observed between the RGS findings and effects of 100 % (Spearman Rank Correlation rho = - 0.66) and 50 % pore water on the development of sea urchin embryos (Spearman Rank Correlation rho = -0.63). There was no indication that the RGS responses correlated with the condition of the benthic community in this investigation, while in more contaminated sediments (eg., San Diego Bay) RGS responses of about 60 µg B(a)P equivalents/ g and higher were found to be related to impacts on benthic community structure (Fairey 1996).

Comparison of Toxicity Test Protocols

Interlaboratory Results

Results of the split sample interlaboratory comparison between the Marine Pollution Studies Laboratory (MPSL) and the Southern California Coastal Waters Research Project (SCCWRP) indicated consistent results in 5 of the 6 samples tested with amphipods *Ampelisca abdita* ; most of the samples were relatively non-toxic **(Figure 30)**. There was a large variation in magnitude of response in the sample from Station No. 85013 (Rhine Channel, Newport Bay), with much lower survival detected at MPSL. This sample had 1.24 mg/L un-ionized ammonia in the overlying water at the end of the MPSL test. EPA reports an "application limit" (NOEC) for un-ionized ammonia for *Ampelisca* of 0.8 mg/L. It is possible the un-ionized ammonia was higher in the *Ampelisca* test at MPSL because these samples were stored longer. The samples tested with *Ampelisca* were stored longer at MPSL because of the necessity of a re-test due to poor control in the initial test, as discussed earlier.

There were large differences between laboratories in response of sea urchin development in porewater from these samples (**Figure 31**). Except for Sample No. 95015, toxicity was generally greater in samples tested by SCCWRP. Total ammonia concentrations were considerably higher in the SCCWRP samples (**Table 18**). Un-ionized ammonia concentrations in the SCCWRP samples were elevated above the effect level at which urchin development is inhibited. The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) from ammonia-spiked toxicity tests at MPSL is approximately 0.05 and 0.06 mg/L, respectively; the EC50 for un-ionized ammonia is 0.07 mg/L (MPSL unpublished data). Two of the samples exceeded the LOEC at MPSL while 5 of 6 samples exceeded the LOEC at SCCWRP

(**Table 18**). It is possible that the longer sediment holding times prior to pore water extraction of the SCCWRP samples resulted in greater ammonia generation (S. Bay - SCCWRP, personal communication). This, in combination with initial pH adjustments at SCCWRP, resulted in higher concentrations of un-ionized ammonia and increased toxicity. At 50% pore water concentration, un-ionized ammonia in the MPSL samples were below concentrations likely to cause toxicity, indicating that toxicity in these samples was due to other factors.

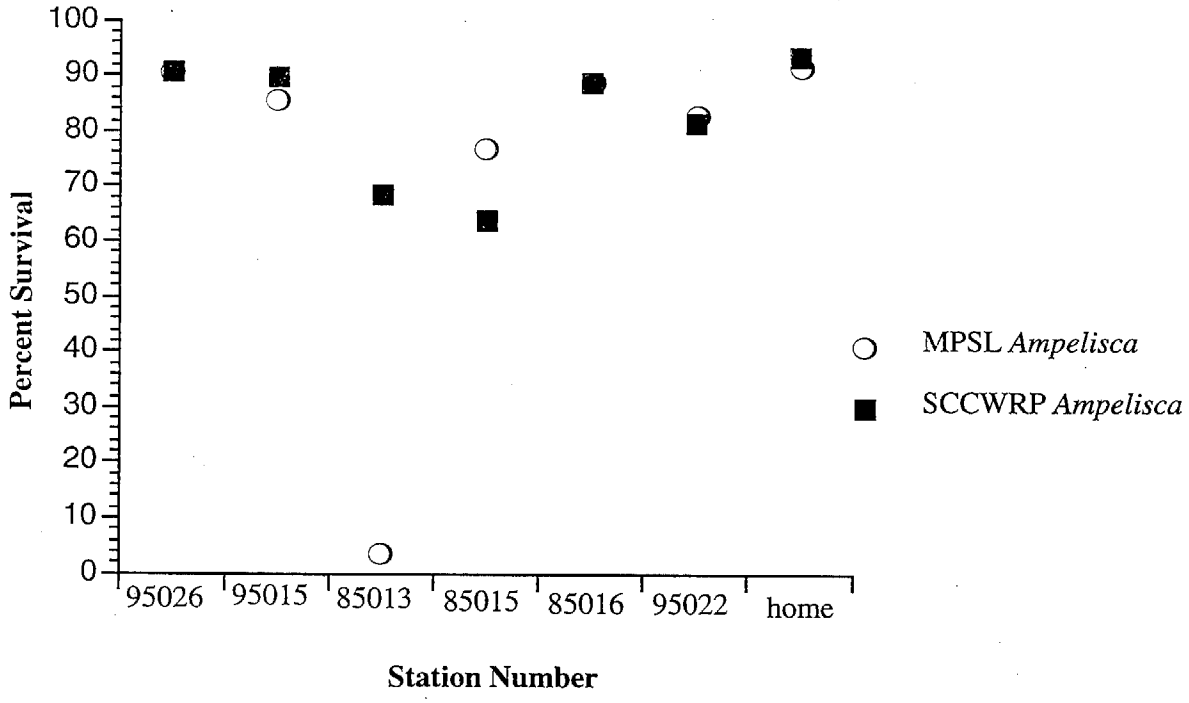


Figure 30. Results of interlaboratory comparison of amphipod survival between MPSL and SCCWRP

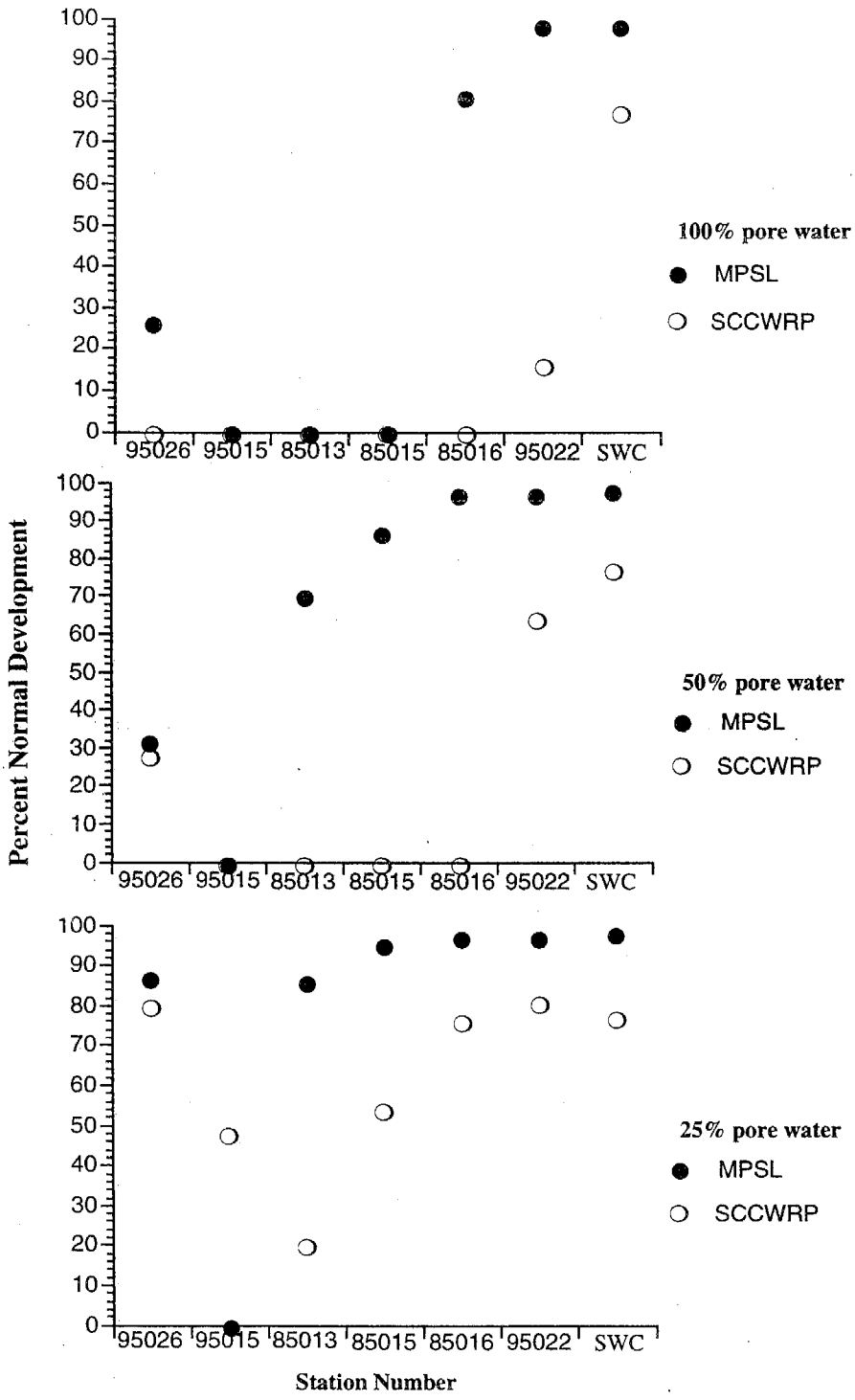


Figure 31. Results of interlaboratory comparison of sea urchin development in porewater between MPSL and SCCWRP. SWC = Sea Water Control.

Table 18. Ammonia concentrations in 100% pore water in interlaboratory test between MPSL and SCCWRP.

	ph		Total ammonia (mg/L)		Un-ionized ammonia* (mg/L)	
Station	MPSL	SCCWRP	MPSL	SCCWRP	MPSL	SCCWRP
85013	8.0	8.1	3.76	7.02	0.08	0.18
85015	8.1	8.2	4.36	5.44	0.12	0.20
85016	7.8	8.0	3.48	4.22	0.05	0.12
95015	7.9	8.1	3.55	5.70	0.06	0.18
95022	7.8	8.0	1.68	1.86	0.02	0.04
95026	7.9	8.0	2.42	3.60	0.04	0.10

* Un-ionized NH_3 NOEC for sea urchin development is 0.05 mg/L;
Un-ionized NH_3 EC50 for sea urchin development is 0.07 mg/L (MPSL unpublished data)

Comparison of Toxicity Results Using Two Amphipod Species

Comparisons between the two amphipod species *Rhepoxynius* and *Ampelisca* indicate that in terms of the number of stations toxic, a greater number of stations were toxic to *Rhepoxynius* (survival ≤ 80% of control value and statistically significant with a t-test; **Figure 32**). While 12% of the stations (3 of 25) were toxic to *Ampelisca,* 40% of the stations were toxic to *Rhepoxynius* (10 of 25). There was concordance between the two species on the presence or absence of toxicity at 18 of 25 stations (72%). At the three stations significantly toxic to both species (85008, 85013, 85014) the magnitude of toxic response was considerably higher for *Ampelisca.* Conversely, at several of the stations determined to be significantly toxic to *Rhepoxynius* but not *Ampelisca*, there were minimal differences in survival between the two species (eg., Stations 85010, 85012, 95014, 95022. 95023). As discussed earlier, it should be noted that un-ionized ammonia concentrations were elevated beyond EPA's application limit for this toxicant (EPA 1994) at 2 of the 3 stations which were significantly toxic to *Ampelisca* . Un-ionized ammonia was probably elevated in the samples re-tested with *Ampelisca* due to longer sediment holding times. These samples were re-tested due to inadequate control survival in the initial test.

Based on the correlations discussed above, possible sources of toxicity to the amphipod *Rhepoxynius* include o'p DDE, zinc, PCB52, un-ionized ammonia, and sediment grain size (**Table 15a-b**). Based on correlations, possible sources of toxicity to the amphipod *Ampelisca* include four metals (mercury, selenium, tin, and zinc), and several PCBs (**Table 16**). Limitations of the correlations are discussed above.

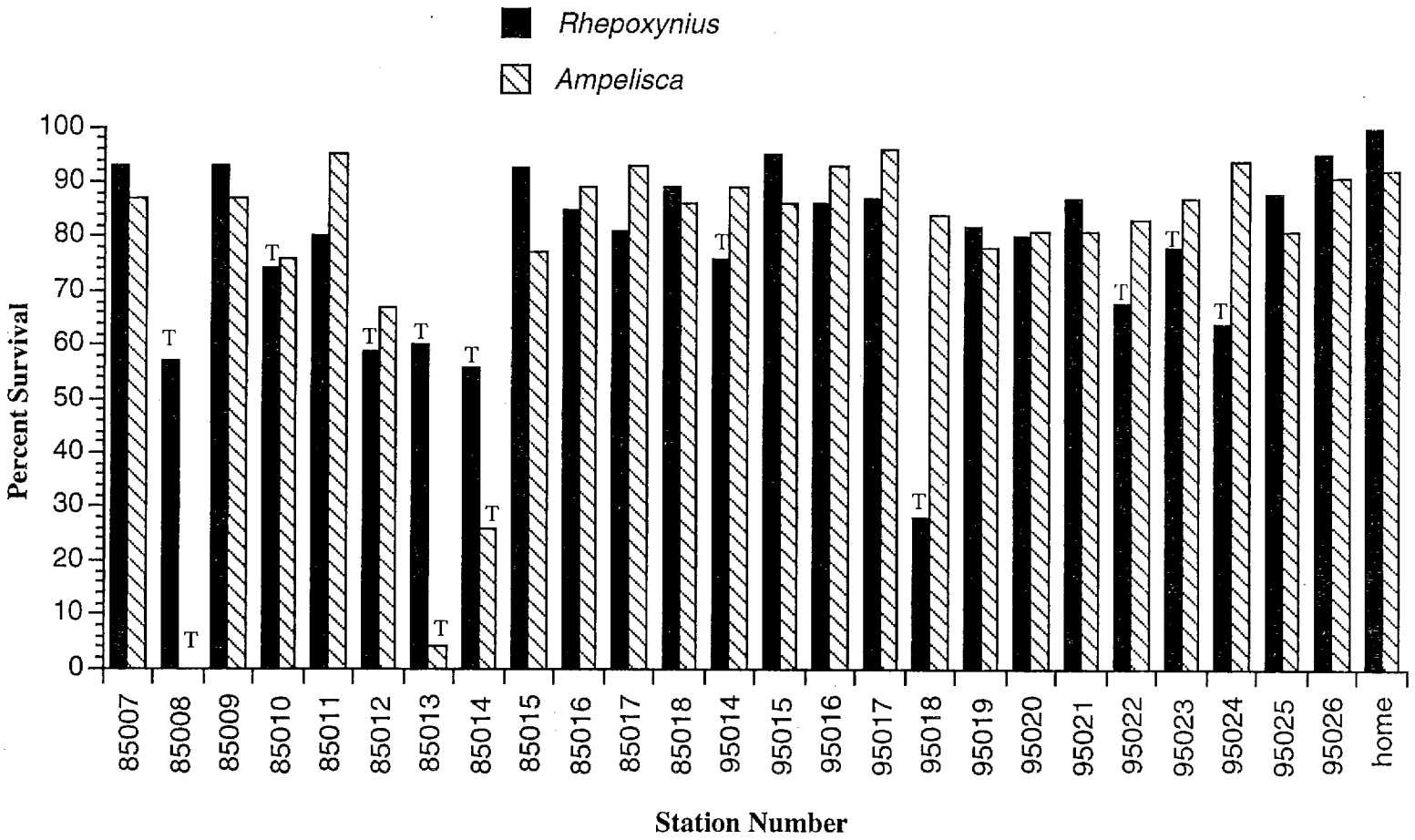


Figure 32. Survival of *Rhepoxynius* and *Ampelisca* at 25 Southern California EMAP stations. T = Toxic (survival < 80% of control value and statistically significant with t-test).

Benthic Community Structure

The complete tabulated results of the benthic community analysis are presented in **Appendix E.** Shown are the number of individuals of each species in each replicate core. A number of summary statistics were calculated for each station, including summaries of total fauna, number of species, and the 4 major phyla (Polychaetes, Crustaceans, Molluscs, and Echinoderms).

A total of almost 20,000 individuals from 168 taxa were identified from the 43 stations analyzed for benthic infauna. Of this total, 90 (53%) were polychaete species, along with 42 crustacean species, 25 molluscs, 2 echinoderms, and 9 other phyla. The entire species list, along with the number of stations of occurrence of each taxa, is shown in **Appendix E.**

Since the purpose of the study was to identify contaminated sites, and not necessarily to do a complete community analysis, generation of a benthic index was considered to be the most critical goal of the benthic work.

Benthic Index

The benthic index used in this study is a refined version of the index used in the San Diego BPTCP report (Fairey et al. 1996). It combines the use of benthic community data with the presence of positive or negative indicator species to give a measure of the relative degree of degradation of the benthic fauna. It does not require the presence of uncontaminated reference stations, and does not refer to data beyond that collected in this study. Other benthic indices often rely on *a priori* assumptions, particularly the presence of uncontaminated reference sites, which can lead to false results if the assumptions are not met.

Community Data

Two aspects of the community data were used in the benthic index: the total number of species, and the number of crustacean species. An increase in species richness is a well accepted indicator of healthy environments (Diaz, 1992). While a variety of indices have been developed to quantify species richness in absolute terms, for a study limited in spatial scale, as was this one, total number of species is an appropriate indicator of community richness.

Crustaceans are generally more sensitive to environmental contaminants than most other components of the infauna, particularly polychaetes and bivalves. Speciose and numerically abundant crustacean faunas on the Pacific coast of the United States are generally only found in

uncontaminated environments, making the number of crustacean species an important indicator of overall environmental health.

Indicator Species

Eleven of the 168 total species were chosen as indicator species. The bioindicators were chosen based on a review of pertinent literature, known habitat preferences and life history, their abundance over all of the stations, and on discussions with experienced ecologists. The 3 negative indicator species are highly opportunistic annelids which thrive in disturbed, polluted, or marginal environments, and are generally not found in mature, undisturbed communities. The 8 positive indicator species consist of 2 bivalves and 6 crustaceans, and are generally not found in polluted habitats. Each indicator species is discussed below:

Negative indicator species

Capitella capitata

The *Capitella* species complex is a cosmopolitan group which lives in a wide range of conditions: fouled or low oxygen, high organic matter and fine sediments. They are abundant around outfalls discharging biological wastes, and have a rapid (1 to 2 month) life cycle. *Capitella* are capable of surviving for days with little or no oxygen, and are often considered the best example of a "weedy", opportunistic species (Reisch and Barnard, 1960).

Streblospio sp.

Streblospio were introduced from the East coast, and are now found in huge numbers on mud flats of bays and estuaries. They exhibit extreme fluctuations in abundance both temporally and spatially. *Streblospio* are deposit feeders on organic aggregates and detritus at the surface, but can also suspension feed. While generally a tube dweller, they can also be mobile. They have an annual life cycle, and no intraspecific competition, so can settle in very high densities (Light, 1980; Levin, 1981).

Oligochaetes

Oligochaetes are a poorly known group typically found in peripheral/disturbed habitats such as under decaying algae on beaches, and in fouled or low oxygen muds of back bays, estuaries, and harbors. They often occur in large masses to exclusion of all or nearly all other macrofauna. In SF Bay they may comprise 100% of the fauna where there is gross pollution (i.e. large amounts of organic material from sewage). If oxygen levels are sufficient, and there is little toxic waste and high bacterial levels, oligochaete levels are high. Given sufficient oxygen, oligochaete densities

become extremely high (Smith and Carlton, 1975; Brinkhurst and Simmons, 1968).

Positive Indicator Species

Monoculodes sp.
Monoculodes is a fossorial oedocerotid amphipod which requires well-oxygenated, clean nearshore sands. They are shallow burrowers which occur at the sand surface/water interface. *Monoculodes* are carnivorous and therefore are probably active and sensitive to sediment surface quality (Mills, 1962; Bousfield, 1970).

Bathyleberis sp.
Bathyleberis is a filter-feeding ostracod which lives in offshore and well oxygenated sands. They may be found in fine sands with organic matter, but require adequate water circulation and relatively pristine conditions, such as well flushed harbors (eg. Half Moon Bay, California; Baker, 1975).

Euphilomedes sp.
Euphilomedes are detritivores, as is typical of myodocopid ostracods. They can have very specific nearshore habitats; several *Euphilomedes* species are zoned relative to each other in response to wave size and sediment stability. However, they are often found in sands with fairly high organic matter, such as moderately distant halos around outfalls (eg., San Francisco and Palos Verdes) probably because of high detritus levels. The Southern California mainland shelf has the most myodocopid species in the west coast of North America (Pearson and Rosenberg, 1978; Fenwick, 1984; Slattery, 1980; Baker, 1975).

Paracereis sp.
Paracereis is an epibenthic herbivorous amphipod found in southern California in clean waters, and sand, and on corals, sponges, and intertidal algae (Menzies, 1962; Schultz, 1969; Schuster, 1987).

Acuminodeutopus sp.
Acuminodeutopus are found in shallow clean, well-oxygenated sands, and also in bay muds. They build tubes, and are early/first colonizers of ray pits and other sand perturbations (Barnard 1961, Barnard and Reish 1959, VanBlaricom 1982).

Tellina sp.
Tellina is a bivalve which inhabits shallow, clean to silty sands of protected waters. Their size

increases with increasing sediment size. While mainly a deposit feeder, they can filter feed in very clean sediment (Barnard 1963; Maurer, 1967).

Eobrolgus sp.

Eobrolgus are typical phoxocephalid amphipods: active, subsurface burrowers in clean well-oxygenated sands, but often associated with fines and some organic matter. They are not common in very fine muds probably because of clogging by particles during burrowing activities. They are carnivorous scavengers. A similar genus, *Rhepoxynius*, is one of the most commonly used bioassay animals for marine sediments (Barnard 1960, 1963; Barnard and Barnard 1982; Oakden, 1984; Slattery, 1980).

Mactra sp.

Mactra is a bivalve found in various sediments including sand and mud. They are common in bays and lagoons of southern California, although not in back-bay environments (Abbott, 1974).

Calculation of Benthic Index

Previous versions of the Benthic Index have used individual impact thresholds for determination of degree of negative impact to Total Fauna and Number of Crustacean Species (Fairey et al. 1996). While these thresholds have been useful, the necessarily arbitrary nature of the selection process introduced potential artifacts for stations whose values for Total Fauna and Total Crustacea approached the threshold value. To address this problem, calculation of the Benthic Index was revised to be based on percentages of the total range. The final threshold value for determination of impacted versus non-impacted sites was based on the overall Benthic Index and selected using best professional judgment. Justification for this threshold of Benthic Index impact is discussed below.

For Total Fauna and Number of Crustacean Species, the total range in these parameters for the 43 stations were determined. For each station, the total number of species and total number of crustacean species were then converted to the percentage of the total range for these parameters (**Table 19**). These two numbers represent two-thirds of the Benthic Index for each station. For the positive and negative indicator indices, the final index was weighted towards presence and absence of key indicator species, with abundance of each species given additional incremental weight. Accordingly, the abundance of each indicator species was transformed using a double square-root transformation to compress the range of values. For each species, the transformed abundance was converted to a percentage of the total range. The percentages of the negative indicator species was summed (**Table 19**, "Neg Sum") and subtracted from the percentages of the

Table 19. Benthic community data showing Total Fauna Index, Crustacean Species Index, Indicator Index, and final Benthic Index combining all three Indices. Stations having final Benthic Index ≤0.30 are considered to be significantly impacted.

STATION		TOTAL FAUNA		CRUSTACEANS		Indicator sp		Indicator	**Benthic**
		# species	indx %	# species	indx %	pos %	neg %	Index	**Index**
San Elijo Lagoon: 18	95023	6	0.12	0	0.00	0.00	0.64	0.11	***0.08***
San Elijo Lagoon: Waste Site	95012	7	0.14	0	0.00	0.00	0.47	0.17	***0.10***
San Elijo Lagoon: 269	95011	2	0.04	0	0.00	0.00	0.17	0.27	***0.10***
San Elijo Lagoon: 24	95010	4	0.08	1	0.07	0.00	0.40	0.19	***0.11***
Los Peñasquitos Lagoon: 331	95007	15	0.3	2	0.13	0.00	0.99	0.00	***0.14***
Santa Margarita Lagoon: 33	95013	7	0.14	2	0.13	0.00	0.35	0.21	***0.16***
Los Peñasquitos Lagoon: 319	95006	12	0.24	2	0.13	0.00	0.54	0.15	***0.17***
Los Peñasquitos Lagoon: 336	95018	12	0.24	3	0.20	0.00	0.70	0.09	***0.18***
Newport Bay Lagoon: Unit I Basin	85018	16	0.32	4	0.27	0.00	0.92	0.02	***0.20***
San Dieguito Lagoon: 306	95024	17	0.34	2	0.13	0.15	0.80	0.17	***0.21***
Dana Point Harbor: 396	95016	11	0.22	3	0.20	0.00	0.00	0.33	***0.25***
Oceanside Harbor: Pendleton	95021	18	0.36	2	0.13	0.00	0.11	0.29	***0.26***
Newport Bay Lagoon: 431	85007	21	0.42	4	0.27	0.14	0.85	0.14	***0.28***
Santa Margarita Lagoon: 48	95025	17	0.34	4	0.27	0.15	0.50	0.26	***0.29***
Newport Bay Lagoon: Unit II Basin	85017	14	0.28	5	0.33	0.09	0.38	0.26	***0.29***
Agua Hedionda Lagoon: 190	95001	19	0.38	2	0.13	0.16	0.09	0.41	**0.31**
Dana Point Harbor: Commercial Basin	95005	15	0.3	5	0.33	0.00	0.11	0.29	**0.31**
Newport Bay Lagoon: 705	85009	16	0.32	6	0.40	0.11	0.40	0.27	**0.33**
Agua Hedionda Lagoon: 179	95014	17	0.34	3	0.20	0.27	0.00	0.51	**0.35**
Oceanside Harbor: Commercial Basin	95020	21	0.42	3	0.20	0.18	0.00	0.45	**0.36**
Dana Point Harbor: 386	95004	16	0.32	6	0.40	0.07	0.00	0.38	**0.37**
Oceanside Harbor: Stormdrains	95022	23	0.46	5	0.33	0.07	0.00	0.38	**0.39**
Agua Hedionda Lagoon: Finger	95003	18	0.36	9	0.60	0.20	0.33	0.35	**0.44**
Oceanside Harbor: 90	95019	20	0.4	7	0.47	0.21	0.00	0.47	**0.45**
Newport Bay Harbor: Newport Island	85014	25	0.5	8	0.53	0.32	0.43	0.40	**0.48**
Oceanside Harbor: 110	95008	32	0.64	5	0.33	0.21	0.00	0.47	**0.48**
Newport Bay Harbor: Rhine Channel	85013	32	0.64	8	0.53	0.09	0.34	0.27	**0.48**
Newport Bay Harbor: Arches	85015	27	0.54	6	0.40	0.36	0.14	0.52	**0.49**
Agua Hedionda Lagoon: 234	95002	23	0.46	5	0.33	0.72	0.11	0.78	**0.52**
Newport Bay Harbor: 1064	85012	38	0.76	5	0.33	0.61	0.10	0.54	**0.54**
Newport Bay: 523	85001	30	0.6	15	1.00	0.74	0.16	0.24	**0.61**
Agua Hedionda Lagoon: 144	95026	27	0.54	9	0.60	0.81	0.23	0.80	**0.65**
Dana Point Harbor: Stormdrain	95017	32	0.64	11	0.73	0.50	0.20	0.60	**0.66**
Newport Bay: 1009	85006	37	0.74	11	0.73	0.36	1.00	0.52	**0.66**
Newport Bay: 949	85005	40	0.8	10	0.67	0.39	0.20	0.64	**0.70**
Newport Bay Harbor: 905	85011	44	0.88	10	0.67	0.39	0.16	0.62	**0.72**
Newport Bay: 616	85002	42	0.84	10	0.67	0.58	0.12	0.77	**0.76**
Newport Bay: 791	85003	46	0.92	12	0.80	1.00	0.00	0.68	**0.80**
Newport Bay Harbor: 819	85010	48	0.96	11	0.73	0.49	0.13	0.71	**0.80**
Newport Bay Lagoon: 670	85008	50	1	13	0.87	0.55	0.44	0.55	**0.80**
Agua Hedionda Lagoon: 212	95015	38	0.76	13	0.87	0.87	0.36	0.79	**0.81**
Newport Bay Harbor: Yachtsman Cove	85016	49	0.98	12	0.80	0.71	0.14	0.76	**0.85**
Newport Bay: 877	85004	35	0.7	13	0.87	0.51	0.09	1.00	**0.86**

positive indicator species (**Table 19**,"Pos Sum"). This value ("Pos-Neg") was then converted into a percentage of the total for each station (Indicator Index %).

The overall Benthic Index was calculated by summing the percentages of the Total Fauna, Crustacean Species, and Indicator Species indices. This resulted in a range in values from 0.08 (Most Impacted) to 0.86 (Least Impacted; **Table 19**).

It is not possible to test the Benthic Index to determine significance levels or confidence levels, or to statistically determine what ranking indicates significant impact. However, since a degree of arbitrarity is incorporated into all determinations of significance, whether statistical or intuitive, this should not be considered a significant drawback. For this study, the threshold for significantly impacted benthic community structure was set at a Benthic Index less than or equal to 0.30. While this threshold is necessarily somewhat arbitrary, it is considered suitable based on the best professional judgment of the benthic ecologists who performed the analysis. Several factors were considered in deriving this threshold: the stations below the threshold have few overall species, few crustacean species, presence of negative indicator species, and absence of positive indicator species. These stations would be considered to be significantly degraded by the vast majority of naturalists familiar with southern California's bays and estuaries. The Benthic Index can be used in combination with chemistry and toxicity test data to provide a "weight-of-evidence" for determination of the most impacted stations (see below).

Fifteen of the 43 stations analyzed for benthic community structure had a Benthic Index less than or equal to 0.30, and were therefore considered to be significantly impacted. Three of the 18 Newport Bay stations had significantly impacted benthic community structure **(Figure 33)**. Two stations were degraded in Dana Point and Oceanside Harbors **(Figure 34)**. Ten of the 15 impacted stations (67%) were in 4 of the coastal lagoons **(Figures 35 and 36)**. All 4 of the stations in San Elijo Lagoon, and all 3 of the stations in Los Peñaquitos Lagoon were significantly impacted.

Correlations of Benthic Community Structure with Chemical Contaminants

Statistical associations between benthic community structure and bulk-phase chemical contamination were determined using Spearman Rank Correlations and by correlating the sub-indices of the Benthic Index with ERM and PEL quotient values. As with the correlations of chemical contaminants and toxicity discussed above, these analyses were conducted using all of the contaminants analyzed. Associations between contaminants and several indicators of benthic community structure were determined before and after normalization with Total Organic Carbon.

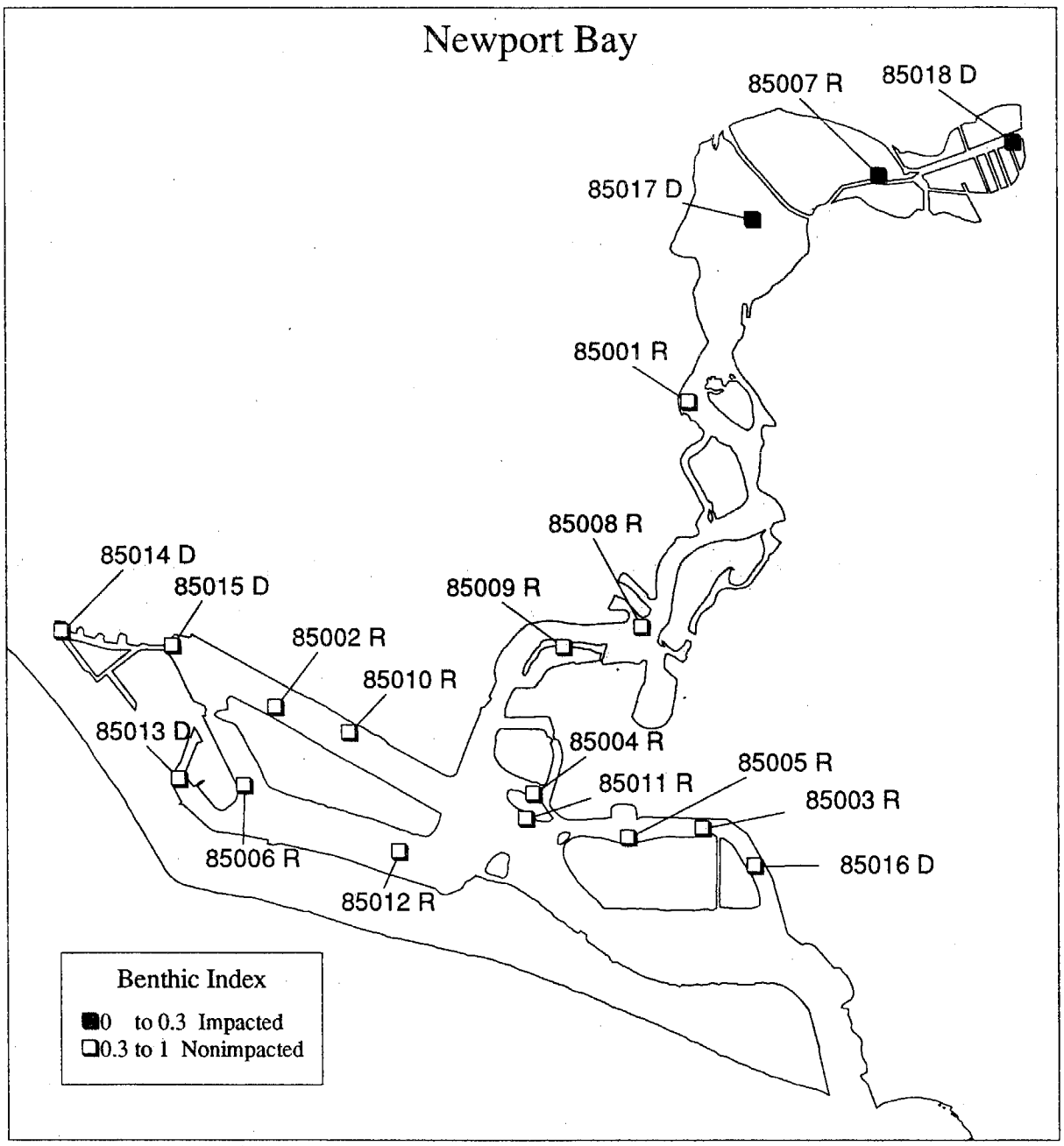


Figure 33. Distribution of stations in Newport Bay demonstrating significant benthic community degradation.

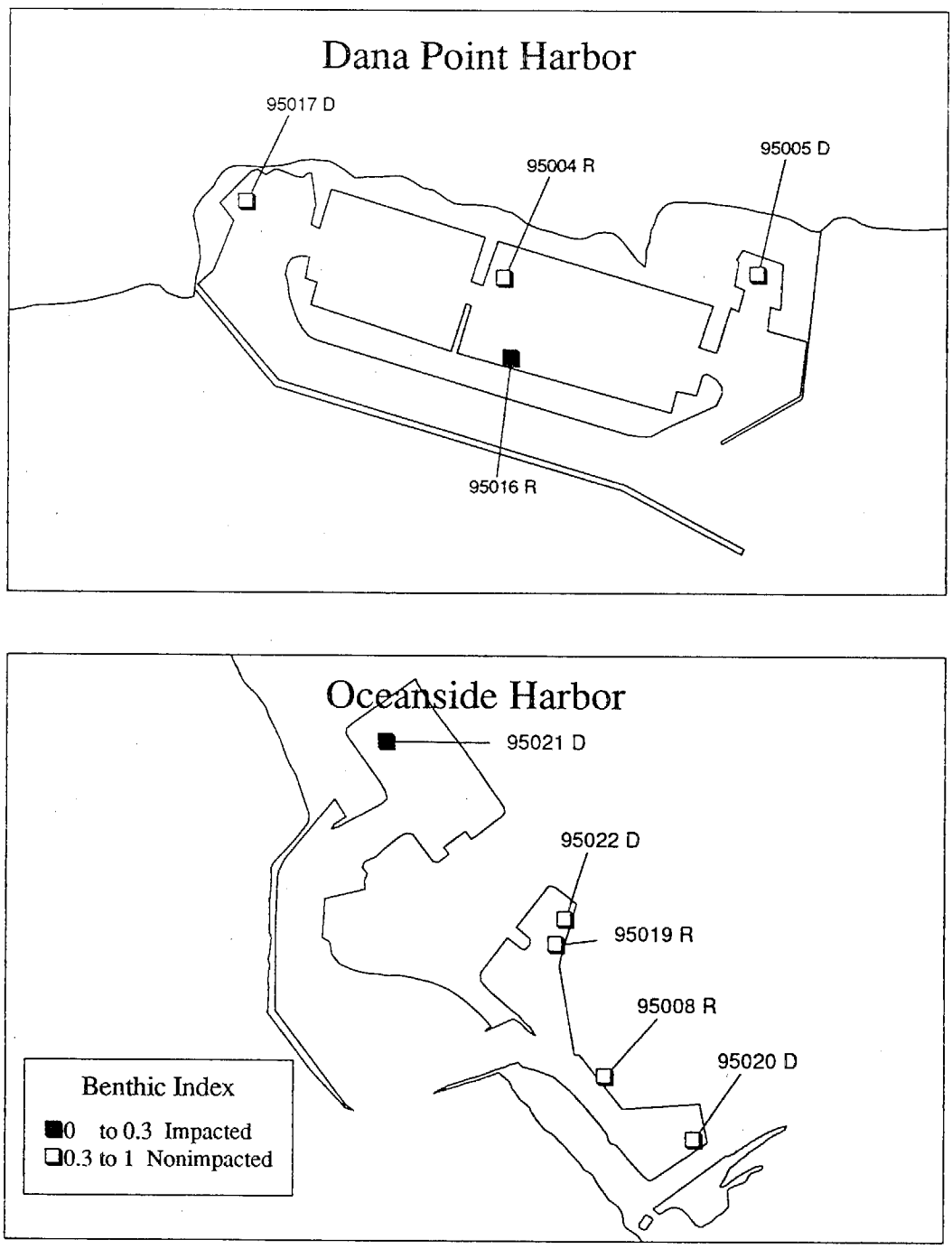


Figure 34. Distribution of stations in Dana Point and Oceanside Harbors demonstrating significant benthic community degradation.

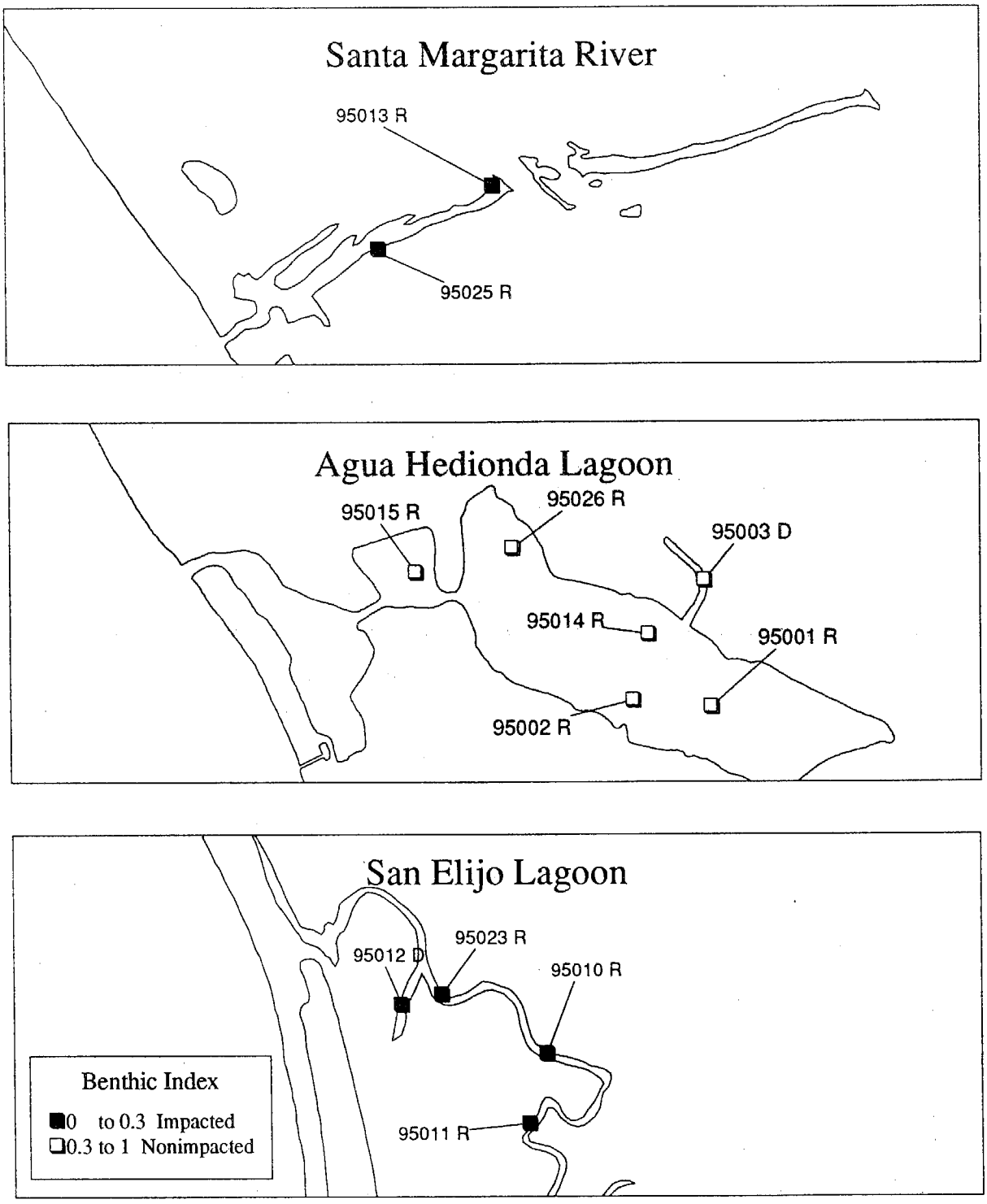


Figure 35. Distribution of stations in Santa Margarita, Agua Hedionda, and San Elijo Lagoons demonstrating significant benthic community degradation.

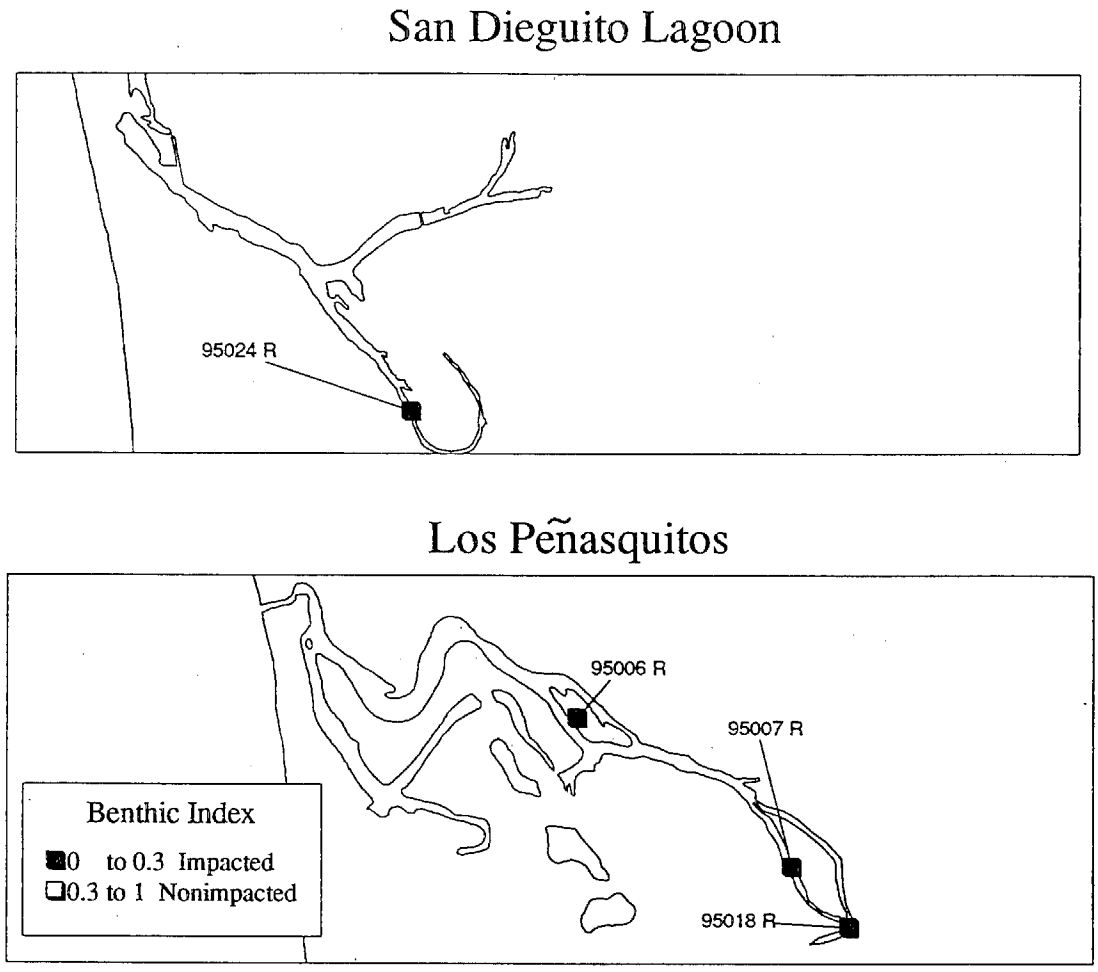


Figure 36. Distribution of stations in Los Peñasquitos and San Dieguito Lagoons demonstrating significant benthic community degradation.

In addition to correlation with the overall Benthic Index presented in **Table 19**, bulk-phase and TOC-normalized contaminants were correlated with Total Number of Species and Number of Crustacean Species at each station. These measures were also correlated with sediment TOC and grain size, interstitial water un-ionized ammonia and hydrogen sulfide. Finally, the Benthic Index was correlated with results of each of the toxicity test protocols.

The results indicated few significant associations. There were no significant correlations between benthic community structure and any of the parameters listed above, except for a positive correlation between the Benthic Index and percent fertilization measured in the sea urchin fertilization protocol (Spearman Rho = 0.564; significant @ alpha =0.0001), and a negative correlation between interstitial water hydrogen sulfide concentrations and the Benthic Index (Spearman rho = -.375; significant @ alpha =0 .05).

Interstitial water hydrogen sulfide concentrations measured in the toxicity exposures may be used to indicate whether anoxic conditions existed at the sampling sites in the absence of *in situ* dissolved oxygen measurements. The four samples with the highest hydrogen sulfide values were from San Elijo Lagoon, which is subject to increased sedimentation, minimal tidal flow, and resultant anoxic conditions (California Coastal Conservancy, 1989). All four stations from this lagoon had significantly impacted benthic community structure (**Figure 35**).

That benthic community structure may be influenced by factors other than the measured chemical contaminants is illustrated by plotting the Total Number of Species, Number of Crustacean Species, and Benthic Index against the distribution of summary ERM Quotients for all 43 samples (**Figure 37**). As noted previously, the majority of samples were relatively uncontaminated; most had average PEL quotients less than 0.6. Despite this, the distribution of benthic community parameters was quite variable ranging from significantly impacted to undisturbed at the least contaminated sites. The Benthic Index did not indicate significant negative impacts at the three Newport Bay sites with the highest ERM quotients (Arches Storm Drain, Rhine Channel, and Newport Island; **Figure 37**). In an analysis of benthic community structure in San Diego Bay, Fairey et al (1996) noted that significant negative impacts on benthic community structure occurred beyond an average ERM quotient of approximately 0.6. The range of average ERM quotients was higher in San Diego Bay, indicating greater contamination (Fairey et al. 1996; Figure 14). This, combined with differences in the types of chemicals driving the high quotients, as well as possible differences in bioavailability, may explain the lack of any threshold effect in the present study.

It should also be noted that many of these sites are heavily influenced by extremes in physical

factors. For example, because all of the coastal lagoons except Agua Hedionda are closed to tidal influences for at least part of the year, these areas undergo significant seasonal fluctuations in salinity, dissolved oxygen concentrations, and temperature. All of these factors may have considerable negative impacts on benthic community structure, and also probably play a role in structuring benthic communities in low water flow areas of the harbors, particularly upper Newport Bay. The lagoon stations were often dominated by negative indicator organisms such as *Capitella,* and oligochaetes. This may reflect the greater tolerance of these species to extremes in environmental factors at these stations.

It should be noted that in addition to the sediment triad data from San Diego Bay discussed in Fairey et al (1996), this study is considered to be a preliminary assessment of the utility of the Benthic Index for assessing the effects of contaminated sediments on benthic community structure. It is recognized that as this approach is applied to future triad data sets generated from the BPTCP, additional validation of the Benthic Index will be performed, and that it may be necessary to modify methods used for calculating the Index as more information becomes available.

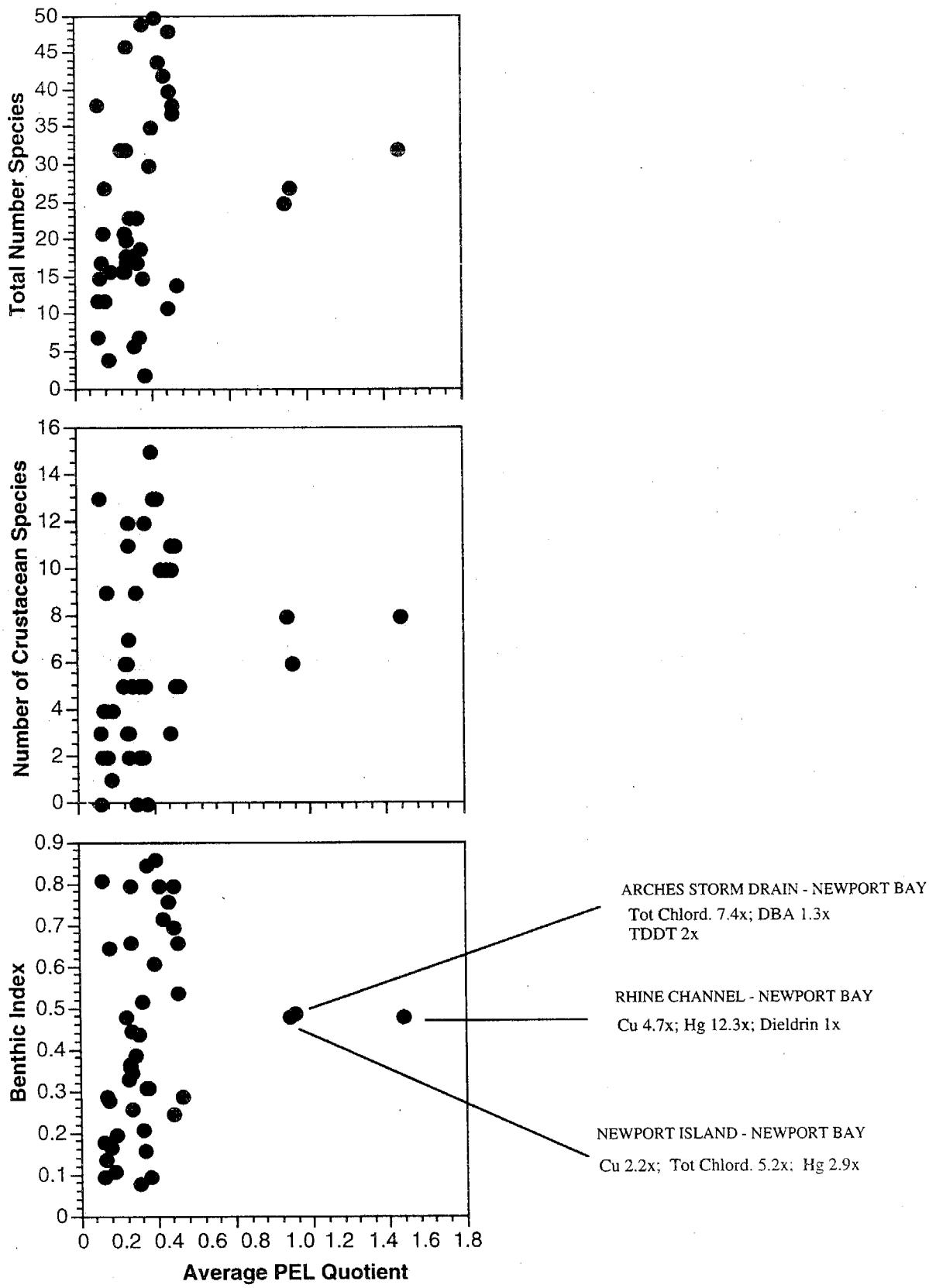


Figure 37. Association between the Benthic Index, Number of Crustacean Species, and Total Number of Species and the average PEL Quotient at 43 EMAP Stations. Also given are the three stations with the highest PEL Quotients, the primary chemicals of concern at these sites, and the levels of PEL exceedances for these chemicals.

Random vs Directed Sampling

Of the 43 stations analyzed in this study, 30 were sampled using the EMAP random sampling procedures. This sampling design (previously described) was used to address the first study objective of investigating the spatial extent of degraded fine grained environment. The remaining 13 samples were selected using a directed sampling design used to address the second study objective of identifying and prioritizing specific individual sites as toxic hot spots. Stations selected using the directed design were those suspected of being contaminated based on their proximity to point source or non-point source discharges or previous information indicating toxicity or the presence of contamination. One of the goals of this investigation was to determine if results differed depending on whether samples were collected using a random or directed design. This was determined by comparing the number of samples having high chemical concentrations or significantly impacted benthic community structure, and the percentage of samples which were toxic for each sampling design and toxicity test protocol. Chemical contamination was compared relative to ERM Quotients, benthic community structure was compared relative to the Benthic Index, and toxicity was compared based on survival $\leq$ 80% of control value and statistical significance with a t-test.

Stations demonstrating the highest chemical contamination based on PEL or ERM quotients were selected using the directed design. For example, the three stations with the highest ERM quotients were all in Newport Bay (Station 85013 - Rhine Channel; Station 85014 - Newport Island; Station 85015 - Unit II Basin; **Figure 37**); all three of these stations were selected using the directed sampling design.

Except for samples tested with *Ampelisca*, the percentage of toxic samples was greater using the random sampling design. The reason for this disparity is unclear. For samples tested with the amphipod *Rhepoxynius,* 60% of the 30 random samples were toxic, 38% of the 13 directed samples were toxic to this species (**Table 20**). Using the 100% pore concentration from samples tested with the sea urchin development protocol, 87% of the random samples were toxic, while 77% of the directed samples were toxic. Using the 50% pore water concentration, 70% of the random samples were toxic, and 46% of the directed samples were toxic. Using the 25% pore water concentration, the percentage of random and directed samples which were toxic were 26% and 15%, respectively. A similar trend occurred using the sea urchin fertilization protocol; 57% of the random samples tested with this protocol were toxic, while 46% of the directed samples were

Table 20. Percentage of toxic samples and degree of chemical contamination using stratified random and directed sampling designs.

Toxicity Assessment Method	Random Samples			Directed Samples		
	Toxic	Non-Toxic	Percentage Toxic	Toxic	Non-Toxic	Percentage Toxic
Rhepoxynius survival	18	12	60%	5	8	38%
Ampelisca survival	1	15	7%	2	8	20%
Strongylocentrotus Development (100% PW)	26	4	87%	10	3	77%
Strongylocentrotus Development (50% PW)	21	9	70%	6	7	46%
Strongylocentrotus Development (25% PW)	13	17	26%	2	11	15%
Strongylocentrotus Fertilization (100% PW)	17	13	57%	6	7	46%
Benthic Community Structure	11	19	37%	4	9	31%
Degree of Chemical Contamination	**Random Samples**			**Directed Samples**		
	Mean ERM Q	**Range ERM Q**		**Mean ERM Q**	**Range ERM Q**	
ERM quotient	0.153	0.66 - 0.322		0.326	0.65 - 1.27	

toxic (**Table 20**). Only 1 of the 15 random samples tested with *Ampelisca* were toxic (7%); 2 of the 10 directed samples were toxic to this species (20%).

Analysis of the benthic community structure indicated minimal differences between the two sampling designs. For this comparison, stations with a benthic index less than or equal to 0.30 were considered to be significantly impacted. Of the 30 random stations assessed for benthic community structure, 11 (37%) had a benthic index ≤ 0.30 (**Table 20**). Benthic community structure was significantly impacted in 4 of the 13 directed stations (31%).

STATION RANKING AND PRIORITIZATION

One goal of this study was to identify those sites considered to be of primary concern in terms of chemical contamination and potential impacts on beneficial uses identified through biological measures. By comparing the relative degree of chemical contamination with different measures of toxic effect, and combining these data with information on benthic community degradation, a weight-of-evidence approach may be employed to identify the most impacted sites.

It is recognized that any conclusions based on interpretation of these data should be considered preliminary because of the limited nature of the data set. As with any study of this scope, it is difficult to identify all variables which may be associated with biological responses at a particular location. For example, our characterization of organic chemical contamination is constrained by the limited number of contaminants measured (**Appendix B**). Samples often contained un-identified organic compounds which were not further characterized due to the limited scope of the study; these could have contributed to the toxicity of the samples. In addition, no measures of interstitial water chemical concentrations were conducted for substances other than ammonia and hydrogen sulfide. Therefore, our ability to characterize bioavailability of the bulk-phase chemicals is limited to TOC normalization. In addition, no measures of Acid Volatile Sulfides and associated metals (AVS-SEM) were made, which limits our ability to predict bioavailability and toxicity of metals. Conclusions regarding benthic community degradation was limited by the lack of *in situ* sediment dissolved oxygen levels.

Because of these limitations, characterization of the most impacted stations must rely, to a certain extent, on a qualitative interpretation of the data. To accomplish this, individual stations were evaluated based on a Triad of measures (*sensu* Chapman et al. 1987): chemical contamination, benthic community structure, and toxicity to amphipods and echinoderms, and a screening test (P450 RGS). These were used to establish a weight-of evidence demonstration of degradation. These data were combined with information on possible inputs as well as past use practices to help

explain the results. The sites were then ranked in order of impact, and prioritized for further investigations. Sites given the highest priority for future investigation had the following characteristics: 1) high chemical contamination with single or multiple compounds, and 2) significant toxicity which could not be attributed solely to un-ionized ammonia or hydrogen sulfide, and 3) benthic community degradation. Samples from sites given the highest priority ranking in this study also demonstrated a response of the RGS assay to PAHs and PCBs. Sites given a moderate priority for future investigation generally had some combination of the three triad measures but not all three. Sites given a low priority generally had lower chemical contamination and toxicity.

All but 1 of the 7 stations with the highest sediment contamination were from Newport Bay. Three stations from Newport Bay (Rhine Channel, Newport Island, and Arches Storm Drain) had the highest PEL/ERM quotients (**Table 21**). Seven of 8 sediments producing the highest induction of the RGS Assay were from Newport Bay (85001-005; 85011-012), and the eighth was from Dana Point Harbor (95016). The induction was likely from the PAH contamination in these sediments, but coplanar PCBs may have contributed to the effects on the CYP1A1 gene. The RGS assay correlated with both PAHs and the Aroclors (1254 and 1260), so it is not possible to separate out the contribution of the two classes of compounds. Analyses of the 12 coplanar PCB congeners possibly present in the samples would aid in determining the contribution of PCBs to the induction of these cells. The remaining stations had relatively lower chemistry quotient values. As discussed earlier, stations with the most impacted benthic community structure were for the most part located in four of the coastal lagoons with a few impacted stations in Newport Bay, and Dana Point and Oceanside Harbors. Although toxicity to sea urchin development was relatively widespread, this was in large part due to high un-ionized ammonia concentrations. Toxicity to sea urchin sperm was less widespread. Toxicity to amphipods (*Rhepoxynius abronius*) was greatest in the 3 Los Peñasquitos Lagoon stations. In addition, there was significant amphipod toxicity (*Rhepoxynius abronius* and *Ampelisca abdita*) at several Newport Bay stations (**Table 21**).

Of the 43 stations sampled, 4 were given the highest priority for further work. These included two stations in Newport Bay: Newport Island (Station No. 85014), and Rhine Channel (Station No. 85013), as well as Station No. 95016 in Dana Point Harbor and Station No. 95024 in San Dieguito Lagoon.

Rhine Channel in Newport Bay (Station No. 85013) had the highest ERM/PEL quotients of all the 43 stations sampled. The high chemistry quotient at this station was driven primarily by copper,

and mercury. This sample also had an elevated TBT concentration, a substance for which neither an ERM or PEL has been established. The Benthic Index from this station indicated moderate impacts (**Table 21**). Toxicity to both amphipod species tested was statistically significant, and was particularly high for the amphipod *Ampelisca abdita*. Although the un-ionized ammonia concentration in the *Rhepoxynius* test was low, toxicity to *Ampelisca* might be attributed to ammonia. The initial un-ionized ammonia concentration was below the application limit for this species at the initiation of the test (0.4 mg/L un-ionized ammonia; EPA, 1994), but the un-ionized ammonia concentrations in overlying water at the end of the 10 day exposure was 1.24 mg/L (**Table 21**). The un-ionized ammonia concentration in pore water was also well above the application limit for sea urchin embryos. It is therefore not possible to eliminate ammonia as a factor in this test.

Table 21. Summary of EMAP southern California bays and estuaries data, and ranking of stations for future investigations.

STANUM	STATION	ID#	% FINES	TOC	ERMQ	PELQ	Chem. Comments	RGS (µg/g) BaPEq	Aa Mean Survival	Ra Mean Survival	Urchin Dev. 100% PW	Urchin Dev. 50% PW	Urchin Dev. 25% PW	Urchin Fert. 100% PW	Benthic Index	Ra OUNH3 (mg/L)	Urch. Dev. IUNH3 (mg/L)	Aa OUNH3 (mg/L)	H2S interstit. (mg/L)	Priority
85014.0	NEWP. BAY (NEWPORT ISLAND	1425	85.4	3.3	0.733	1.039	Cu2xPQ; Chl5xQP;Hg3xPQ		26.0	56.0	0.0	0.0	62.0	96.4	0.48	0.11	0.26	0.42	.018D	High
85013.0	NEWP. BAY (RHINE CHANNEL)	1424	64.7	2.0	1.270	1.684	Cu 4.7xPQ; Hg12.3xEQ		4.0	60.0	0.0	70.0	86.3	93.4	0.48	0.18	0.10	1.24		High
95016.0	DANA POINT HARBOR (396)	1415	93.5	1.9	0.322	0.579	Cu3.8xPQ; TChl 1.7xPQ	15.50	93.0	86.0	75.0	96.0	96.3	0.7	0.25	0.01	0.03	0.02		High
95024.0	SAN DIEGUITO LAGOON (306)	1435	59.8	0.8	0.174	0.307	Dieldren 3xPQ	8.10	93.8	64.0	17.1	90.3	98.4	0.0	0.21	0.05	0.07	0.03		High
85015.0	NEWPORT BAY (ARCHES St. Dr.)	1426	44.2	3.8	0.668	0.972	Chl7.4xPQ;DBA1.3xPQ		77.0	92.5	0.3	87.3	95.1	92.1	0.49	0.08	0.15	0.10		Moderate
95006.0	LOS PENASQUITOS (319)	1385	57.3	1.2	0.093	0.126	None	12.60		23.0	42.3	92.2	92.8	0.0	0.17	0.00	0.12			Moderate
95018.0	LOS PENASQUITOS (336)	1417	94.9	1.1	0.077	0.097	None	1.80	84.0	28.0	0.0	84.2	96.8	94.7	0.18	0.08	0.17	0.14		Moderate
85001.0	NEWPORT BAY (523)	1387	81.4	1.4	0.180	0.283	TCHL=1xPQ	16.20		29.0	0.0	0.0	0.0	47.2	0.61	0.59	0.36		.032D,F	Moderate
95007.0	LOS PENASQUITOS (331)	1386	82.2	1.0	0.080	0.105	None	2.20		42.0	91.8	93.4	94.5	32.3	0.14	0.05	0.03			Moderate
95002.0	AGUA HEDIONDA LAGOON (234	1381	96.2	1.8	0.123	0.185	TDDT 1.3XPQ	5.20		50.0	5.7	0.0	51.2	93.4	0.52	0.15	0.01			Moderate
85008.0	NEWPORT BAY (670)	1419	65.5	1.9	0.175	0.267	TChl 1.5xPQ	10.90	0.0	57.0	0.0	0.0	0.0	0.0	0.80	1.58	0.35	1.99		More info
85002.0	NEWPORT BAY (616)	1388	64.0	1.3	0.239	0.340	Hg 1xPQ	21.70		57.5	0.0	0.0	57.6	92.6	0.76	0.09	0.03			Moderate
85012.0	NEWPORT BAY (1064)	1423	98.8	1.7	0.212	0.316	TDDT2.5xEQ	14.40	67.0	59.0	2.0	43.5	23.3	86.1	0.54	0.06	0.06	0.27		Moderate
85005.0	NEWPORT BAY (949)	1391	97.4	1.8	0.244	0.359	PPDDE2.3xEQ; TChl1xPQ	22.80		63.0	0.0	0.0	21.6	96.1	0.70	0.19	0.03			Moderate
95012.0	SAN ELIJO LAGOON (WST SITE)	1396	40.3	1.1	0.065	0.100	None			63.0	0.0	36.3	91.1	0.0	0.10	0.19	0.12		.414D,F	Moderate
95004.0	DANA POINT HARBOR (386)	1383	54.0	1.1	0.166	0.294	Cu 1.7xPQ	12.50		67.0	24.7	0.0	86.4	93.5	0.37	0.01	0.02			Moderate
95022.0	OCEANSIDE HARBOR(ST. DR.)	1433	87.9	1.1	0.183	0.284	Cu 1.3xPQ		83.0	68.0	98.0	97.0	96.7	65.3	0.39	0.03	0.03	0.01		Moderate
95001.0	AGUA HEDIONDA LAGOON (190	1380	99.2	2.4	0.126	0.187	TDDT1.5xPQ	2.50		85.0	43.3	1.7	78.2	67.7	0.31	0.36	0.01		.012D	Moderate
95011.0	SAN ELIJO LAGOON (269)	1395	71.5	2.7	0.103	0.153	None	6.60		70.0	0.0	38.5	83.3	0.0	0.10	0.12	0.25		.090D,F	Moderate
85011.0	NEWPORT BAY (905)	1422	95.0	1.5	0.200	0.295	TDDE 1.7xEQ; TChl1.5xPQ	18.40	95.0	80.0	0.0	0.0	3.3	95.0	0.72	0.02	0.08	0.04		Moderate
95005.0	DANA POINT HARBOR.	1384	96.4	1.6	0.178	0.285	Cu 1.3xPQ			73.0	0.0	0.0	58.1	78.5	0.31	0.02	0.02			Moderate
95013.0	SANTA MARGARITA RIVER	1397	89.9	1.4	0.116	0.180	TDDT 1.6xPQ	4.60		73.0	91.7	62.1	80.9	50.9	0.16	0.01	0.02			Moderate
85010.0	NEWPORT BAY (819)	1421	98.6	2.5	0.216	0.329	TChl 1.2xPQ; TTDDT1.8xEQ	9.30	76.0	74.0	0.0	0.0	50.1	71.7	0.80	0.06	0.08	0.04		Moderate
85007.0	NEWPORT BAY (431)	1418	16.1	0.3	0.070	0.100	PPDDT3.8xPQ	3.30	87.0	93.0	0.0	0.0	0.0	0.0	0.28	0.12	0.53	0.25	.017D	Moderate
85009.0	NEWPORT BAY (705)	1420	47.7	0.9	0.131	0.209	PPDDE2.4xEQ;TDDT1.9xEQ	4.90	87.0	93.0	0.0	0.7	51.1	0.0	0.33	0.17	0.48	0.27		Moderate
95003.0	AGUA HEDIONDA LAGOON.	1382	98.2	2.4	0.144	0.216	PPDDE 1.4xEQ; TDDT 1xEQ			93.0	1.6	76.2	77.3	0.0	0.44	0.13	0.01			Moderate
95014.0	AGUA HEDIONDA LAGOON (179	1413	84.6	1.5	0.107	0.161	TDDT 1xPQ	6.70	89.0	76.0	55.6	94.7	91.7	61.4	0.35	0.01	0.02	0.04		Low
95023.0	SAN ELIJO LAGOON (18)	1434	74.9	3.0	0.181	0.304	None	13.00	87.0	78.0	0.0	0.3	29.1	0.0	0.08	0.12	0.36	0.40	.064D,F	Low
85006.0	NEWPORT BAY (1009)	1392	54.7	1.1	0.318	0.426	Hg 2.5xPQ	8.50		79.0	0.0	0.0	23.2	93.7	0.66	0.15	0.07			Low
95008.0	OCEANSIDE HARBOR (110)	1393	82.2	1.3	0.128	0.214	None	8.70		79.0	0.0	0.0	69.7	94.5	0.48	0.01	0.03			Low
95010.0	SAN ELIJO LAGOON (24)	1394	81.8	2.7	0.088	0.147	None	5.80		80.0	0.0	0.7	55.6	0.0	0.11	0.16	0.16		.166D,F	Low
95020.0	OCEANSIDE HARBOR .	1431	69.1	1.3	0.157	0.262	Cu 1.1xPQ		81.0	80.0	81.3	96.5	94.6	78.2	0.36	0.03	0.04	0.01		Low
85017.0	NEWPORT BAY (UNIT II BASIN)	1428	62.5	1.9	0.256	0.373	TChl 2.5xPQ		93.0	81.0	0.0	1.3	79.8	95.9	0.29	0.06	0.27	0.14		Low
95019.0	OCEANSIDE HARBOR (90)	1430	79.3	2.5	0.158	0.262	TChl 1.1xPQ	7.50	78.0	82.0	91.0	96.1	94.6	65.7	0.45	0.03	0.05	0.01		Low
85016.0	NEWPORT BAY (Y'MANS COVE)	1427	27.8	0.6	0.163	0.247	None		89.0	85.0	80.9	97.4	97.1	86.3	0.85	0.01	0.06	0.04		Low
85003.0	NEWPORT BAY (791)	1389	32.8	0.7	0.147	0.212	PPDDE 1xEQ	19.30		72.0	0.0	0.0	1.7	91.1	0.80	0.02	0.06			Low
95017.0	DANA POINT HARBOR(ST.DR.)	1416	70.0	1.0	0.169	0.280	None		96.0	87.0	67.3	95.8	94.1	66.8	0.66	0.02	0.03	0.04		Low
95021.0	OCEANSIDE HARBOR	1432	95.3	0.7	0.153	0.234	None		81.0	87.0	36.0	93.0	95.2	61.5	0.26	0.03	0.08	0.01		Low
95025.0	SANTA MARGARITA RIVER (48)	1436	65.7	0.8	0.077	0.123	None	1.70	81.0	88.0	0.0	0.0	70.5	0.0	0.29	0.04	0.12	0.05		Low
85018.0	NEWPORT BAY (UNIT I BASIN)	1429	29.3	0.4	0.093	0.152	None		86.0	89.0	0.0	0.0	1.8	28.9	0.20	0.09	0.70	0.15		Low
85004.0	NEWPORT BAY (877)	1390	67.5	1.1	0.198	0.290	TDDT 1.5xEQ	16.20		70.0	0.0	0.0	33.8	91.9	0.86	0.02	0.03			Low
95015.0	AGUA HEDIONDA LAGOON (212	1414	30.0	0.6	0.066	0.103	None	2.30	86.0	95.0	0.0	0.0	0.0	95.8	0.81	0.05	0.09	0.13		Low
95026.0	AGUA HEDIONDA LAGOON (144	1412	62.5	1.0	0.076	0.117	None	6.20	91.0	95.0	26.0	31.5	87.0	73.9	0.65	0.02	0.03	0.02		Low

PQ or EQ= PEL or ERM Quotients for indicated chemicals. Shading indicates significant toxicity or benthic degradation. Shading in NH3 and H2S indicates toxic concentration to indicated species.
Under H2S column, D or F = toxicity to sea urchin Development or Fertilization, respectively. Consult Appendix A for list of abbreviations.

Newport Island (Station No. 85014) also had relatively high chemical contamination coupled with significant toxicity to amphipods and sea urchins. Three chemicals had elevated concentrations at this station: copper, total chlordane, and mercury. This sample was significantly toxic to both amphipod species. The un-ionized ammonia concentration in the sea urchin development test was above the effect level for this species. The Benthic Index indicated moderate impacts at this site.

Station No. 396 in Dana Point Harbor had elevated TBT, copper and total chlordane concentrations. This station was significantly toxic to sea urchin fertilization and had a Benthic Index indicating significant impacts. Station No. 1435 in San Dieguito Lagoon demonstrated elevated dieldrin concentrations, coupled with significant toxicity to amphipods (*Rhepoxynius abronius*) and sea urchin fertilization. The Benthic Index at this station also demonstrated significant impacts.

The remaining station having the highest chemical contamination was Arches Storm Drain in Newport Bay (Station No. 85015). This station had particularly high total chlordane concentrations. However, this sample was relatively non-toxic to amphipods, and toxicity to sea urchin embryos was apparently due to high ammonia. The Benthic Index at this site indicated moderate impacts. It should be noted that this station had a relatively high TOC value (3.8% TOC), which could have effectively reduced bioavailability of neutral organic compounds such as chlordane.

Several of the coastal lagoon stations were significantly toxic to amphipods and sea urchins and demonstrated significantly impacted benthic community structure. Most of these stations however, were not highly contaminated by the compounds analyzed. For example, the 3 stations from Los Penasquitos Lagoon (Station No.s 95006, 95007, and 95018) produced the lowest survival of amphipods (*Rhepoxynius abronius*) of any of the stations tested. Two of these stations were significantly toxic using the sea urchin fertilization test, which, unlike the sea urchin development test, is not influenced by elevated un-ionized ammonia concentrations. The Benthic Index was 0.17, 0.14 and 0.18, for Stations 95006, 95007, and 95018, respectively, indicating significant impacts to benthic community structure. Thus, the toxicity test and benthic community data indicate negative impacts at these stations. The chemistry data, however, indicate minimal contamination. None of these stations had chemical concentrations exceeding the ERM or PELs for the compounds analyzed, and two (95007, and 95018) had no ERL or TEL exceedances. Although impacts on benthic community structure might be associated with high sedimentation, low dissolved oxygen, and extremes in salinity at these sites, these factors are mitigated in the laboratory exposures through aeration of the test containers and test water salinity adjustment.

Evidence indicates that these sites are impacted and require additional efforts to explain the observed results. This might be addressed through application of Toxicity Identification Evaluations (TIEs) coupled with expanded organic chemistry analysis.

CONCLUSIONS

1. By combining resources in a cooperative agreement between the SWRCB, NOAA, and EPA , this study achieved the combined program objectives of the State Water Resources Control Board's Bay Protection and Toxic Cleanup Program, NOAA's Status and Trends Program, and EPA's Environmental Monitoring and Assessment Program.

2. Using a weight-of-evidence approach based on the Sediment Quality Triad, measures of chemical contamination, toxicity, and benthic community structure were completed at 43 stations to determine relative degradation in selected Southern California bays, estuaries and lagoons. When combined with measures of other sediment characteristics such as grain size, TOC, un-ionized ammonia, and hydrogen sulfide, these measures were useful for prioritizing sites for further investigations.

The data set was limited by lack of the following information: sediment Acid-Volatile Sulfides and Simultaneously Extracted Metals (AVS-SEM), which limited conclusions regarding metal bioavailability; lack of *in situ* measures of dissolved oxygen concentrations, which limited conclusions regarding effects of anoxia on benthic community structure. Additional un-measured factors which may have influenced benthic community structure included seasonal variations in salinity and temperature.

3. Degree of chemical contamination was assessed using two sets of sediment quality guidelines: the ERL/ERM guidelines developed by NOAA (Long et al., 1995), and the TEL/PEL guidelines developed for the State of Florida (MacDonald, 1996). Relative to these guidelines, Total DDT, Total Chlordane, Copper, Mercury, and Zinc were found to be the chemicals or chemical groups of greatest concern. Chemical contamination in the bays and estuaries studied was generally considered to be low in most areas and moderate in a few areas relative to other more highly industrialized areas.

4. In this study, 30 of the 43 stations sampled were selected using a stratified random (EMAP)

sampling design intended to assess the spatial extent of toxicity. The remaining 13 samples were selected using a directed point sampling design intended to investigate potential toxic hotspots. Using toxicity information from the randomly selected stations, 58% of the total randomly-sampled study area were significantly toxic to *Rhepoxynius abronius*. Using the sea urchin development test, 91, 83, and 51% of the randomly-sampled study area was significantly toxic using 100, 50, and 25% pore water concentrations, respectively. Forty-three percent of the randomly-sampled study area was toxic to sea urchin fertilization using 100% pore water.

5. Exceedances of toxicity thresholds were determined using two approaches: the first approach compared sample toxicity to a laboratory negative control; the Reference Envelope Approach compared sample toxicity to a reference population. Using the t-test-control, 53% of the 43 solid-phase samples tested with the amphipod *Rhepoxynius abronius* were significantly toxic. Using the t-test-control approach, 81% and 53% of the 43 interstitial water samples tested were toxic to sea urchin (*Strongylocentrotus purpuratus*) development and fertilization, respectively. The reference envelope approach was a more conservative indicator of toxicity. Six sites were considered to be adequate reference sites based on lack of chemical contamination and un-degraded benthic community structure. Using this approach 12% of the 43 solid-phase samples tested with the amphipod *Rhepoxynius abronius* were significantly toxic, and 47% of the 43 interstitial water samples tested were toxic to sea urchin (*Strongylocentrotus purpuratus*) fertilization. A reference envelope threshold could not be calculated for *Ampelisca* survival because of the limited size of the data set. A reference envelope could not be calculated for sea urchin development because of high variability in this test at the selected reference sites.

6. Strong correlations were found in the relationship between bulk sediment concentrations of PAHs and Aroclors (1254, 1260) and the responses of the screening test, P450 RGS. This cellular response would be expected from the CYP1A1 inducing compounds included in these mixtures. The RGS assay results also showed a significant negative correlation with the development of urchin embryos exposed to 50 and 100% pore water. These data suggest that some of the compounds detected by the RGS assay may be responsible for the adverse affects on development of echinoderm embryos. Survival of the amphipod (*Rhepoxynius abronius*) was negatively associated with DDE, PCB52, un-ionized ammonia, two metals, and fine-grained sediment. *Ampelisca* survival was negatively associated with PCBs and several metals. Sea urchin embryo development was negatively associated with two metals, chlordanes, and DDT compounds. There was a strong negative correlation between sea urchin embryo development and interstitial water un-ionized ammonia concentrations.

7. Benthic community structure was assessed using a Benthic Index, calculated based on measures of the Total Number of Fauna, Number of Crustacean Species, and Numbers of Positive and Negative Indicator Species. Using this index, 15 of the 43 stations sampled (35%) were considered to be significantly degraded; 10 of the 15 degraded stations were located in 4 of the coastal lagoons sampled. Benthic community degradation was not strongly associated with measured bulk-phase chemicals. The Benthic Index was negatively correlated with interstitial water hydrogen sulfide concentrations, indicating that sediment anoxia influenced benthic community structure, particularly in the coastal lagoons.

8. Interlaboratory comparisons of solid-phase samples between the Marine Pollution Studies Laboratory (MPSL) and the Southern California Coastal Water Research Project (SCCWRP) using the amphipod *Ampelisca abdita* demonstrated comparable results for all but one sample. Interlaboratory comparisons of interstitial water toxicity using the sea urchin development test with *Strongylocentrotus purpuratus* were less consistent. Higher toxicity in the samples tested at SCCWRP were associated with greater un-ionized ammonia concentrations.

9. Comparisons of the two amphipods (*Rhepoxynius abronius* and *Ampelisca abdita*) using the 30 randomly selected samples showed lower survival, overall, using *Rhepoxynius*. While 12% of the samples tested were significantly toxic to *Ampelisca* based on a t-test comparison to the negative control value, 40% of the samples were significantly toxic to *Rhepoxynius*.

10. Results using the 30 stratified random samples generally demonstrated greater toxicity but comparable benthic community degradation when compared to the 13 samples selected using the directed point sampling design. Samples having the greatest chemical contamination were selected using the directed point sampling design.

11. All measures of sediment contamination and degradation proved useful in this study. Stations recommended for further investigation were prioritized to help direct future investigations by State and Regional Water Board staff. Each station receiving a high, moderate or low priority ranking met one or more of the criteria under evaluation for determining hotspot status in the Bay Protection Toxic Cleanup Program. Those meeting all of the criteria were designated with the highest priority for future investigation.

Four stations were given the highest priority ranking: two were in Newport Bay (Station No.s 85013 and 85014) and one each was designated with the highest ranking in Dana Point Harbor (No. 95016) and San Dieguito Lagoon (95024). Twenty-one stations were designated with

moderate rankings, and 17 stations were designated with the lowest ranking. One station was not ranked because more information is needed to rank it.

Future actions, if any, at sites receiving the highest priority ranking will be left to staff of the appropriate Regional Water Quality Control Boards (Santa Ana Region and San Diego Region). Additional information might be necessary to determine areal extent of contamination and associated effects, spatial and temporal variability of contaminant effects, and causes of toxicity (such as those identified through Toxicity Identification Evaluations - TIEs). Any site remediation such as source control and/or toxic hotspot cleanup will be dictated by regional board staff.

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