SEDIMENT CHEMISTRY, TOXICITY AND BENTHIC COMMUNITY CONDITIONS IN SELECTED WATER BODIES OF THE SANTA ANA REGION

FINAL REPORT

California State Water Resources Control Board
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EXECUTIVE SUMMARY

The following report describes and evaluates chemical and biological data collected from the Santa Ana Region between September 1992 and August 1997. The study was conducted as part of the Bay Protection and Toxic Cleanup Program, a legislatively mandated program designed to assess the degree of chemical pollution and associated biological effects in California's bays and estuaries. The workplan for this study resulted from a cooperative agreement between the State Water Resources Control Board (SWRCB) and the National Oceanic and Atmospheric Administration (NOAA). Monitoring and reporting aspects of the study were conducted by the Environmental Services Division, of the California Department of Fish and Game, and its subcontractors.

Using a weight-of-evidence approach, various components of the Sediment Quality Triad were measured at 96 stations to determine the relative degradation in selected Southern California water bodies. All stations received toxicity analyses, 57 stations received sediment chemistry analyses, and 37 stations received benthic analyses. The Santa Ana Region (Region 8) was divided into three distinct water bodies to aid in data interpretation. Multiple stations were sampled from 12 sites in Anaheim Bay, 8 sites in Huntington Harbor and 22 sites in Newport Bay.

Degree of chemical contamination was assessed using sediment quality guidelines (ERL/ERM) developed by NOAA (Long et al., 1995). Stations were defined as having elevated chemistry if the mean ERM quotients were greater than 0.500, if more than five ERM guideline values were exceeded, or if individual chemicals were at concentrations high enough to likely be associated with biological effects. Five stations had elevated chemistry: one from Anaheim Bay (82030.0), one from Huntington Harbor (80028.3) and three from Newport Bay (85013.0, 85014.0, 85015.0). Relative to the chemistry guidelines, p,p'DDE, total chlordane, total PCB, copper, mercury, and zinc were found to be the chemicals or chemical groups of greatest concern.

Determinations of the statistical significance of toxicity test results were assessed using the t-test/Minimum Significant Difference (MSD) approach to compare sample toxicity to a laboratory negative control. A sample was considered toxic if: 1) there was a significant difference in mean organism response between a sample and the control as determined using a separate-variance t-test, and 2) if the mean organism response in the toxicity test was less than the MSD value as a percent of the control. Using the t-test/MSD approach, 41% of the 96 solid-phase samples tested with amphipods (*Eohaustorius* and *Rhepoxynius*) were significantly toxic. Ninety-five percent of the 56 interstitial water samples tested at 100% concentration were significantly toxic in larval development (abalone and purple urchin) tests.

There were several negative associations between toxicity test results and chemical compounds measured in bulk-phase samples. Amphipod survival from the entire region was negatively correlated with several metals and fine-grained sediments. Newport Bay amphipod survival was negatively correlated with metals, total chlordane and total PCB. Purple urchin larval development in 100% porewater was correlated with several metals, total chlordane, several DDT metabolites, tributyltin and total PCB. There was a strong negative correlation between sea urchin embryo development and pore water un-ionized ammonia concentrations.

Benthic community structure was assessed using a Relative Benthic Index (RBI) calculated based on measures of the total number of fauna, number of crustacean species, and numbers of positive and negative indicator species. The RBI ranged from 0.00 (degraded) to 1.00 (undegraded). Based on this index, 4 of the 37 stations sampled for benthic structure (11%) were significantly degraded. All four stations were from central Newport Bay (85005.0, 85010.0, 85011.0, 85012.0). Benthic community degradation was significantly correlated with several metals, several DDT metabolites and fine-grained sediments.

Principle Components Analysis (PCA) indicated significant relationships between RBI, amphipod survival and fine-grained sediments. PCA also revealed significant associations between *Ampelisca* survival and chemicals exceeding ERM guideline values in Newport Bay. Urchin development in porewater was also significantly associated with chemicals that had exceeded ERM guidelines (total chlordane, p,p'DDE and Zn).

All stations were categorized to help direct future investigations by State and Regional Water Board staff. Each station was placed in one of eight categories based on the degree of elevated chemical contamination, recurrent toxicity and degraded benthos. Categories ranged from Category 1, which included stations with elevated chemistry, recurrent toxicity and degraded benthos, to Category 8, which were reference stations.

There were no stations listed in Categories 1 through 3. One station from Anaheim Bay was listed in Category 4 (82030.0), and four stations were listed in Category 5. These two categories included stations with elevated chemistry and varied biological impacts. Category 5 stations included Upper Huntington Harbor (80028.3), and three from Newport Bay (85013.0, 85014.0, and 85015.0). The remaining stations were listed under Category 6, biological impact with no elevated chemistry, and Category 7, no biological impact or elevated chemistry.

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TABLE OF CONTENTS

EXECUTIVE SUMMARY I

ACKNOWLEDGEMENTS III

TABLE OF CONTENTS IV

LIST OF FIGURES V

LIST OF TABLES VI

LIST OF APPENDICES VII

LIST OF ABBREVIATIONS VIII

INTRODUCTION 1

PURPOSE 1
PROGRAMMATIC BACKGROUND AND NEEDS 1
STUDY AREA 2

METHODS 7

SAMPLING DESIGN 7
SAMPLE COLLECTION AND PROCESSING 9
TRACE METALS ANALYSIS OF SEDIMENTS 15
ACID VOLATILE SULFIDE AND SIMULTANEOUSLY EXTRACTED METALS – AVS-SEM 17
TRACE ORGANIC ANALYSIS OF SEDIMENTS (PCBS, PESTICIDES, AND PAHS) 17
TOTAL ORGANIC CARBON ANALYSIS OF SEDIMENTS 22
GRAIN SIZE ANALYSIS OF SEDIMENTS 23
TOXICITY TESTING 24
BENTHIC COMMUNITY ANALYSIS 34
DATA ANALYSIS 38
WEIGHT-OF-EVIDENCE AND CATEGORIZATION OF SITES 45
QUALITY ASSURANCE/QUALITY CONTROL 48

RESULTS AND DISCUSSION 48

CHEMISTRY DATA 48
TOXICITY TESTING 62
BENTHIC COMMUNITY ANALYSIS 81
PRINCIPAL COMPONENTS ANALYSIS RESULTS 85
STATION CATEGORIZATION 87

CONCLUSIONS 94

REFERENCES 95

APPENDICES 105

LIST OF FIGURES

FIGURE 1. LOCATION OF REGION 8 STUDY AREAS....4
FIGURE 2A-B. STATION LOCATIONS FOR SITES IN ANAHEIM BAY AND HUNTINGTON HARBOR....5
FIGURE 2C. STATION LOCATIONS FOR SITES IN NEWPORT BAY....6
FIGURE 3. SEDIMENT-WATER INTERFACE EXPOSURE SYSTEM....32
FIGURE 4. FREQUENCY OF STATIONS EXCEEDING ERL OR ERM SEDIMENT QUALITY GUIDELINES....50
FIGURE 5A-B. SPATIAL DISTRIBUTION OF ERM QUOTIENTS IN ANAHEIM BAY AND HUNTINGTON HARBOR....54
FIGURE 5C. SPATIAL DISTRIBUTION OF ERM QUOTIENTS IN NEWPORT BAY....55
FIGURE 6A-B. SPATIAL DISTRIBUTION OF TOTAL CHLORDANE IN ANAHEIM BAY AND HUNTINGTON HARBOR....57
FIGURE 6C. SPATIAL DISTRIBUTION OF TOTAL CHLORDANE IN NEWPORT BAY....58
FIGURE 7A-B. SPATIAL DISTRIBUTION OF P,P'DDE IN ANAHEIM BAY AND HUNTINGTON HARBOR....59
FIGURE 7C. SPATIAL DISTRIBUTION OF P,P'DDE IN NEWPORT BAY....60
FIGURE 8. SPATIAL DISTRIBUTION NEWPORT BAY SELECTED METALS AND TOTAL PCB CONCENTRATIONS....61
FIGURE 9A-B. SPATIAL DISTRIBUTION OF AMPHIPOD TOXICITY IN ANAHEIM BAY AND HUNTINGTON HARBOR....68
FIGURE 9C. SPATIAL DISTRIBUTION AMPHIPOD TOXICITY IN NEWPORT BAY....69
FIGURE 10A-B. SPATIAL DISTRIBUTION OF POREWATER TOXICITY IN ANAHEIM BAY AND HUNTINGTON HARBOR....74
FIGURE 10C. SPATIAL DISTRIBUTION OF POREWATER TOXICITY IN NEWPORT BAY....75
FIGURE 11. ASSOCIATION OF UN-IONIZED AMMONIA CONCENTRATION AND PURPLE URCHIN DEVELOPMENT....79
FIGURE 12. ASSOCIATION OF UN-IONIZED AMMONIA CONCENTRATION AND *AMPELISCA* SURVIVAL....79
FIGURE 13A. NEWPORT BAY AMPHIPOD TOXICITY VERSUS ERM QUOTIENT....80
FIGURE 13B. REGION WIDE AMPHIPOD TOXICITY VERSUS ERM QUOTIENT....80
FIGURE 13C. REGION WIDE PURPLE URCHIN TOXICITY VERSUS ERM QUOTIENT....80
FIGURE 14A-B. SPATIAL DISTRIBUTION OF BENTHIC INDICES IN ANAHEIM BAY AND HUNTINGTON HARBOR....83
FIGURE 14C. SPATIAL DISTRIBUTION OF BENTHIC INDICES IN NEWPORT BAY....84

LIST OF TABLES

TABLE 1. SUMMARY OF REGION 8 SAMPLING DESIGN AND SITES SAMPLED 8
TABLE 2. DRY WEIGHT TRACE METAL MINIMUM DETECTION LIMITS (MDL).. 16
TABLE 3. DRY WEIGHT MINIMUM DETECTION LIMITS OF CHLORINATED PESTICIDES. 18
TABLE 4. DRY WEIGHT DETECTION LIMITS OF NIST PCB CONGENERS. 19
TABLE 5. DRY WEIGHT MINIMUM DETECTION LIMITS FOR ADDITIONAL PCB CONGENERS. 19
TABLE 6. DRY WEIGHT MINIMUM DETECTION LIMITS OF CHLORINATED TECHNICAL GRADE MIXTURES..... 20
TABLE 7. DRY WEIGHT MINIMUM DETECTION LIMITS OF POLYAROMATIC HYDROCARBONS IN TISSUE..... 20
TABLE 8. DRY WEIGHT MINIMUM DETECTION LIMITS OF ORGANOMETALIC COMPOUNDS..... 20
TABLE 9. COMPARISON OF SEDIMENT SCREENING LEVELS DEVELOPED BY NOAA AND THE STATE OF FLORIDA..... 40
TABLE 10. UPPER PERCENTILE CONCENTRATIONS OF BPTCP CHEMICALS FOR WHICH THERE ARE NO ERL OR ERM SEDIMENT GUIDELINE VALUES..... 41
TABLE 11. MINIMUM SIGNIFICANT DIFFERENCES USED TO CALCULATE SIGNIFICANT TOXICITY IN BPTCP TOXICITY TEST PROTOCOLS. 43
TABLE 12. UNIONIZED AMMONIA AND HYDROGEN SULFIDE EFFECTS THRESHOLDS FOR BPTCP TOXICITY TESTS..... 43
TABLE 13. ANAHEIM BAY CHEMISTRY RESULTS..... 51
TABLE 14. HUNTINGTON HARBOR CHEMISTRY RESULTS.. 52
TABLE 15. NEWPORT BAY CHEMISTRY RESULTS.. 53
TABLE 16. CONCENTRATIONS OF SELECTED TRACE METALS IN POREWATER, AND SEM/AVS IN STATION 85013.0... 62
TABLE 17. CONCENTRATIONS OF SELECTED TISSUE CONTAMINANTS FROM STATION 85013.0. 62
TABLE 18. TOXICITY OF ANAHEIM BAY SEDIMENTS TO *RHEPOXYNIUS* AMPHIPODS. 65
TABLE 19. TOXICITY OF HUNTINGTON HARBOR SEDIMENTS TO *RHEPOXYNIUS* AMPHIPODS. 66
TABLE 20. TOXICITY OF NEWPORT BAY SEDIMENTS TO *RHEPOXYNIUS*, *EOHAUSTORIUS* AND *AMPELISCA*. 67
TABLE 21. TOXICITY OF ANAHEIM BAY POREWATER TO ABALONE AND PURPLE URCHIN LARVAL DEVELOPMENT..... 71
TABLE 22. TOXICITY OF HUNTINGTON HARBOR POREWATER TO ABALONE AND PURPLE URCHIN LARVAL DEVELOPMENT..... 72
TABLE 23. TOXICITY OF NEWPORT BAY POREWATER TO PURPLE URCHIN LARVAL DEVELOPMENT AND FERTILIZATION..... 73
TABLE 24. SPEARMAN RANK CORRELATION RESULTS FOR SELECTED TOXICANTS SIGNIFICANTLY CORRELATED WITH AMPHIPOD TOXICITY (*EOHAUSTORIUS* AND *RHEPOXYNIUS*) RESULTS FROM SPECIFIC WATER BODIES..... 77
TABLE 25. SPEARMAN RANK CORRELATION RESULTS FOR SELECTED TOXICANTS SIGNIFICANTLY CORRELATED WITH AMPHIPOD (*EOHAUSTORIUS* AND *RHEPOXYNIUS*) AND URCHIN DEVELOPMENT TOXICITY RESULTS FROM THE ENTIRE REGION. 78
TABLE 26. SUMMARY OF ANAHEIM BAY BENTHIC COMMUNITY INDICES. 81
TABLE 27. SUMMARY OF HUNTINGTON HARBOR BENTHIC COMMUNITY INDICES..... 81
TABLE 28. SUMMARY OF NEWPORT BAY BENTHIC COMMUNITY INDICES..... 82
TABLE 29. SPEARMAN RANK CORRELATION RESULTS FOR SELECTED TOXICANTS SIGNIFICANTLY CORRELATED WITH BENTHIC INDICES. 85
TABLE 30. RESULTS OF PRINCIPLE COMPONENTS ANALYSIS..... 86
TABLE 31. CATEGORIZATION OF REGION 8 STATIONS BASED ON CHEMISTRY, TOXICITY AND BENTHIC ANALYSIS..... 89

LIST OF APPENDICES

Appendix A: Data Base Description
Appendix B: Sampling Data
Appendix C: Analytical Chemistry Data
- Section 1: Trace Metal Concentrations
- Section 2: Trace Metal Concentrations in Porewater
- Section 3: Acid Volatile Sulfides and Simultaneous Extracted Metals Concentrations
- Section 4: Pesticide Concentrations
- Section 5: PCB and Arochlor Concentrations
- Section 6: PAH Concentrations
- Section 7: Chemistry Summations and Quotients
- Section 8: Fish Tissue Chemical Concentrations

Appendix D: Sediment Grain Size and Total Organic Carbon Analyses
Appendix E: Toxicity Test Data
- Section 1: *Rhepoxynius abronius* Survival in Sediment
- Section 2: *Eohaustorius estuarius* Survival in Sediment
- Section 3: *Haliotis rufescens* Larval Shell Development in Subsurface Water
- Section 4: *Haliotis rufescens* Larval Shell Development in Porewater
- Section 5: *Strongylocentrotus purpuratus* Fertilization in Porewater
- Section 6: *Strongylocentrotus purpuratus* Development in Porewater
- Section 7: *Strongylocentrotus purpuratus* Development in Intact Sediment Cores
- Section 8: *Mytilus* Larval Shell Development in Porewater
- Section 9: *Neanthes arenaceodentata* Survival in Sediment
- Section 10: *Neanthes arenaceodentata* Weight Change in Sediment
- Section 11: *Ampelisca abdita* Survival in Sediment
- Section 12: *Ceriodaphnia dubia* Survival in Intact Sediment Cores
- Section 13: *Hyalella azteca* Survival in Sediment
- Section 14: Toxicity Test Quality Assurance Data

Appendix F: Benthic Community Data

LIST OF ABBREVIATIONS

AA	Atomic Absorption
ASTM	American Society for Testing Materials
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
EqP	Equilibrium Partitioning Coefficient
ERL	Effects Range Low
ERM	Effects Range Median
ERMQ	Effects Range Median Summary Quotient
FAAS	Flame Atomic Absorption Spectroscopy
GC/ECD	Gas Chromatograph Electron Capture Detection
GFAAS	Graphite Furnace 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
SCCWRP	Southern Calif. Coastal Waters Research Project
SEM-AVS	Simultaneously Extracted Metals-Acid Volatile Sulfide
SJSUF	San Jose State University Foundation
SPARC	Scientific Planning and Review Committee
SQC	Sediment Quality Criteria
SWRCB	State Water Resources Control Board
T	Temperature
TBT	Tributyltin
TEL	Threshold Effects Level
TFE	Tefzel Teflon®
TIE	Toxicity Identification Evaluation
TOC	Total Organic Carbon
TOF	Trace Organics Facility
UCSC	University of California Santa Cruz
U.S. EPA	U.S. Environmental Protection Agency
WCS	Whole core squeezing

Units

1 part per thousand (ppt) = 1 mg/g

1 part per million (ppm) = 1 mg/kg, 1 µg/g sediment

1 part per billion (ppb) = 1 µg/kg, 1 ng/g sediment

INTRODUCTION

In 1989, the California State legislature established the Bay Protection and Toxic Cleanup Program (BPTCP). One of the primary activities of the BPTCP is monitoring and assessment of sediments in selected California bays and estuaries. The assessment strategy has generally relied upon application of various components of the Sediment Quality Triad in a weight-of-evidence approach to hot spot determination (Chapman et al., 1987).

In 1992, the State Water Resources Control Board (SWRCB) and the National Oceanic and Atmospheric Administration (NOAA) entered into a three-year cooperative agreement to assess the potential adverse biological effects in selected coastal bays and harbors in Southern California (Fairey et al., 1996; Anderson et al., 1997). This report includes results from the first year of this cooperative agreement, which included studies conducted in Anaheim Bay, Huntington Harbor, and the Seal Beach vicinity. In addition, this report contains results of subsequent BPTCP monitoring and assessment studies conducted throughout the Santa Ana Region including the Newport Bay vicinity.

Purpose

Studies were performed in Anaheim Bay, Huntington Harbor, Bolsa Chica, Seal Beach and Newport Bay. The objectives of the study were as follows:

1. Characterize the magnitude and relative spatial distribution of toxicant-associated bioeffects in the above listed water bodies.
2. Determine relationships between concentrations and mixtures of sediment-associated toxicants and the occurrence and severity of bioeffects.
3. Distinguish more severely impacted sediments from less severely impacted sediments.
4. Use a weight-of-evidence approach based on the Sediment Quality Triad to rank and prioritize candidate hot spots for future work.

Programmatic Background and Needs

This study was part of a cooperative agreement between NOAA and the SWRCB and was implemented through the BPTCP. 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.

Although the State Water Board and NOAA have common programmatic needs, they are not identical. NOAA is mandated by Congress to conduct a program of research and monitoring on marine pollution. Much of this research is being conducted through the National Status and Trends (NS&T) Program and the Coastal Ocean Program. The NS&T Program performs regional intensive studies of the magnitude and extent of toxicant-associated bioeffects in selected coastal embayments and estuaries. The areas chosen for these regional studies are those in which the contaminant concentrations indicate the greatest potential for biological effects.

These biological studies augment the regular chemical monitoring activities of the Program, and provide a means of estimating the 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. The BPTCP has four major goals: (1) provide protection of present and future beneficial uses of the bays and estuarine waters of California; (2) identify and characterize toxic hot spots; (3) plan for toxic hot spot cleanup or other remedial or mitigation actions; (4) develop prevention and control strategies for toxic pollutants that will prevent creation of new toxic hot spots or the perpetuation of existing ones within the bays and estuaries of the State.

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, Monterey County. Trace metals analyses were performed by CDFG personnel at the trace metal analytical 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

The BPTCP examined three distinct water bodies in the Santa Ana Region: Anaheim Bay/Seal Beach Naval Weapons Reserve, Huntington Harbor/Bolsa Chica, and Newport Bay (Figure 1). Anaheim Bay and Huntington Harbor are connected via a man-made channel, which was constructed in the late 1800's, but Newport Bay is a distinct water body. Descriptions of the specific water bodies follow.

Anaheim Bay and Huntington Harbor

The Anaheim Bay/Huntington Harbor complex is located on the northern edge of the Orange County coast, approximately 20 miles southeast of Los Angeles. The complex consists of inner and outer Anaheim Bay, Huntington Harbor, and several ecologically significant wetlands such as the Anaheim Bay National Wildlife Refuge and Bolsa Chica Ecological Reserve.

The U.S. Navy controls access through the outer bay (Figure 2a) which serves as the main entrance to the U.S. Naval Weapons Station, Seal Beach. The Navy also operates and manages the National Wildlife Refuge, which is located on their property. Besides the Naval property, the only developed area is a 55-acre partially developed parcel called Sunset Aquatic Regional Park. The area surrounding Huntington Harbor area is primarily residential with small boat marina

activity (Figure 2b). Huntington Harbor has one boatyard facility located in the harbor. The Santa Ana Regional Water Quality Control Board currently regulates boatyard dischargers under a general Boatyard NPDES permit. Land use around the Bolsa Chica Ecological Reserve is primarily oil production with some residential areas.

The inner section of Anaheim Bay and Huntington Harbor receive very little tidal flushing because of the 600-foot wide shipping channel connecting the outer and inner bays and the constriction at the Pacific Coast Highway Bridge. Culverts and tide gates further restrict tidal flow into the wildlife refuge. Outer Bolsa Bay is connected directly to Huntington Harbor and is the only section of the Bolsa Chica Reserve directly open to tidal influence. Inner Bolsa Bay and the rest of the reserve have a tidal regime controlled by flood gates. Because of the muted tidal flow, freshwater inputs have significant impacts on water quality.

Two major storm drains enter the Anaheim Bay/Huntington Harbor complex. The Bolsa Chica flood control channel enters lower Huntington Harbor, and the East Garden Grove Wintersburg flood control channel enters outer Bolsa Bay. These channels, as well as their tributaries, convey runoff from the northern portion of the heavily urbanized Orange County into Huntington Harbor. Inputs of stormwater and urban nuisance flows via these channels are potentially significant sources of pollutant loadings and are being addressed through the county's urban runoff/stormwater permit. Because of metals and pesticide input from urban runoff, and non-point source pollutants, water quality in this area is categorized as impaired by the Regional Water Quality Control Board

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 (Figure 2c). 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, restaurants, marinas, and light marine industry such as boatyards and fuel docks. The Newport Bay watershed encompasses 154 square miles with San Diego Creek being 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 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.

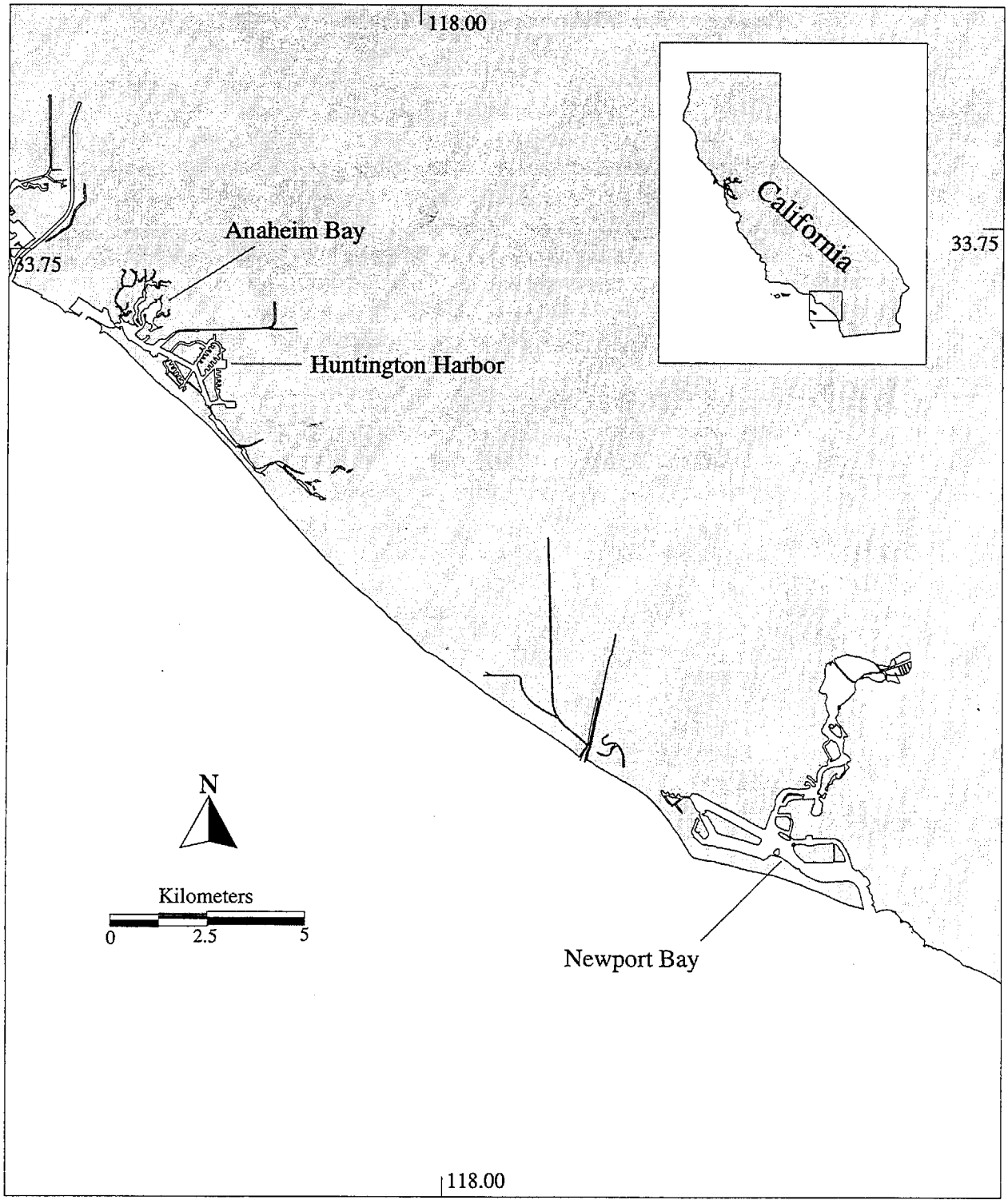
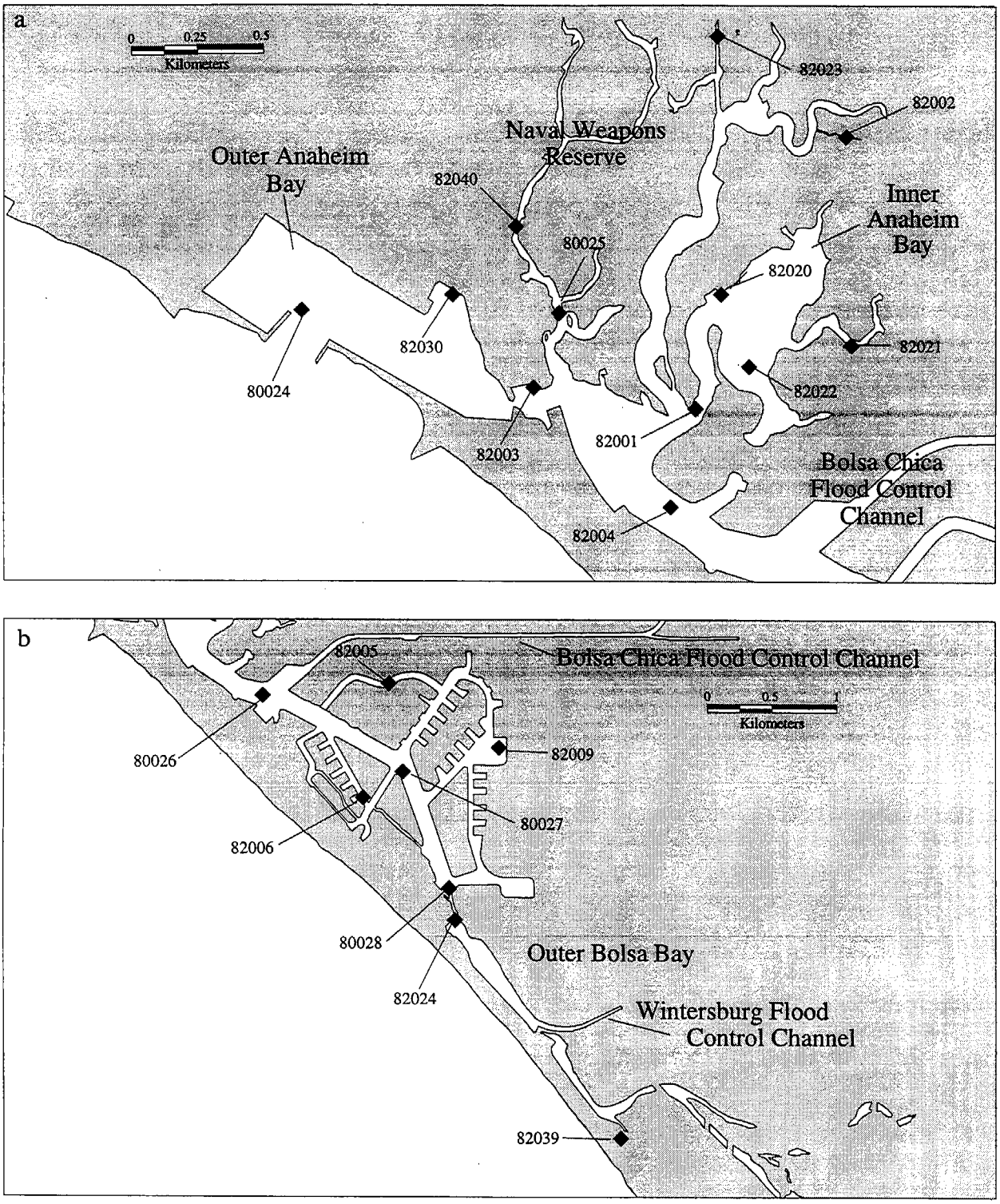


Figure 1. Locations of Region 8 study areas.



Figures 2a and 2b. Station locations for sites in Anaheim Bay and Huntington Harbor.

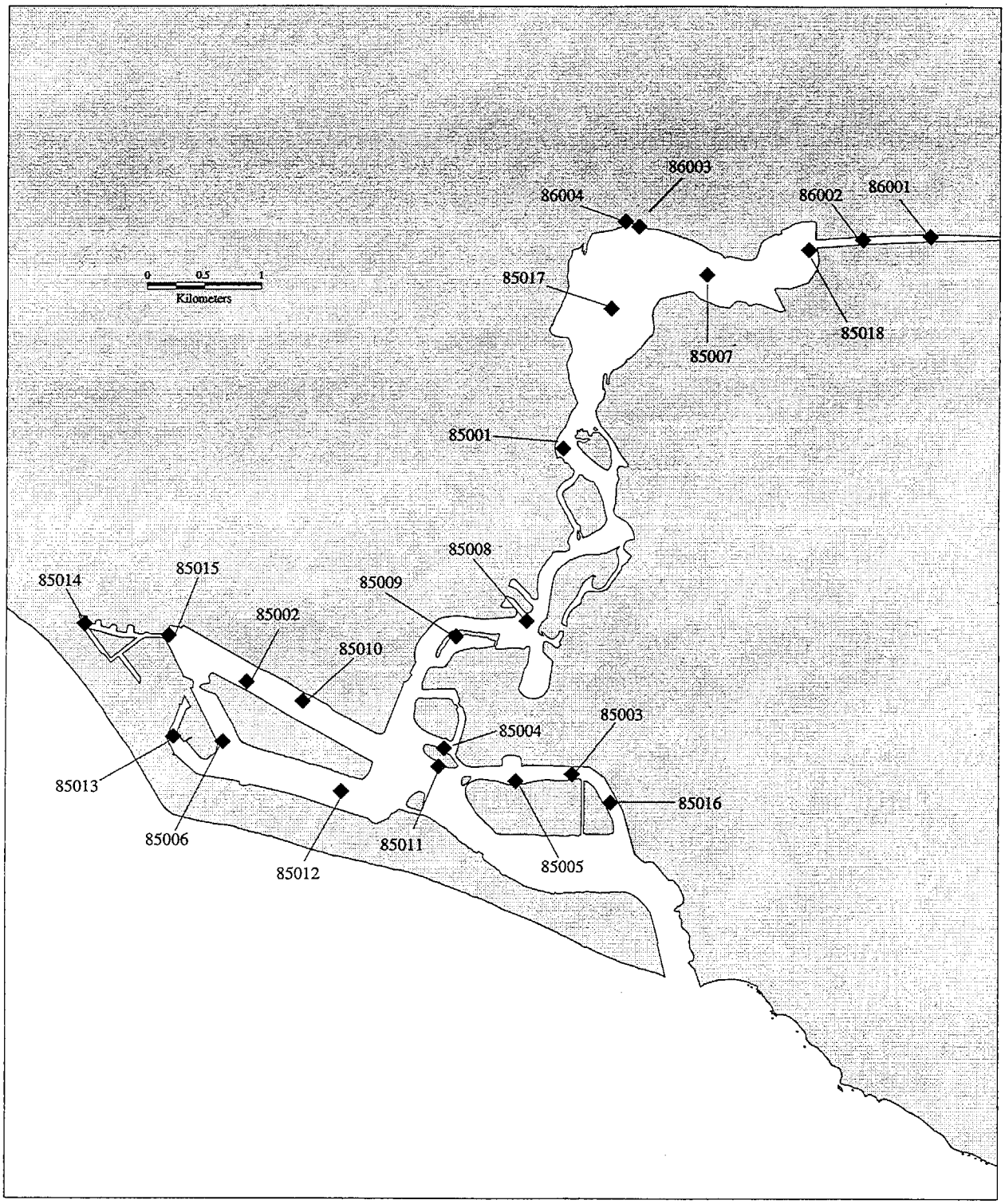


Figure 2c. Station locations for sites in Newport Bay.

METHODS

Sampling Design

Sampling for the Santa Ana Region was conducted in 14 separate sampling periods (Legs), over a five-year period from September 1992 to August 1997 (Table 1). In general, the BPTCP monitoring strategy was designed to proceed in two phases with an initial screening phase followed by confirmation studies. Screening studies typically consisted of some component(s) of the Sediment Quality Triad (Toxicity, Chemistry, and/or Benthics after Chapman et al., 1987), and confirmation studies were designed to include additional toxicity monitoring, as well as chemistry, and benthic community structure as warranted. The initial Legs of the Santa Ana Region monitoring (Legs 4 and 5) were conducted as a cooperative monitoring study between the BPTCP and the NOAA Status and Trends program, as described above. Later Legs combined screening surveys in water bodies not recently monitored, confirmation studies at stations previously demonstrating toxicity or high chemistry, and surveys to locate appropriate reference sites for inclusion in reference envelope determinations (not included in this region).

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. Of the 96 samples collected, 66 were collected from directed point sampled stations and 30 were collected from randomly sampled stations. Samples were collected for screening during 1992 and 1993, while confirmation samples were collected from 1994 to 1997. Samples collected in Newport Bay as part of the Southern California EMAP study were considered part of the screening phase.

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

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

The random sample design was implemented in Newport Bay as part of the Southern California EMAP study. The following method was used to locate the random sampling stations. A grid of hexagons was laid down over a topographic map of the area demarcating the suitable sampling area. Each hexagon was used to locate a single random point. The points within the area were counted, and a selection probability for the area was computed by dividing the desired number of points in the area by the total number of points. 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 the bay.

This phase of work was intended to give a broad assessment of toxicity throughout the Santa Ana Region using multiple test species and toxicity endpoints. Chemical analysis was performed on selected samples in which toxicity results prompted further analysis. Stations that met certain criteria during the screening phase, or during the random sampling phase, were then selected for a second round of sampling, termed the "confirmation phase". During this phase additional toxicity monitoring, chemical analysis, or benthic analysis was performed. Evidence from this two step process was used to establish a higher level of certainty for the ranking of stations.

From the combined sampling designs, a total of 96 samples were collected from 52 sites in the Santa Ana Region. Site locations that were sampled more than once were always resampled at the original location using navigational equipment and lineups. Bioassay tests, grain size and total organic carbon analyses were performed on all 96 samples. Trace metal analysis and trace synthetic organic analysis was performed on 57 samples. Benthic community analysis was performed on 36 samples.

Table 1. Summary of Region 8 sampling design and sites sampled

Leg	Date	Screening/ Confirmation	Sampling Design	Sites Sampled
4	9/15/92	screening	directed - triangle format around site – stations 100 meters apart	80024.1, 80024.2, 80024.3, 80026.1, 80026.2, 80026.3, 80027.1, 80027.2, 80027.3, 80028.1, 80028.2, 80028.3
5	10/13/92	screening	directed - triangle format around site – stations 100 meters apart	80025.1, 80025.2, 80025.3
9	12/9/92	screening	directed - single site	82001.0, 82002.0, 82003.0, 82004.0, 82005.0, 82006.0, 82009.0, 82020.0, 82021.0, 82022.0, 82023.0, 82024.0, 82030.0, 82039.0, 82040.0
17	4/19/93	screening	directed - single site	82020.0, 82023.0, 82024.0, 82030.0
19	5/28/93	screening	directed - single site	80024.3, 82002.0, 82009.0
25	2/3/94	confirmation	directed - triangle format around station – sub-replicates 50 meters apart	82030.0
26	2/14/94	confirmation	directed - triangle format around station – sub-replicates 50 meters apart	82001.0, 82002.0, 82023.0, 82040.0,
29	3/31/94	confirmation	directed - triangle format around station – sub-replicates 20 to 40 meters apart	80024.3, 80027.3, 80028.3
30	4/18/94	confirmation	directed - triangle format around station – sub-replicates 20 to 40 meters apart	82005.0, 82030.0, 82039.0
32	5/22/94	confirmation	directed - single site	82030.0
34	9/8/94	screening	random - EMAP methods	85001.0, 85002.0, 85003.0, 85004.0, 85005.0, 85006.0
36	9/26/94	screening	random - EMAP methods	85007.0, 85008.0, 85009.0, 85010.0, 85011.0, 85012.0, 85013.0, 85014.0, 85015.0, 85016.0, 85017.0, 85018.0
45	6/24/96	confirmation	directed - single site	85001.0, 85013.0
54	8/22/97	confirmation	directed - single site	85001.0, 86001.0, 86002.0, 86003.0, 86004.0

Sample Site Selection

Over the course of the program sites were sampled in three different ways. In the first screening legs, individual sites consisted of three field replicates, referred to as stations. Each station was located approximately 100 meters apart at the points of a triangle centered over the site. Sites are recognized by a 5-digit number, with a decimal place indicating the station (80024.1 = site 80024, station 1). More detailed information on spatial distributions of chemical pollution and toxicity were required for individual stations. In these cases, additional sub-replicates were sampled around one of the field replicates, or points of the triangle. These sub-replicates were sampled in a tight group around the station location and located approximately 50 meters apart. In some cases, particularly confirmation legs, no field replication was included in the sampling design. In this report, unless otherwise stated, all stations are treated separately for discussion of spatial distribution of chemical pollution and bioeffects. Areal extent of pollution and bioeffects around a particular site are inferred from field replicate data only when sufficient information is available. The Magellan Global Positioning System and reference photographs were used to precisely locate the sites for repeat visits. Table 1 summarized BPTCP sampling legs, dates, methods and sites for the Santa Ana Region.

Sample Collection and Processing

Summary of Methods

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

Cleaning Procedures

All sampling equipment (*i.e.*, containers, container liners, scoops, and 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 grab) was cleaned by using the following sequential process: two-day soak and wash in Micro® detergent, three tap-water rinses, three deionized water rinses, a three-day soak in 10% HCl, three ASTM Type II Milli-Q® water rinses, air dry, three petroleum ether rinses, and air dry.

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

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

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

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

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

Sediment Sample Collection

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

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

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

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

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

Intact sediment cores were sampled directly Van Veen grab sampler at selected stations for later sediment-water interface toxicity tests. Cores were 7.5 cm in diameter, and sampled to a depth

of 5 cm. Cores were removed from the sampler by sealing the bottom of the core by hand, and then sealing first the bottom, then the top with polyethylene caps. The bottom caps were then wrapped with parafilm® to prevent leakage, and the cores were stored upright in a cooler. Intact cores were refrigerated in the dark until used in toxicity tests. Sediment-water interface test methods are described below.

Subsurface water samples were collected by attaching a polyethylene water sample bottle to the frame of the grab. As the jaws of the grab closed to collect a sediment sample, a stopper was pulled from the sample bottle, and it filled. The water sample was consequently collected approximately 0.5 meters above the sediment surface. Samples were transferred to pre-cleaned, labeled sample bottles and placed in coolers.

Fish Tissue Sampling

Fish species targeted for collection were selected and prioritized based on relative abundance of species of interest; species behavior (e.g., feeding behavior); and habitat range; frequency of consumption by anglers; likelihood of contaminant accumulation based on tissue lipid content. Composite tissue samples were necessary to maximize the number of stations and fish species on which chemical analysis could be performed. The number of fish required to complete a composite was five for larger fish and fifteen for smaller fish. Fish species collected and number of fish needed to complete a composite were as follows:

1. White Croaker *(Genyonemus lineatus)* (5 per composite)
2. White Surfperch *(Phanerdon furcatus)* (5 per composite)
3. Shiner Surfperch *(Cymatogaster aggregata)* (15 per composite)
4. Topsmelt *(Atherinops affinis)* (15 per composite)

Collected samples were wrapped in chemically cleaned Teflon® sheeting, to prevent trace metal and trace organic contamination, and frozen for transportation to the laboratory. Dissections and muscle tissue sample preparations were performed using non-contaminating methods in a clean room environment (Stephenson et al., 1994). Equal weight samples were taken from each fish using Teflon® forceps to provide a composite total of approximately 125 grams. All composites were homogenized and homogenate splits were taken for each chemical analysis.

Muscle tissue (i.e.- fillets) of white croaker were analyzed with skin on, while topsmelt and perch were analyzed whole body (i.e.- head, guts, tail removed). The decision to analyze tissue filets or whole body was based on the manner that the particular fish was most commonly cooked and eaten.

All sample composites were analyzed for, PAHs, PCB congeners, pesticides, percent moisture and percent lipid. A more detailed description of these methods can be found in the California State Mussel Watch Program Ten Year Data Summary Report (Phillips, 1988) and the California Bay Protection and Toxic Cleanup Program Quality Assurance Project Plan (Stephenson et al., 1994).

The U.S. EPA document used to design the study, Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories-Volume 1-Fish Sampling and Analysis (U.S. EPA, 1995a), was also used to develop the contaminant screening values used in this study. In developing the screening values (SVs) for a number of noncarcinogenic and carcinogenic compounds, risk-based dose response variables were used. These variables were used in the following equations to calculate the SVs used in this study:

For Noncarcinogens: $SV = (RfD * BW)/CR$
For Carcinogens: $SV = [(RL/SF)*BW]/CR$

where
SV = Screening Value (μg/g)
RfD = Oral reference dose (μg/g/d)
RL = Maximum acceptable risk level (dimensionless)
SF = Oral slope factor $(\mu g/g/d)^{-1}$
BW = Body Weight (kg)
CR = Consumption rate of tissue (g/d)

Body weight (BW), consumption rate (CR) and risk level (RL) have been held constant for all calculations in this document. Body weight was chosen at 70 kg, which is the mean body weight for the average male adult population (U.S. EPA, 1990). Consumption rate was chosen at 6.5 grams per day (one meal a month) which is the estimate of the average consumption of fish and shellfish from marine, estuarine and fresh waters by the general adult population (U.S. EPA, 1990). The risk level (RL) was chosen at 10^{-5} as recommended by the EPA Office of Water for the calculation of screening values. In simple terms, this means that if a person weighing 70 kg consumed 6.5 grams of fish per day with the same concentration of contaminant, for 70 years, the increased risk would be at most one additional cancer death per 100,000 persons. Values used for oral RfD and SF were those suggested for use by the EPA (U.S. EPA, 1995a). Screening values could not be calculated for all chemicals analyzed in this study since reliable information on the toxicity or carcinogenic potency of chemicals is not available for all analytes. RfD and SF information that has been developed to date is available in the EPA's Integrated Risk Information System (IRIS, 1992). This system is continuously updated, as information becomes available, so calculations of screening values for additional chemicals may be possible in the future.

The screening values calculated from the constants selected above are used to help identify potential chemicals of concern and should not be treated as health risk thresholds. Comparisons of sample tissue levels with screening values are meant to provide guidance to further investigations of contaminant levels in southern California fish tissues. They should not be construed as regulatory action levels or be used as definitive answers to questions concerning the safety of fish consumption. Health risk concerns will be reviewed and, if necessary, warnings issued, by the California Office of Environmental Health Hazard Assessment (OEHHA).

Transport of Samples

Six-liter sample containers were packed (three to an ice chest) with enough ice to keep them cool for 48 hours. Each container was sealed in clean, large plastic bags closed with a cable tie to

prevent contact with other samples or ice or water. Ice chests were driven back to the laboratory by the sampling crew or flown by air freight within 24 hours of collection.

Homogenization and Aliquoting of Samples

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

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

Procedures for the Extraction of Pore Water

In sampling Legs 1 through 23 the BPTCP used whole core squeezing (WCS) to extract pore water. Pore water sampled after Leg 23 was extracted using centrifugation. Sediment samples were stored on ice at 4°C prior to the extraction process.

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

Pre-cleaned Teflon® scoops were used to transfer sediment from sample containers into high-speed one-liter polycarbonate centrifuge jars, which 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 using pre-cleaned polyethylene siphons. While decanting, care was taken to avoid floating debris, fauna, shell fragments or other solid material. After transfer into

final sample containers, porewater was immediately refrigerated at 4°C. Samples were refrigerated, not frozen, and toxicity testing was initiated within 24 hours of extraction of the final samples.

To avoid contamination, all sample containers, centrifuge jars, filters and squeezer surfaces in contact with the sample were plastics (acrylic, polycarbonate, PVC, and TFE) and cleaned with previously discussed clean techniques. All pore water extraction procedures were performed using trace metal and trace organic clean techniques in a positive pressure clean room with filtered air to prevent airborne contamination.

Chain of Records & Custody

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

Authorization/Instructions to Process Samples

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

Trace Metals Analysis of Sediments

Trace Metals analyses were conducted at the California Department of Fish and Game's (CDFG) Trace Metals Facility at Moss Landing, CA. Table 2 indicates the trace metals analyzed and lists method detection limits for sediments. These methods were modifications of those described by Evans and Hanson (1993), as well as those developed by the CDFG (California Department of Fish and Game, 1990). Samples were selected for chemical analyses by SWRCB staff based on results from toxicity tests.

Analytes and Detection Limits

Table 2. Dry Weight Trace Metal Minimum Detection Limits (MDL). Note that all tissue MDLs are reported in dry weight units because wet weight MDLs are based on percent moisture of the sample.

Analytes	MDL µg/g dry Sediment	MDL µg/g dry Tissue	MDL µg/L Water
Silver	0.002	0.01	0.001
Aluminum	1	1	NA
Arsenic	0.1	0.25	0.1
Cadmium	0.002	0.01	0.002
Copper	0.003	0.1	0.04
Chromium	0.02	0.1	0.05
Iron	0.1	0.1	0.1
Mercury	0.03	0.03	NA
Manganese	0.05	0.05	NA
Nickel	0.1	0.1	0.1
Lead	0.03	0.1	0.01
Antimony	0.1	0.1	NA
Tin	0.02	0.02	NA
Selenium	0.1	0.1	NA
Zinc	0.05	0.05	0.02

Sediment Digestion Procedures

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

Tissues Digestion Procedures

A three gram aliquot of tissue was placed in a pre-weighed Teflon® vessel, and three mLs of concentrated 4:1 nitric:perchloric acid mixture was added. Samples then were capped and heated on hot plates for five hours. Caps were tightened and heated in a vented oven at 130°C for four hours. Samples were allowed to cool and 15 mLs of Type II water was added to the vessels. The solution was then quantitatively transferred to a pre weighed 30 ml polyethylene (HDPE) bottle and taken up to a final weight of 20 g with Type II water.

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 "trace 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.

Acid Volatile Sulfide and Simultaneously Extracted Metals – AVS-SEM

This procedure determines the concentration of acid volatile sulfide (AVS) and the concentrations of selected metals that are solubilized during the acidification process (simultaneously extracted metal, SEM). The AVS/SEM procedure followed methods described by Allen et al. 1993. AVS in the samples was first converted to hydrogen sulfide by acidification with hydrochloric acid at room temperature. The hydrogen sulfide was purged from the samples and trapped in an aqueous solution of sodium hydroxide. Sulfide concentrations were then determined spectrophotometrically by reaction with amine sulfuric acid and ferric chloride reagents to form methylene blue. The SEM are selected metals liberated from the sediment during the acidification. The concentrations of these metals were measured in the remaining acid after filtration of the sample. If the molar concentration of AVS exceeds the combined molar concentration of the simultaneously extracted metals in anoxic sediments, then the metals are assumed to be bound as metal sulfides and are therefore not bioavailable.

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

Analytical sets of 12 samples were scheduled such that extraction and analysis will occur within a 40-day window. The methods employed by the UCSC-TOF were modifications of those described by Sloan et al. (1993). Tables 3 through 8 indicate the pesticides, PCBs, and PAHs currently analyzed and list method detection limits for sediments on a dry weight basis.

Analytes and Detection Limits

Table 3. Dry Weight Minimum Detection Limits of Chlorinated Pesticides.

Analytes †	Database Abbreviation	MDL ng/g dry Sediment	MDL ng/g dry Tissue	MDL ng/L Water
Fraction #1 Analytes †				
Aldrin	ALDRIN	0.5	1.0	2.0
alpha-Chlordene	ACDEN	0.5	1.0	1.0
gamma-Chlordene	GCDEN	0.5	1.0	1.0
o,p'DDE	OPDDE	1.0	3.0	1.0
o,p'DDT	OPDDT	1.0	4.0	2.0
Heptachlor	HEPTACHLOR	0.5	1.0	2.0
Hexachlorobenzene	HCB	0.2	1.0	1.0
Mirex	MIREX	0.5	1.0	1.0
Fraction #1 & #2 Analytes †,‡				
p,p'DDE	PPDDE	1.0	1.0	0.5
p,p'DDT	PPDDT	1.0	4.0	2.0
p,p'DDMU	PPDDMU	2.0	5.0	5.0
trans-Nonachlor	TNONA	0.5	1.0	1.0
Fraction #2 Analytes ‡				
cis-Chlordane	CCHLOR	0.5	1.0	1.0
trans-Chlordane	TCHLOR	0.5	1.0	1.0
Chlorpyrifos	CLPYR	1.0	4.0	4.0
Dacthal	DACTH	0.2	2.0	2.0
o,p'DDD	OPDDD	1.0	5.0	5.0
p,p'DDD	PPDDD	0.4	3.0	3.0
p,p'DDMS	PPDDMS	3.0	20	20
p,p'Dichlorobenzophenone	DICLB	3.0	25	25
Methoxychlor	METHOXY	1.5	15	15
Dieldrin	DIELDRIN	0.5	1.0	1.0
Endosulfan I	ENDO_I	0.5	1.0	1.0
Endosulfan II	ENDO_II	1.0	3.0	3.0
Endosulfan sulfate	ESO4	2.0	5.0	5.0
Endrin	ENDRIN	2.0	6.0	6.0
Ethion	ETHION	2.0	NA	NA
alpha-HCH	HCHA	0.2	1.0	1.0
beta-HCH	HCHB	1.0	3.0	3.0
gamma-HCH	HCHG	0.2	0.8	1.0
delta-HCH	HCHD	0.5	2.0	2.0
Heptachlor Epoxide	HE	0.5	1.0	1.0
cis-Nonachlor	CNONA	0.5	1.0	1.0
Oxadiazon	OXAD	6	NA	NA
Oxychlordane	OCDAN	0.5	0.2	1.0

† The quantitation surrogate is PCB 103. ‡ The quantitation surrogate is d8-p,p'-DDD

Table 4. Dry Weight Detection Limits of NIST PCB Congeners.

Analytes †	Database Abbreviation	MDL ng/g dry sediment	MDL ng/g dry tissue	MDL ng/L water
2,4'-dichlorobiphenyl	PCB08	0.5	1.0	1.0
2,2',5-trichlorobiphenyl	PCB18	0.5	1.0	1.0
2,4,4'-trichlorobiphenyl	PCB28	0.5	1.0	1.0
2,2',3,5'-tetrachlorobiphenyl	PCB44	0.5	1.0	1.0
2,2',5,5'-tetrachlorobiphenyl	PCB52	0.5	1.0	1.0
2,3',4,4'-tetrachlorobiphenyl	PCB66	0.5	1.0	1.0
2,2',3,4,5'-pentachlorobiphenyl	PCB87	0.5	1.0	1.0
2,2',4,5,5'-pentachlorobiphenyl	PCB101	0.5	1.0	1.0
2,3,3',4,4'-pentachlorobiphenyl	PCB105	0.5	1.0	1.0
2,3',4,4',5-pentachlorobiphenyl	PCB118	0.5	1.0	1.0
2,2',3,3',4,4'-hexachlorobiphenyl	PCB128	0.5	1.0	1.0
2,2',3,4,4',5'-hexachlorobiphenyl	PCB138	0.5	1.0	1.0
2,2',4,4',5,5'-hexachlorobiphenyl	PCB153	0.5	1.0	1.0
2,2',3,3',4,4',5-heptachlorobiphenyl	PCB170	0.5	1.0	1.0
2,2',3,4,4',5,5'-heptachlorobiphenyl	PCB180	0.5	1.0	1.0
2,2',3,4',5,5',6-heptachlorobiphenyl	PCB187	0.5	1.0	1.0
2,2',3,3',4,4',5,6-octachlorobiphenyl	PCB195	0.5	1.0	1.0
2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	PCB206	0.5	1.0	1.0
2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl	PCB209	0.5	1.0	1.0

† PCB 103 is the surrogate used for PCBs with 1 - 6 chlorines per molecule. PCB 207 is used for all others.

Table 5. Dry Weight Minimum Detection Limits for additional PCB congeners.

Analytes †	Database Abbreviation	MDL ng/g dry sediment	MDL ng/g dry tissue	MDL ng/L water
2,3-dichlorobiphenyl	PCB5	0.5	1.0	1.0
4,4'-dichlorobiphenyl	PCB15	0.5	1.0	1.0
2,3',6-trichlorobiphenyl	PCB27	0.5	1.0	1.0
2,4,5-trichlorobiphenyl	PCB29	0.5	1.0	1.0
2,4',4-trichlorobiphenyl	PCB31	0.5	1.0	1.0
2,2,'4,5'-tetrachlorobiphenyl	PCB49	0.5	1.0	1.0
2,3',4',5-tetrachlorobiphenyl	PCB70	0.5	1.0	1.0
2,4,4',5-tetrachlorobiphenyl	PCB74	0.5	1.0	1.0
2,2',3,5',6-pentachlorobiphenyl	PCB95	0.5	1.0	1.0
2,2',3',4,5-pentachlorobiphenyl	PCB97	0.5	1.0	1.0
2,2',4,4',5-pentachlorobiphenyl	PCB99	0.5	1.0	1.0
2,3,3',4',6-pentachlorobiphenyl	PCB110	0.5	1.0	1.0
2,2',3,3',4,6'-hexachlorobiphenyl	PCB132	0.5	1.0	1.0
2,2',3,4,4',5-hexachlorobiphenyl	PCB137	0.5	1.0	1.0
2,2',3,4',5',6-hexachlorobiphenyl	PCB149	0.5	1.0	1.0
2,2',3,5,5',6-hexachlorobiphenyl	PCB151	0.5	1.0	1.0
2,3,3',4,4',5-hexachlorobiphenyl	PCB156	0.5	1.0	1.0
2,3,3',4,4',5'-hexachlorobiphenyl	PCB157	0.5	1.0	1.0
2,3,3',4,4',6-hexachlorobiphenyl	PCB158	0.5	1.0	1.0
2,2',3,3',4,5,6'-heptachlorobiphenyl	PCB174	0.5	1.0	1.0
2,2',3,3',4',5,6-heptachlorobiphenyl	PCB177	0.5	1.0	1.0
2,2',3,4,4',5',6-heptachlorobiphenyl	PCB183	0.5	1.0	1.0
2,3,3',4,4',5,5'-heptachlorobiphenyl	PCB189	0.5	1.0	1.0
2,2',3,3',4,4',5,5'-octachlorobiphenyl	PCB194	0.5	1.0	1.0
2,2',3,3',4,5',6,6'-octachlorobiphenyl	PCB201	0.5	1.0	1.0
2,2',3,4,4',5,5',6-octachlorobiphenyl	PCB203	0.5	1.0	1.0

† PCB 103 is the surrogate used for PCBs with 1 - 6 chlorines per molecule. PCB 207 is used for all others.

Table 6. Dry Weight Minimum Detection Limits of Chlorinated Technical Grade Mixtures.

Analytes †	Database Abbreviation	MDL ng/g dry sediment	MDL ng/g dry tissue	MDL ng/L water
Toxaphene ‡	TOXAPH	50	100	100
Polychlorinated Biphenyl Aroclor 1248	ARO1248	5	100	100
Polychlorinated Biphenyl Aroclor 1254	ARO1254	5	50	50
Polychlorinated Biphenyl Aroclor 1260	ARO1260	5	50	50
Polychlorinated Terphenyl Aroclor 5460†	ARO5460	10	100	100

† The quantitation surrogate is PCB 207. ‡ The quantitation surrogate is d8-p,p'-DDD

Table 7. Dry Weight Minimum Detection Limits of Polyaromatic Hydrocarbons in Tissue.

Analytes †	Database Abbreviation	MDL ng/g dry Sediment	MDL ng/g dry Tissue	MDL ng/L Water
Naphthalene	NPH	5	10	30
2-Methylnaphthalene	MNP2	5	10	30
1-Methylnaphthalene	MNP1	5	10	30
Biphenyl	BPH	5	10	30
2,6-Dimethylnaphthalene	DMN	5	10	30
Acenaphthylene	ACY	5	10	30
Acenaphthene	ACE	5	10	30
2,3,5-Trimethylnaphthalene	TMN	5	10	30
Fluorene	FLU	5	10	30
Dibenzothiophene	DBT	5	10	30
Phenanthrene	PHN	5	10	30
Anthracene	ANT	5	10	30
1-Methylphenanthrene	MPH1	5	10	30
Fluoranthrene	FLA	5	10	30
Pyrene	PYR	5	10	30
Benz[a]anthracene	BAA	5	10	30
Chrysene	CHR	5	10	30
Tryphenylene	TRY	5	10	30
Benzo[b]fluoranthrene	BBF	5	10	30
Benzo[k]fluoranthrene	BKF	5	10	30
Benzo[e]pyrene	BEP	5	10	30
Benzo[a]pyrene	BAP	5	10	30
Perylene	PER	5	10	30
Indeno[1,2,3-cd]pyrene	IND	5	15	45
Dibenz[a,h]anthracene	DBA	5	15	45
Benzo[ghi]perylene	BGP	5	15	45
Coronene	COR	5	15	45

† See QA report for surrogate assignments.

Table 8. Dry Weight Minimum Detection Limits of Organometalic Compounds.

Analyte †	Database Abbreviation	MDL ng/g dry Sediment	MDL ng/g dry Tissue	MDL ng/L Water
Tributyltin	TBT	13	20	1

Sediment Extraction

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

Tissue Extraction

Samples were removed from the freezer and allowed to thaw. A 5-gram sample of tissue was removed for chemical analysis and an independent 5-gram aliquot was removed for dry weight determinations. The dry weight sample was placed into a pre-weighed aluminum pan and dried at 110°C for 24 hours. The dried sample was reweighed to determine the sample's percent moisture. The analytical sample was extracted twice with methylene chloride using a Tekmar Tissumizer. Prior to extraction, sodium sulfate and extraction surrogates were added to the sample and methylene chloride.

The two extraction aliquots were combined and brought to 100 mL. A 25-mL aliquot was decanted through a Whatmann 12.5 cm #1 filter paper into a pre-weighed 50-mL flask for lipid weight determination. The filter was rinsed with ~15 mL of methylene chloride and the remaining solvent was removed by vacuum-rotary evaporation. The residue was dried for 2 hours at 110°C and the flask was re-weighed. The change in weight was taken as the total methylene chloride extractable mass. This weight then was used to calculate the samples "percent lipid".

Organic 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 contained > 90% of p,p'DDE and < 10% of p,p'DDT. Fraction 2 (F2) analytes were eluted with 100% methylene chloride. The two fractions were exchanged into hexane and concentrated to 500 µL using a combination of rotary evaporation, controlled boiling on tube heaters, and dry nitrogen blow downs.

F1 and F2 fractions were analyzed on Hewlett-Packard 5890 Series gas chromatographs utilizing capillary columns and electron capture detection (GC/ECD). A single 2 µL splitless injection was directed onto two 60 m x 0.25 mm i.d. columns of different polarity (DB-17 & DB-5, J&W Scientific) using a glass Y-splitter to provide a two dimensional confirmation of each analyte. Analytes were quantified using internal standard methodologies. The extract's PAH portion was

eluted through a silica/alumina column with methylene chloride. It then underwent additional cleanup using size-exclusion high-performance liquid chromatography (HPLC/SEC). The collected PAH fraction was exchanged into hexane and concentrated to 250 µL in the same manner as the CH fractions.

Total Organic Carbon Analysis of Sediments

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

Sub-samples were treated with two, 5 mL additions of 0.5 N, reagent grade HCl to remove inorganic carbon (CO^{-3}), agitated, and centrifuged to a clear supernatant. 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 suppurate, and decanted. Two sample washings were required to remove weight determination and analysis interferences.

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

A modification of the high temperature combustion method, utilizing a Weatstone bridge current differential was used in a commercially available instrument, (Control Equipment Co., 440 Elemental Analyzer) to determine carbon and nitrogen concentrations. The manufactures suggested procedures were followed. The methods are comparable to the validation study of USEPA method MARPCPN I. Two to three 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 are 0.2 ug/mg carbon and 0.01 ug/mg nitrogen dry weight.

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

Quality control was tested by the analysis of National Research Council of Canada Marine Sediment Reference Material BCSS-1 at the beginning and end of each sample analysis set (20-30 individual machine analyses). All analyzed values were within suggested criteria of ± 0.09% carbon (2.19% Average). Nitrogen was not reported on the standard data report, but was accepted at ± 0.008% nitrogen (0.195% Average) from the EPA study. Quality assurance was monitored by re-calibration of the instrument every twenty samples and by the analysis of a standard as a unknown and comparing known theoretical percentages with resultant analyzed

percentages. Acceptable limits of standard unknowns were less than ± 2%. Duplicate or triplicate sample analysis variance (standard deviation/mean) greater than 7% is not accepted. Samples were re-homogenized and re-analyzed until the variance between individual runs fell below the acceptable limit of 7.0%.

Grain Size Analysis of Sediments

Sample Splitting and Preparation

The procedure used combined 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 disappear. The amount and concentration of dispersant used was recorded on the data sheet for each sample. Sample beakers were placed in an ultrasonic cleaner for 15 minutes for disaggregation. Sediment dispersant slurry was poured into a 63 μm (ASTM #230, 4 phi) stainless steel or brass sieve in a large glass funnel suspended over a 1L hydrometer cylinder by a ring stand. All fine sediments were washed through the sieve with water. Fine sediments were captured in a 1L-hydrometer cylinder. Coarse sediments remaining in sieve were collected and returned to the original sample beaker for quantification.

Dry Sieve Analysis (coarse fraction)

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

Hydrometer Analysis (Fine Fraction)

Hydrometers used for the analysis were precalibrated using the techniques of Lewis (1984). A reference cylinder was filled with water and 100 ml of dispersant solution. Prior to the analysis, a hydrometer reading was taken for Cc, the composite correction for temperature, dispersing agent, and the meniscus.

For each of the sample cylinders, the volume was raised to 1000 ml using tap water. The hydrometer number was recorded, the temperature was noted, and the sample added and stirred for 1 minute. Hydrometer readings were taken at 1 minute, 3 minutes, 10 minutes, 30 minutes, 90 minutes, 4.5 hours and 24 hours. If the water temperature had changed by greater than 2°C then hydrometer corrections were remeasured. The colloidal weight was determined by subtracting the other fractions from the total weight.

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.

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.

Sediment Samples

Bedded sediment samples were transported to MPSL from the sample-processing laboratory at Moss Landing in ice chests at 4°C. Transport time was one hour. Samples were held at 4°C and all tests were initiated within 14 days of sample collection, unless otherwise noted in the Quality Assurance Appendix. All sediment samples were handled according to procedures described in ASTM (1992) and BPTCP Quality Assurance Project Plan (Stephenson et al., 1994). Samples were removed from refrigeration the day before the test, and loaded into test containers. Water quality was measured at the beginning and end of all tests. At these times pH, temperature, salinity, and dissolved oxygen were measured in overlying water from all samples to verify that water quality criteria were within the limits defined for each test protocol. Total ammonia concentrations were measured in overlying water and also interstitial water after Leg 30. Sulfide measurements were taken in interstitial water after Leg 30 and in overlying water between Legs 30 through 41. Hydrogen sulfide samples were preserved with zinc acetate and stored in the dark until time of measurement.

Pore Water Samples

Once at MPSL, frozen porewater samples were stored in the dark at -12°C until required for testing. Experiments performed by the U.S. National Biological Survey have shown no effects of freezing porewater upon the results of toxicity tests (Carr et al., 1995). Unfrozen pore water samples were stored in the dark, at 4°C. Porewater samples were stored frozen between Legs 4 and 23, and were stored refrigerated after Leg 31. 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. Total ammonia and sulfide concentrations were also measured. 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‰, 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.

Subsurface Water Samples

Abalone, mussel and urchin embryo-larval development tests were performed on water column samples collected with the modified Van Veen grab. Subsurface water samples were held in the dark at 4°C until testing. Toxicity tests were initiated within 14 days of the sample collection date. Water quality parameters, including ammonia and sulfide concentrations, were measured in one replicate test container from each sample in the overlying water as described above. Measurements were taken at the beginning and end of all tests.

Measurement of Ammonia and Hydrogen Sulfide

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

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

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

Total sulfide concentrations were measured using an Orion Model 94-16 Silver/Sulfide Electrode, except that samples tested after February, 1994, were measured on a spectrophotometer using a colorimetric method (Phillips et al. 1997). The concentration of hydrogen sulfide was derived from the concentration of total sulfide by using the following equation (ASCE 1989):

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

where temperature and salinity dependent $pK_a°$ values were taken from Savenko (1977). The method detection limit for total sulfide was 0.1 mg/L for the electrode method, and 0.01 mg/L for

the colorimetric method. Values and corresponding detection limits for unionized ammonia and hydrogen sulfide were an order of magnitude lower than those for total ammonia and total sulfide, respectively. Care was taken with all sulfide and ammonia samples to minimize volatilization by keeping water quality sample containers capped tightly until analysis.

Marine and Estuarine Amphipod Survival Tests

Solid-phase sediment sample toxicity was assessed using the 10-day amphipod survival toxicity test protocols outlined in EPA 1994. All *Eohaustorius* and *Rhepoxynius* were obtained from Northwestern Aquatic Sciences in Yaquina Bay, Oregon. Animals were separated into groups of approximately 100 and placed in polyethylene boxes containing Yaquina Bay collection site sediment, then shipped on ice via overnight courier. Upon arrival at Granite Canyon, *Eohaustorius* were acclimated to 20‰ (T=15°C), and *Rhepoxynius* were acclimated to 28‰ (T=15°C). Once acclimated, the animals were held for an additional 48-hours prior to addition to the test containers. All *Ampelisca* were obtained from East Coast Amphipods in Wickford, RI. *Ampelisca* were shipped on ice via overnight courier in polyethylene jars containing Rhode Island collection site sediment. Upon arrival at Granite Canyon, *Ampelisca* were acclimated slowly (<2‰ per day) to 28‰ seawater (T=20°C). Once acclimated, 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 2 cm of sediment and filled to the 700-ml line with control seawater adjusted to the appropriate salinity 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 any 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 control 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 Rhode Island home sediment for *Ampelisca* and Yaquina Bay home sediment for *Eohaustorius* and *Rhepoxynius* 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 seawater, diluted to the appropriate salinity was compared to all cadmium concentrations. Amphipod survival for each replicate was calculated as:

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

Ceriodaphnia dubia Water Flea Acute Survival Test

Aquatic toxicity of freshwater samples was assessed using the Cladoceran water flea (*Ceriodaphnia dubia*) acute survival test. Details of the test protocol are given in the MPSL Standard Operating Procedure for *Ceriodaphnia dubia* that follows EPA freshwater acute methods (EPA 1993).

Ceriodaphnia neonates (<24 h) were obtained from in house cultures or from Toxscan Laboratories (Watsonville, CA). Neonates were isolated from cultures or obtained from Toxscan on Day 0 of the test. All dilution water was prepared according to EPA (1993). Porewater test containers were 50-mL glass beakers containing 15 mL of test solution. Each test container was inoculated with 5 or 8 neonates depending on availability. The laboratory negative control consisted of EPA dilution water. After an exposure period of 96 hours neonates were counted. A positive control reference test was conducted concurrently with the test using a dilution series of copper chloride as the reference toxicant.

Ceriodaphnia dubia Water Flea Acute Survival Test at the Sediment-Water Interface

The toxicity of solid-phase freshwater sediments was assessed using the water flea (*Ceriodaphnia dubia*) acute survival test at the sediment-water interface. Details of the test protocol are given in the MPSL Standard Operating Procedure for *Ceriodaphnia dubia* that follows EPA freshwater acute methods (EPA 1993).

Ceriodaphnia neonates (<24 h) were obtained from in house cultures or from Toxscan Laboratories (Watsonville, CA). Neonates were isolated from cultures or obtained from Toxscan on Day 0 of the test. All dilution water was prepared according to EPA (1993). Sediment-water interface test containers consisted of a polycarbonate tube with a 25-µm screened bottom placed so that the screen was within 1 cm of the surface of an intact sediment core (Anderson et al. 1996). Dilution water was poured into the screen tube at the surface of each core and allowed to equilibrate for 24 hours before the start of the test. Each test container was inoculated with 5 or 8 neonates depending on availability. The laboratory negative control consisted of Yaquina Bay amphipod home sediment from Northwestern Aquatic Sciences. After an exposure period of 96 hours, screens were removed from the intact cores, and neonates were counted. A positive control reference test was conducted concurrently with the test using a dilution series of copper chloride as the reference toxicant.

Haliotis rufescens Abalone Embryo-Larval Development Test

The red abalone (*Haliotis rufescens*) embryo-larval development test was conducted on pore water and subsurface water samples. Details of the test protocol are given in EPA 1995. A brief description of the method follows.

Adult male and female abalone were induced to spawn separately using a dilute solution of hydrogen peroxide in seawater. Fertilized eggs were distributed to the test containers within one hour of fertilization. Test containers were polyethylene-capped, seawater leached, 20-ml glass scintillation vials containing 10 mLs of sample. Each test container was inoculated with 100

embryos (10/mL). Samples that were tested at multiple concentrations were diluted 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, and a brine control with all samples that require brine adjustment. Tests were conducted at ambient seawater salinity (33±2‰). A 48-h positive control reference test was conducted concurrently with each pore water test using a dilution series of zinc sulfate as a reference toxicant.

After a 48-h exposure period, developing larvae were fixed in 5% buffered formalin. All larvae in each container were examined using an inverted light microscope at 100x to determine the proportion of veliger larvae with normal shells, as described in EPA 1995. Percent normal development was calculated as:

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

***Hyalella azteca* Amphipod Survival Test**

These amphipod tests followed ASTM (1993) procedures for *Hyalella azteca*. All *Hyalella* were obtained from Northwestern Aquatic Sciences (NWAS) in Yaquina Bay, Oregon. Animals were separated into groups of approximately 1000 and placed in polyethylene cubitainers containing NWAS laboratory water, then shipped via overnight courier. Upon arrival at Granite Canyon, the amphipods were acclimated to Granite Canyon well water (T=25°C). Once acclimated, the animals were held for an additional 48-h prior to addition to the test containers.

Test containers were one-liter glass jars containing 2 cm of sediment and filled to the 700-mL line with Granite Canyon well water. Test sediment and overlying water were allowed to equilibrate for 24 hours, then 20 amphipods were placed in each beaker along with well water to fill each test container to the one-liter line. Test chambers were gently aerated and continuously illuminated.

Five replicates of each sample were tested for 10 days. In addition, a negative sediment control consisting of 5 replicates of Yaquina Bay home sediment was included with each set of samples tested. Test containers were fed slurry of crushed alfalfa pellets three times per week (ASTM 1993). After 10 days, samples 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. In these tests, amphipod mortality was recorded in three replicates of four cadmium concentrations after a 96-hour water-only exposure. A dilution water control consisting of Granite Canyon well water was included in each test. Amphipod survival for each replicate was calculated as:

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

Mytilus spp. Embryo-Larval Development Test

The bay mussel (*Mytilus* spp.) embryo-larval development test was conducted on pore water and subsurface water samples. Details of the test protocol are given in EPA 1995. A brief description of the method follows.

Adult male and female mussels were induced to spawn separately using temperature shock by raising the ambient temperature by 10°C. Fertilized eggs were distributed to the test containers within four hours of fertilization. Test containers were polyethylene-capped, seawater leached, 20-ml glass scintillation vials containing 10 mLs of sample. Each test container was inoculated with 150 to 300 embryos (15-30/mL) consistent among replicates and treatments within a test set. Samples that were tested at multiple concentrations were diluted 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 28±2‰. A 48-h positive control reference test was conducted concurrently with each test using a dilution series of cadmium chloride as a reference toxicant.

After a 48-h exposure period, developing larvae were fixed in 5% buffered formalin. All larvae in each container were examined using an inverted light microscope at 100x to determine the proportion of normal live prossidoconch larvae, as described in EPA 1995. Percent normal live larvae was calculated as:

$$\frac{\text{(Number of normal larvae)}}{\text{(Initial embryo density)}} \times 100$$

Neanthes arenaceodentata Polychaete Survival and Growth Test

The *Neanthes* test followed procedures described in Puget Sound Protocols (1992). Emergent juvenile *Neanthes arenaceodentata* (2-3 weeks old) were obtained from Dr. Donald Reish of California State University, Long Beach. Worms were shipped in seawater in plastic bags at ambient temperature via overnight courier. Upon arrival at MPSL, worms were allowed to acclimate gradually to 28‰ salinity (<2‰ per day, T=15°C). Once acclimated, the worms were maintained at least 48 hours, and no longer than 10 days, before the start of the test.

Test containers were one-liter glass beakers or jars containing 2 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, but the presence of any predators was noted and recorded on the data sheet at the conclusion of the test. Test sediment and overlying water were allowed to equilibrate for 24 hours, after which 5 worms 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. Worms were fed TetraMin® every 2 days, and overlying water was renewed every 3 days. Water quality parameters were measured at the time of renewals.

After 20 days, samples were sieved through a 0.5-mm Nitex screen, and the number of surviving worms recorded. Surviving worms from each replicate were wrapped in an piece of pre-weighed aluminum foil, and placed in a drying oven until reaching a constant weight. Each foil packet was then weighed to the nearest 0.1 mg. Worm survival and mean weight/worm for each replicate was calculated as follows:

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

$$\text{Mean weight per worm} = \frac{\text{(Total weight - foil weight)}}{\text{(Number of surviving worms)}} \times 100$$

***Strongylocentrotus purpuratus* Sea Urchin Embryo-Larval Development Test**

The sea urchin (*Strongylocentrotus purpuratus*) larval development test was conducted on pore water samples. Details of the test protocol are given in EPA 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, seawater leached, 20-ml glass scintillation vials containing 10 mLs of sample. Each test container was inoculated with approximately 250 embryos (25/ml). Pore water samples from Legs 34 and 36 were tested at three concentrations: 100, 50 and 25%, each having three replicates. Samples from Legs 17 and 19 were tested at 100 and 50% porewater with three replicates and samples from Legs 9 and 45 were tested at 100% only with 5 replicates. Pore water samples were diluted 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, and a brine control with all samples that require brine adjustment. Tests were conducted at ambient seawater salinity (33±2‰). A 96-hour 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-hour 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 EPA 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:

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

***Strongylocentrotus purpuratus* Sea Urchin Embryo-Larval Development Test at the Sediment-Water Interface**

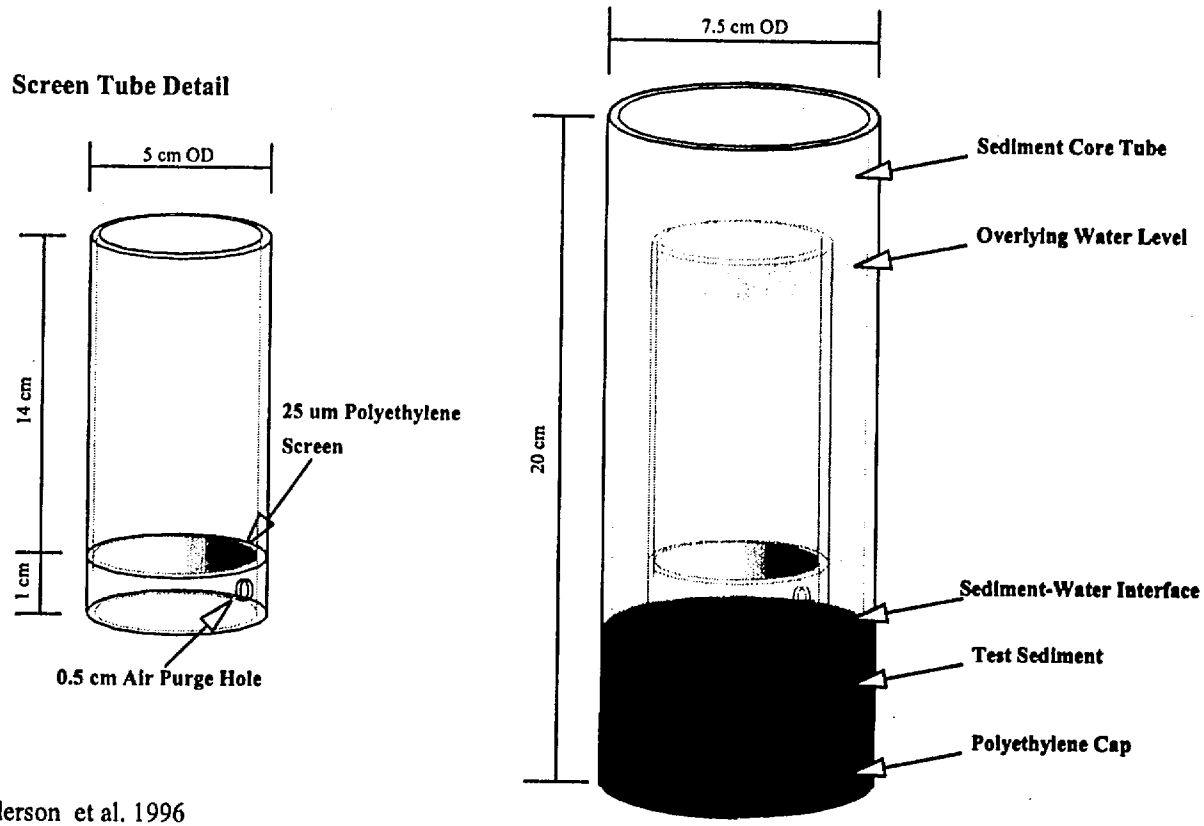
The purple sea urchin (*Strongylocentrotus purpuratus*) embryo/larval development test at the sediment-water interface was conducted on intact core sediment samples taken with minimal disturbance from the Van Veen grab sampler. Details of the test protocol are given in the MPSL Standard Operating Procedure, which follows the EPA methods manual (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 until testing. Adult sea urchins were held in complete darkness to preserve gonadal condition. On the day of the test, urchins were induced to spawn in air by injection with 0.5 mL of 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 the test containers within one hour of fertilization. Sediment-water interface test containers consisted of a polycarbonate tube with a 25-µm screened bottom placed so that the screen was within 1 cm of the surface of an intact sediment core (Figure 3, Anderson et al. 1996). Seawater at ambient salinity was poured into the core tube and allowed to equilibrate for 24 hours before the start of the test. After inserting the screen tube into the equilibrated cores, each tube was inoculated with approximately 250 embryos. The laboratory control consisted of Yaquina Bay amphipod home sediment from Northwestern Aquatic Sciences. Tests were conducted at ambient seawater salinity ± 2‰. Ambient salinity at Granite Canyon is usually 32 to 34‰. A positive control reference test was conducted concurrently with the test using a dilution series of copper chloride as a reference toxicant.

After an exposure period of 96 hours, larvae were fixed in 5% buffered formalin. One hundred larvae in each container were examined under an inverted light microscope at 100x to determine the proportion of normally developed larvae as described in EPA 1995. Percent normal development was calculated as:

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

Sediment-Water Interface Exposure System*



* After Anderson et al. 1996

Figure 3. Sediment-Water Interface (SWI) Exposure System: 7.5 cm polycarbonate sample core with 5 cm screen tube.

Strongylocentrotus purpuratus 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, seawater leached, 20-ml glass scintillation vials containing 5 mLs of pore water. Porewater samples that were tested at three concentrations (100, 50 and 25%, Legs 17, 19 and 34) were diluted with one micron-filtered Granite Canyon seawater. Porewater from Legs 9 and 36 were tested at 100% only. 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:

$$\frac{\text{(Number of fertilized eggs)}}{\text{(Number of eggs observed)}} \times 100$$

Test Acceptability and Evaluation

Quality Assurance/Quality Control (QA/QC) guidelines for the toxicity tests used in the BPTCP project are summarized in the BPTCP Quality Assurance Project Plan (Stephenson et al., 1994). Test acceptability criteria from published protocols were evaluated for all tests. Quality assurance checklists were compiled that noted compliance for all tests with each of these criteria. Evaluation codes were assigned to each deviation from QA/QC guidelines, and can be summarized as follows:

- -3: sample has minor exceedances of QA criteria that are unlikely to affect assessments.
- -4: sample meets or exceeds control criteria requirements.
- -5: data has exceedances, but are generally usable for most assessments and reporting purposes.
- -6: sample has major exceedances of control criteria requirements and the data is not usable for most assessments and reporting purposes.

It is recommended that if assessments are made that are especially sensitive or critical, the QA evaluations be consulted before using the data. Test data judged to be unacceptable are not reported, and samples from unacceptable tests are retested if necessary.

Benthic Community Analysis

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. Samples were selected for benthic community analysis by SWRCB staff based on results from toxicity tests.

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

Relative Benthic Index

Benthic samples were sieved, sorted and the number of individuals of each species in each replicate core were identified. 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).

The Relative Benthic Index (RBI) used in this study utilizes the above summarized fauna information in a refined version of the benthic index presented in the San Diego BPTCP report (Fairey et al. 1996). It is based on simple, realistic natural history concerning responses of marine benthic communities to anthropogenic and natural disturbances. The community patterns used in the index include number of species (all taxa, only molluscs, and only crustaceans), the number of crustacean individuals, and the number of individuals of selected species that are indicators of relatively disturbed and undisturbed benthic habitats. The RBI is developed for particular areas by selecting different indicator species. It does not require the presence of uncontaminated reference stations, and does not refer to data beyond that collected in each study. Often the evaluation of community degradation depends on comparisons to uncontaminated reference sites which are difficult to locate and vary for reasons that are unknown and unrelated to contamination.

Number of Species

The number of species often decreases with severe disturbances (Oliver et al. 1977, Oliver et al. 1980, Lenihan and Oliver 1995) and is the best indicator of biodiversity, particularly when species are sampled in relation to habitat area (Hurlbert 1971, Jumars 1975, Jumars 1976, Abel and Walters 1979). Therefore, the first community parameter in the RBI is the total number of species found in a standard sample of habitat area. Among the more numerous large taxonomic groups, crustaceans are generally more sensitive to environmental contaminants and other

anthropogenic disturbances than other components of the infauna, particularly polychaetes (Pearson and Rosenberg 1978, Reish et al. 1980, Thistle 1981, Swartz et al. 1986, Stull et al. 1986, Oliver et al. 1977, Lenihan and Oliver 1995, Lenihan et al. 1995). Speciose and numerically abundant crustacean faunas on the Pacific coast of the United States are generally only found in uncontaminated environments (Barnard 1963), making the number of crustacean species an important indicator of overall environmental health. To a lesser degree, the number of mollusk species also increase with decreasing environmental stress (Stull et al. 1986, Swartz et al. 1986, Oliver et al. 1977), and are also included in the RBI. Polychaetes, crustaceans, and molluscs are the three dominant groups of benthic macro-invertebrates from many nearshore communities (Oliver et al. 1980). Unlike the crustaceans and molluscs many of the most opportunistic species are polychaete (Grassle and Grassle 1974, McCall 1977, Oliver et al. 1977, Pearson and Rosenberg 1978, Reish et al. 1980, Sanders et al. 1980, Santos and Simon 1980, Thistle 1981, Rhoads et al. 1982, Lenihan and Oliver 1995). As a result, the number of polychaete species was not used in the RBI, because they do not clearly indicate relatively disturbed or undisturbed habitats.

Number of Individuals

An increase in the number of crustacean individuals is indicative of relatively healthy environments (Stull et al. 1986, Swartz et al. 1986, Oliver et al. 1977, Lenihan and Oliver 1995). Occasionally individual crustacean species can be abundant in disturbed habitats (Vetter 1995, Okey 1997), but less so than other major taxonomic groups, such as polychaete worms (Pearson and Rosenberg 1978, Grassle and Grassle 1974, Oliver et al. 1977). Therefore, the number of individuals of crustaceans is used in the RBI, but not the number of individuals in any other major taxonomic group.

Indicator Species

The population sizes of selected indicator species are more strongly associated with benthic habitats that are disturbed or undisturbed than the number of species or the number of crustacean individuals (Grassle and Grassle 1974, Oliver et al. 1977, Davis and Spies 1980, Westin 1990, Lenihan and Oliver 1995, Okey 1997). Therefore, five species were used in the RBI as indicators of highly disturbed or undisturbed benthic communities and habitats. The number and identity of indicator species can change from one regional study site to another. Selection of indicator species was based on known responses to anthropogenic and other disturbances (Grassle and Grassle 1974, McCall 1977, Oliver et al. 1977, Pearson and Rosenberg 1978, Davis and Spies 1980, Sanders et al. 1980, Santos and Simon 1980, Thistle 1981, Lenihan and Oliver 1995, Okey 1997). Selection was also based on life history traits (Grassle and Grassle 1974, Oliver et al. 1977, Rhoads et al. 1978, Rhoads and Boyer 1982, Lenihan and Oliver 1995) and abundance patterns along environmental gradients and among the study stations (Oliver et al. 1980, Stull et al. 1986, Swartz et al. 1986, Weston 1990). The two negative indicator species are highly opportunistic annelids which thrive in disturbed, polluted, or marginal environments, and are generally not found in less disturbed communities. The three positive indicator species are generally not found in polluted habitats and are characteristic of regions where anthropogenic and other severe disturbances do not play major roles in structuring communities. Each indicator species is discussed below:

Negative indicator species

Capitella capitata

The *Capitella* species complex is a cosmopolitan group that lives in a wide range of conditions including fouled or low oxygen, high organic matter and fine sediments. They have a rapid (1 to 2 month) life cycle, and are abundant around outfalls discharging biological wastes. *Capitella* are capable of surviving for days with little or no oxygen, and are often considered the best example of a "weedy", opportunistic species (Grassle and Grassle 1974, Grassle and Grassle 1976, Oliver et al. 1977, McCall 1977, Pearson and Rosenberg 1978, Lenihan and Oliver 1995, Okey 1995 and many others).

Oligochaetes

Oligochaetes are a poorly known group typically found in peripheral/disturbed habitats such as under decaying algae on beaches, and in the fouled or low oxygen sediments of back bays, estuaries and harbors (Brinkhurst and Simmons 1968, Pearson and Rosenberg 1978, Brinkhurst and Cook 1980). They often occur in large masses with nearly no other macrofauna. In San Francisco 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 densities become extremely high (Smith and Carlton, 1975; Brinkhurst and Simmons, 1968). Oligochaetes are also well known indicators of relatively degraded freshwater ecosystems (Brinkhurst and Simmons 1968, Pearson and Rosenberg 1978, Brinkhurst and Cook 1980).

Positive Indicator Species

Acuminodeutopus sp.

Acuminodeutopus is found in shallow clean, well-oxygenated sands, and in relatively clean bay sediments. They build tubes, and are early/first colonizers of ray pits and other relatively small-scale perturbations. *Acuminodeutopus* live in sedimentary habitats that are less strongly influenced by large-scale physical and chemical disturbances and more by smaller-scale biological disturbances such as ray feeding (Barnard 1961, Barnard and Reish 1959, VanBlaricom 1982).

Monoculodes

Monoculodes is a fossorial oedocerotid amphipod that requires well-oxygenated, clean sediment (Oliver et al. 1980). They are shallow burrowers that 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, Bousfield 1996). They can also colonize relatively small open patches in sandy habitats (Oliver et al. 1977), and have been selected as sensitive species to use in bioassays (Lenihan et al. 1995).

SEDIMENT CHEMISTRY, TOXICITY AND BENTHIC COMMUNITY CONDITIONS IN SELECTED WATER BODIES OF THE SANTA ANA REGION

FINAL REPORT

California State Water Resources Control Board
Division of Water Quality
Bay Protection and Toxic Cleanup Program

National Oceanic and Atmospheric Administration
Coastal Monitoring and Bioeffects Assessment Division
Bioeffects Assessment Branch

Regional Water Quality Control Board
Santa Ana Region

California Department of Fish and Game
Marine Pollution Studies Laboratory

University of California, Santa Cruz
Institute of Marine Sciences

San Jose State University
Moss Landing Marine Laboratories

August, 1998

Tellina

Tellina live in clean, well-oxygenated sands of shallow water (Oliver et al. 1980). Species in Southern California attain great enough densities to be a major component of the shallow water, benthic infaunal community (Barnard 1963). They are not known to be early colonists in disturbed sedimentary habitats (Oliver et al. 1977).

Calculation of Relative 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, total molluscs and total crustacea approached the threshold value. To address this problem, calculation of the RBI was revised and is now based on percentages of the total range. The final threshold value for determination of impacted versus non-impacted sites was based on the overall RBI and selected using best professional judgment. Justification for this critical threshold value of the RBI is discussed below.

For total fauna, number of mollusk species and number of crustacean species, the maximum and minimum values in these parameters over all the stations were determined. For each station, the total number of species, total mollusk species, and total number of crustacean species were then converted to the percentage of the total range for these parameters. The number of crustacean individuals at each station is similarly converted to a percentage of the total range, and is added to the total fauna, mollusk, and crustacean species numbers. The community numbers thus represent two thirds of the RBI 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 transformed values of the negative indicator species were summed and subtracted from the sum of the values for the positive indicator species.

The overall RBI was calculated by summing the values of the Total Fauna, Total Molluscs, Crustacean Species, and Indicator Species, and standardizing it to the total range. This resulted in a range in values from 0.00 (Most Impacted) to 1.00 (Least Impacted).

Use of Relative Benthic Index

It is not possible to compare directly RBI values between different regions. The high and low ranges of values vary based on the extreme values within each data set. In addition, different indicator species are often used in different regions. What the RBI does provide is the relative "health" of each of the stations in a given data set compared to the other stations in the same data set.

The RBI does not indicate causality. While a low RBI value could be the result of chemical toxicity, it also could be the result of other types of anthropogenic disturbance, such as dredging, or could result from a variety of natural disturbances, such as freshwater runoff, temperature stratification, or storm impacts.

It is not possible to test the RBI 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 RBI 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 significantly degraded by the vast majority of naturalists familiar with the region's bays and estuaries. The RBI can be used in combination with chemistry and toxicity test data to provide a "weight-of-evidence" for determination of the most impacted stations.

Data Analysis

Analysis of Chemistry Data

Comparisons with Sediment Quality Guideline Values

Bioavailability is the key to understanding the relationship between sediment chemistry and biological impacts. However, it was not possible to use TIEs, bioaccumulation analyses, or other specialized methods to evaluate bioavailability on the large number of samples evaluated in BPTCP studies to date. In order to assess large numbers of samples for their potential to impact biological resources, we compared sediment chemical concentrations to published guideline values derived from studies of approximately one thousand samples collected nationwide. These studies have used empirical observation of large data sets containing matching chemistry and biology data to provide guidance for evaluating the probability that measured contaminant concentrations might contribute to observed biological effects (MacDonald 1994, Long et al. 1995). While the reported guideline values were derived from sediments containing mixtures of chemicals, they were calculated individually for each chemical. Their application may be confounded in sediments where biological responses are affected by synergistic or antagonistic interactions among multiple compounds, by unmeasured or unidentified compounds, or by unconsidered physical factors.

The National Status and Trends Program has evaluated chemical and toxicological evidence from a number or laboratory, field, and modeling studies to establish three ranges of chemical concentrations which are either rarely, sometimes, or usually associated with biological effects. 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.

3) 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 that were associated with toxic response. These data were used to determine the lowest 10th percentile of ranked data where chemical concentration was associated with an effect (Effects Range - Low, or ERL). Chemical concentrations below the ERL are expected to rarely affect organisms. 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 (1994) 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 9.

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. While neither of these methods is advocated over the other in this report, we have presented only ERM comparisons to simplify the many presentations of the data. Long, Field, and MacDonald (1998) found that the predictive ability of ERMs was slightly greater than that of PELs in a recent evaluation of additional sediment data.

It should be noted that the degree of confidence that MacDonald (1994) and Long et al. (1995) had in their respective numerical guidelines varied considerably among the different chemicals. 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. Swartz et al. (1994) have recently revised guidelines for DDT and its metabolites to derive Sediment Effect Concentrations (SECs) for these compounds. In this report the SEC for Total DDT (100 μg DDT per Kg organic carbon) is used instead of the ERM for Total DDT.

Table 9. Comparison of sediment screening levels developed by NOAA and the State of Florida.

	State of Florida (1)		NOAA (2,3)	
SUBSTANCE	TEL	PEL	ERL	ERM
Total PCB (ng/g- dry weight)	21.550	188.79	22.70	180.0
PAH (ng/g- dry weight)				
Acenaphthene	6.710	88.90	16.00	500.0
Acenaphthylene	5.870	127.89	44.00	640.0
Anthracene	46.850	245.00	85.30	1100.0
Fluorene	21.170	144.35	19.00	540.0
2-methylnaphthalene	20.210	201.28	70.00	670.0
Naphthalene	34.570	390.64	160.00	2100.0
Phenanthrene	86.680	543.53	240.00	1500.0
Total LMW-PAHs	311.700	1442.00	552.00	3160.0
Benz(a)anthracene	74.830	692.53	261.00	1600.0
Benzo(a)pyrene	88.810	763.22	430.00	1600.0
Chrysene	107.710	845.98	384.00	2800.0
Dibenz(a,h)anthracene	6.220	134.61	63.40	260.0
Fluoranthene	112.820	1493.54	600.00	5100.0
Pyrene	152.660	1397.60	665.00	2600.0
Total HMW-PAHs	655.340	6676.14	1700.00	9600.0
Total PAHs	1684.060	16770.54	4022.00	44792.0
Pesticides (ng/g- dry weight)				
p,p'DDE	2.070	374.17	2.20	27.0
p,p'DDT	1.190	4.77	n/a	n/a
Total DDT	3.890	51.70	1.58	100.0 (4)
Lindane	0.320	0.99	n/a	n/a
Chlordane	2.260	4.79	2.00	6.0
Dieldrin	0.715	4.30	n/a	8.0
Endrin	n/a	n/a	n/a	45.0
Metals (mg/kg- dry weight)				
Arsenic	7.240	41.60	8.20	70.0
Antimony	n/a	n/a	2.00	25.0
Cadmium	0.676	4.21	1.20	9.6
Chromium	52.300	160.40	81.00	370.0
Copper	18.700	108.20	34.00	270.0
Lead	30.240	112.18	46.70	218.0
Mercury	0.130	0.70	0.15	0.7
Nickel	15.900	42.80	20.90	51.6
Silver	0.733	1.77	1.00	3.7
Zinc	124.000	271.00	150.00	410.0

(1) D.D. MacDonald, 1994; (2) Long et al., 1995; (3) Long and Morgan, 1990;
(4) Swartz et al., 1994

Non-Guideline Chemicals

For the purposes of categorizing chemical contamination in this data set, the NOAA ERM and ERL guidelines were used. To evaluate chemicals for which no ERM guidelines have been calculated, concentrations of specific chemicals were compared to the range of chemical concentrations in the BPTCP database. This database contains concentrations of approximately 120 analytes measured in sediments collected in the majority of California bays, estuaries, lagoons and near coast areas. The following information was described for each chemical: the Method Detection Limit (MDL), the highest value in the dataset, and the 90th and 95th percentile thresholds for each chemical (Table 10). For the purposes of station categorization, chemicals for which no sediment quality guideline values have been calculated were compared to the 90th and 95th percentile thresholds, and to the range of concentration measured throughout the state for comparison. Stations with chemical concentrations greater than the 90th percentile thresholds are noted in Table 31.

Table 10. Upper percentile concentrations of BPTCP chemicals for which there are no ERL or ERM sediment guideline values.

Chemical Name	MDL	Highest Value	90th % Threshold	95th % Threshold
Aluminum	1	165,000	83,000	101,000
Iron	0.1	336,300	55,300	59,900
Manganese	0.05	1190	630	682
Selenium	0.1	35.7	1.09	1.9
Tin	0.02	92.9	9.03	12
Aldrin	0.5	8.2	4.7	8.2
Chloropyrifos	1	78	28	44.4
Dacthal	0.2	25.2	7.51	19
p,p'Dichlorobenzophenone	3	63.3	30.6	35.2
Endosulfan I	0.5	19.6	13.4	19.6
Endosulfan II	1	59.8	10.4	13.8
Endosulfan Sulfate	2	163	21	45.6
Ethion	2	36.4	36.4	36.4
alpha-HCH	0.2	292	26.1	292
beta-HCH	1	56.8	56.8	56.8
delta-HCH	0.5	99.4	14.4	99.4
Heptachlor	0.5	15.8	4.5	7.3
Heptachlor Epoxide	0.5	17.8	2.5	3.1
Hexachlorobenzene	0.2	59.7	3.63	7.07
Methoxychlor	1.5	131	55.3	78.6
Mirex	0.5	103	2.6	3.74
Oxadiazon	6	114	45.8	114
Oxychlordane	0.5	30.3	10.7	12.3
Toxaphene	50	3,200	3,200	15,700
Tributyltin	0.003	6.21	0.422	0.724
Mean ERM Quotient	NA	4.37	1.11	1.4

ERM Quotients

The effects-based numerical guidelines listed previously may also be used to assess the relative degree of contamination at these stations. In order to compare contamination using these guidelines, chemical summary quotients (ERMQ) were calculated for all of the compounds for which these values exist. These are summations of chemical concentrations of the chemicals listed in Table 10, divided by their respective ERM 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. Chemical summary quotients are reported as average quotient values. The ERMQ was calculated by summing ERM quotient values for the following chemicals: Antimony, Cadmium, Chromium, Copper, Lead, Mercury, Silver, Zinc, Total DDT (after value of Swartz et al., 1994), Total Chlordane, Dieldrin, Endrin, Total PCBs, LMW PAHs, and HMW PAHs. This sum was then divided by the total number of analyte quotients (15) to give an ERMQ 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.

Statistical Analysis of Toxicity Test Data

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 separate-variance t-test, and 2) mean organism response in the toxicity test, as a percent of the control, was less than the Minimum Significant Difference (MSD) value as a percent of the laboratory control value.

Statistical significance in t-tests is determined by dividing an expression of the difference between sample and control by an expression of the variance among replicates. We used a "separate variance" t-test that adjusted the degrees of freedom to account for variance heterogeneity among samples. If the difference between sample and control is large relative to the variance among replicates, then the difference is determined to be significant. In many cases, however, low between-replicate variance will cause a comparison to be considered significant, even though the magnitude of the difference can be small. These samples were identified as "significantly toxic" in this report in order to acknowledge the statistical difference, although it is recognized that the magnitude of toxicity in some cases may not have been biologically meaningful. A second tier of "significant toxicity" was considered in order to identify those samples where the toxic response was considered to be more biologically meaningful. This involved the Minimum Significant Difference (MSD) value specific to each toxicity test protocol. The magnitude of difference that can be identified as significant is termed the Minimum Significant Difference, which is dependent on the selected alpha level, the level of between-replicate variation, and the number of replicates specific to the experiment. With the number of replicates and alpha level held constant, the MSD varies with the degree of between-replicate variation. The "detectable difference" inherent to the toxicity test protocol can be determined by identifying the magnitude of difference that can be detected by the protocol 90%

of the time (Schimmel et al. 1994, Thursby and Schlekat, 1993). This is equivalent to setting the level of statistical power at 0.90 for these comparisons. This is accomplished by determining the MSD for each t-test conducted, ranking them in ascending order, and identifying the 90th percentile MSD, the MSD that is larger than or equal to 90% of the MSD values generated.

Thursby et al. (1997) identify a value of 80% of the control as the detectable difference for the *Ampelisca* test, and similar values have been derived for BPTCP test data. Current BPTCP detectable difference (90th percentile MSD) values are listed in Table 11.

Table 11. Minimum Significant Differences used to calculate significant toxicity in BPTCP toxicity test protocols.

Test Species	MSD	% of control	N	Reference
Ampelisca	20	80		Thursby 1997
Ceriodaphnia Survival	20	80		Thursby 1997
Ceriodaphnia SWI	20	80		Thursby 1997
Eohaustorius Survival	25	75	385	MPSL*
Hyalella Survival	20	80		Thursby 1997
Abalone Development (5 reps)	10	90	131	MPSL*
Abalone Development (3 reps)	36	64	336	MPSL*
Mytilus Development	20	80	223	MPSL*
Neanthes Survival	36	64	335	MPSL*
Neanthes Weight	56	44	335	MPSL*
Rhepoxynius Survival	23	77	720	MPSL*
Purple Urchin Development (5 reps)	22	78	309	MPSL*
Purple Urchin Development (3 reps)	45	55	630	MPSL*
Purple Urchin Fertilization	12	88	79	MPSL*
Purple Urchin SWI	41	59	109	MPSL*

*MPSL unpublished data.

Effects of Unionized Ammonia and Hydrogen Sulfide

Toxicity results were screened against known application limits for unionized ammonia and hydrogen sulfide (Table 12). Toxicity test ammonia and sulfide concentrations above the application limits were taken into consideration when examining toxicity test results.

Table 12. Unionized ammonia and hydrogen sulfide effects thresholds for BPTCP toxicity tests.

Species	Unionized Ammonia (mg/L)	Limit Definition	Reference
Ampelisca	0.4	Application Limit	EPA 1994
Eohaustorius	0.8	Application Limit	EPA 1994
Red Abalone	0.05	NOEC	MPSL
Mytilus	0.15	LOEC	Tang et al. 1997
Neanthes	1.25	LOEC	Dillon 1993
Rhepoxynius	0.4	Application Limit	EPA 1994
Purple Urchin Development	0.07	NOEC	Bay et al. 1993
Purple Urchin Fertilization	>1.4	NOEC	Bay et al. 1993

Species	Hydrogen Sulfide (mg/L)	Limit Definition	Reference
Eohaustorius	0.114	LOEC	Knezovich et al., 1996
Mytilus	0.0053	LOEC	Knezovich et al., 1996
Rhepoxynius	0.087	LOEC	Knezovich et al., 1996
Purple Urchin Development	0.0076	LOEC	Knezovich et al., 1996
Purple Urchin Fertilization	0.007-0.014	NOEC	Bay et al., 1993

Multivariate and Univariate Techniques for Comparison of Chemistry and Toxicity Data

While the main objective of this study was to identify stations of concern, the data were also evaluated to investigate whether certain individual chemicals were found to be associated with biological impacts. These preliminary evaluations were made using Principal Components Analysis (a multivariate technique) followed by Correlation analysis (a univariate technique). This identification of chemicals that were associated with toxicity does not in itself prove cause and effect, but it allows the suggestion of hypotheses regarding the chemical causes of biological impacts, hypotheses that can later be tested with TIEs and other more extensive toxicological methods.

Principle Components Analysis

Because many chemicals tend to co-vary in sediments, Principal Components Analysis (PCA) was used to investigate relationships between chemistry, toxicity, and benthic indicators prior to conducting simple correlation analyses. The PCA was treated as exploratory in nature; therefore, data were not screened for sample size, normality, linearity, outliers or multicolinearity.

Principal components were extracted using SYSTAT statistics software (v. 7.0.1 for Windows; SPSS, 1997). The analysis was run with a correlation matrix and varimax rotation, and included any factors which accounted for greater than 10% of the total variance. A component loading cutoff value of 0.40 was used in selecting variables for inclusion into factors, based on suggestions by Tabachnick and Fidell (1996) that a cut-off of at least 0.32 be used, and that component loadings of greater than 0.45 are considered fair or better.

Correlation Analysis

Compounds determined by PCA to have a negative relationship with biological indicators (e.g. increasing concentration associated with decreasing survival) were selected for univariate correlation analysis. In order to examine associations between levels of these pollutants in sediments and the response observed in toxicity tests, Spearman rank correlation coefficients (Rho) were calculated using SYSTAT software. Since the response of the control groups for each toxicity test was both acceptable and consistent, the sediment toxicity test data were not normalized to control results. Rho values, corrected for ties, were determined for each toxicity test and each pollutant or pollutant class, and these Rho values were compared to tables at the

appropriate n value to determine the level of statistical significance associated with the observed correlation.

Weight-of-Evidence and Categorization of Sites

Toxicological, chemical, and ecological measures were combined to provide a weight-of-evidence categorization of sediment quality at each site. This approach is consistent with generally accepted methods of sediment quality assessment, such as the commonly used "sediment quality triad" described by Chapman et al. (1987). The three primary measures in the triad approach are sediment chemical analysis, toxicity testing, and benthic community analysis. All of these measures have their advantages and drawbacks, but together they can be used to effectively characterize sediment quality. In the Santa Ana region, toxicity testing was used as the primary screening tool in the first round of sampling. Stations that produced toxic samples or had been shown in previous studies to have elevated chemistry, bioaccumulation, or other measures of pollution were then resampled and analyzed for toxicity, chemistry, and, to a lesser extent, benthic community structure.

Use of Threshold Values

Using the data collected in this study, stations were categorized based on chemical concentrations, the severity of biological impacts, and the completeness of sample characterization. The conceptual framework for categorizing stations is provided in the listing below. In order to categorize stations, it was necessary to define terms such as "elevated chemistry", "sample toxicity" or "degraded benthos" for a large number of samples. To be consistent, thresholds were established for this purpose. Those thresholds are defined below in the description of the first category. Toxicity thresholds were based on the t-test plus detectable difference criteria as defined above. Benthic community degradation was defined as a Relative Benthic Index ≤ 0.30, based on the best professional judgement of the ecologists who developed the index. Elevated chemistry was defined as 6 or more chemicals exceeding ERM guidelines, a mean ERMQ above 0.5, or one or more chemicals at concentrations high enough to likely be associated with biological effects, based on best professional judgement. The mean ERMQ value of 0.5 was based on an evaluation by Long and MacDonald (in press) that indicated at least 50% of samples in a nationwide evaluation exhibited toxicity when this value was exceeded. The BPTCP has calculated mean ERMQ values using a different suite of chemicals than used by Long and MacDonald (in press). The primary differences being that Long and MacDonald (in press) used a number of individual PAHs and the DDT ERM, whereas the BPTCP used only the summary low and high molecular weight PAHs (2 values) and the DDT value of Swartz et al. (1994). When the mean ERMQ values, as calculated by the BPTCP, were compared with amphipod toxicity in the statewide BPTCP database, 62% of the samples with mean ERMQs greater than 0.5 were found to be toxic to amphipods.

These chemistry, toxicity, and benthic community threshold values were derived to allow a consistent interpretation of data from samples throughout the Region and state. It is important to note that while these threshold values were selected based on the best available information and best professional judgement of the authors, they are by nature discretionary. Chemical

biological impacts. The thresholds and station characterizations used here are not intended to be absolute. They are intended to aid in the screening of data collected from a large number of locations, in order to support management decisions. In some cases additional studies may be undertaken to further evaluate the sites of concern identified in this Region-wide assessment. As more data become available through additional studies, more accurate site-specific characterizations of sediment quality may result.

Weight-of-Evidence Categorization Criteria

Category 1:
Stations with elevated chemistry*, recurrent toxicity**, and degraded benthos***.

Category 2:
Stations with elevated chemistry, one (of one) toxicity hit, and degraded benthos. (only one sample tested and significant toxicity indicated)

Category 3:
Stations where muscle or whole body tissue residues in resident, non-migratory organisms exceed levels established by the FDA or NAS for protection of human health or wildlife. Organisms may be either deployed or collected from resident populations. (FDA and NAS values given in SWRCB FED on Guidance for THS Cleanup Plans, page xxiii)

Category 4:
Stations with elevated chemistry and one measure of biological impact. (with no data available for the second biological indicator):

a. Stations with elevated chemistry, degraded benthos, and no available toxicity data.
b. Stations with elevated chemistry, recurrent toxicity and no available benthic data.
c. Stations with elevated chemistry, toxicity in a single sample and no available Benthics data (only one toxicity sample tested).

Category 5:
Stations with elevated chemistry and mixed results from biological indicators.

a. Stations with elevated chemistry, degraded benthos, and multiple toxicity tests with some toxic and some non-toxic.
b. Stations with elevated chemistry, degraded benthos, and toxicity data indicating samples were non-toxic.
c. Stations with elevated chemistry, recurrent toxicity and data indicating non-degraded benthos.
d. Stations with elevated chemistry, toxicity in a single sample and data indicating non-degraded benthos (only one toxicity sample tested).
e. Stations with elevated chemistry, data indicating non-degraded benthos and multiple toxicity tests with some toxic and some non-toxic.

Category 6
Stations with measured biological impact but no indication of elevated chemistry.

a. Stations with recurrent toxicity, and degraded benthos, but no chemistry data available.
b. Stations with recurrent toxicity, and degraded benthos, and elevated NH_3 or H_2S ****, but no other elevated chemistry.
c. Stations with recurrent toxicity, and degraded benthos, but existing chemistry data has fewer than six chemicals measured at elevated concentrations.
d. Stations with a single indicator of biological effect (either recurrent toxicity or degraded benthos), but existing chemistry data has fewer than six chemicals measured at elevated concentrations.
e. Stations with a single toxic sample, but existing chemistry data has fewer than six chemicals measured at elevated concentrations.

Category 7
Stations with no measured toxicity, benthic degradation or elevated chemistry.

Reference Stations
These should be selected using best professional judgement of available information, including grain size, salinity, chemistry, benthic ecology, and toxicity data, as well as station location relative to pollutant sources. The parameter to be compared to reference (e.g., toxicity) should not be the primary measure used in reference site selection.

Ranking within these major categories were determined by the actual data values, such as 20% survival was ranked above 55% survival, etc. Best professional judgement was necessary to balance chemical versus biological data values.

*Elevated Chemistry was indicated by:

1. A guideline ERM quotient (ERMQ) above 0.5, indicating a mixture of pollutants, or
2. Six or more chemicals having concentrations above guideline (ERM) values, or
3. One or more individual chemicals at concentrations high enough to likely be associated with biological effects, based on best professional judgement.

Additional chemicals without sediment quality guidelines associated with them are also examined for additional evidence of chemical contamination. These chemicals are noted in Table 31.

**Recurrent toxicity is indicated when at least two samples collected at different times from a station are determined to be significantly toxic (as defined by t-test and MSD) by any of the BPTCP toxicity test protocols.

***Degraded benthos are indicated by a Relative Benthic Index score of 0.30 or less, or by best professional judgement of a qualified benthic ecologist.

****Elevated concentrations of NH_3 or H_2S thought to have resulted from human activity may be considered equivalent to elevated concentrations of other anthropogenic chemicals for ranking purposes, based on best professional judgement. In cases where NH_3 and H_2S are thought to

result from natural processes, high concentrations may be considered as interferences in toxicity or benthic assessments.

Chemistry, toxicity, benthic, bioaccumulation or other data from previous studies may be considered as part of any of the scenarios described above.

Quality Assurance/Quality Control

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. This document describes procedures within the program, which ensure data quality and integrity. Quality assurance procedures follow those of the NS&T Program to ensure comparability with other NOAA survey areas nationwide. In addition, individual laboratories prepare quality assurance evaluations of each discrete set of samples analyzed and authorized by task order. These documents were submitted to the California Department of Fish and Game for review, then forwarded to the State Water Resources Control Board for further review.

RESULTS AND DISCUSSION

Chemistry Data

Discussion of Data Relative to QA Criteria

All chemistry data were evaluated for acceptability using the Quality Assurance guidelines presented in the BPTCP Quality Assurance Project Plan (Stephenson et al., 1994). Most of the data reported here met test acceptability standards for each analysis procedure. Departures from acceptability standards are summarized in Appendix E. There were minor deviations of quality assurance criteria that generally included blank responses falling outside of control chart guidelines. In the cases of these minor deviations the reported chemical concentration has been corrected based on the blank response.

Discussion of Chemical Mixtures

The analytical results for specific analytes and analyte classes used in the BPTCP are listed in Appendix C. These results were compared with the NOAA's ERL and ERM levels, and the frequency of guideline exceedances for the Santa Ana region is shown in Figure 4. The Santa Ana region was divided into three distinct water bodies: Anaheim Bay/Seal Beach Naval Weapons Reserve, Huntington Harbor/Bolsa Chica, and Newport Bay. Based on exceedances of chemical guideline values, chemicals of concern were noted for each water body. In addition to individual ERM exceedances, chemical summary quotients (ERMQs) were used to rank stations by chemical load within water bodies (Tables 13 through 15). Not all stations had chemical analysis conducted during every visit therefore, all sampling events for a given station are grouped together for reference. Stations that did not have any chemical analysis conducted, are grouped at the bottom of the tables. The ERMQs are mapped in Figures 5a through 5c to depict areal extent of ERM exceedances.

grouped at the bottom of the tables. The ERMQs are mapped in Figures 5a through 5c to depict areal extent of ERM exceedances.

Anaheim Bay Naval Reserve (82030.0, ERMQ = 0.597) and Outer Anaheim Bay (80024.0, ERMQ = 0.210) had the highest ERMQ values in the northern water body (Table 13). The elevated ERMQs for these stations were based on the ERM exceedances of total chlordane and p,p'DDE. Total chlordane at Anaheim Bay Naval Reserve was in the top 10% of samples measured for the BPTCP. The exceedance of the ERM guidelines for total chlordane and p,p'DDE also contributed to Huntington Harbor's highest ERMQ values. Huntington Harbor had higher ERMQs than Anaheim Bay and exhibited a clear chemical gradient from the upper to the lower harbor (Table 14). Exceedances of total chlordane and p,p'DDE occurred along the main channel of Huntington Harbor and extended into Outer Anaheim Bay. No other chemicals exceeded sediment guidelines in the samples measured.

Newport Bay had the highest ERMQ values of any regional water body (Table 15). Exceedances of ERM guidelines for copper, mercury, zinc and total PCBs contributed to high ERMQs for Rhine Channel (85013.0) and Newport Island (85014.0). Mercury exceedances also occurred at Stations 85002.0 and 85006.0, both in close proximity to Rhine Channel and Newport Island stations. Mercury, copper and tributyltin concentrations at Rhine Channel station were in the top 5% of concentrations measured in the BPTCP. Exceedances of total chlordane and p'p-DDE occurred at various stations throughout Newport Bay.

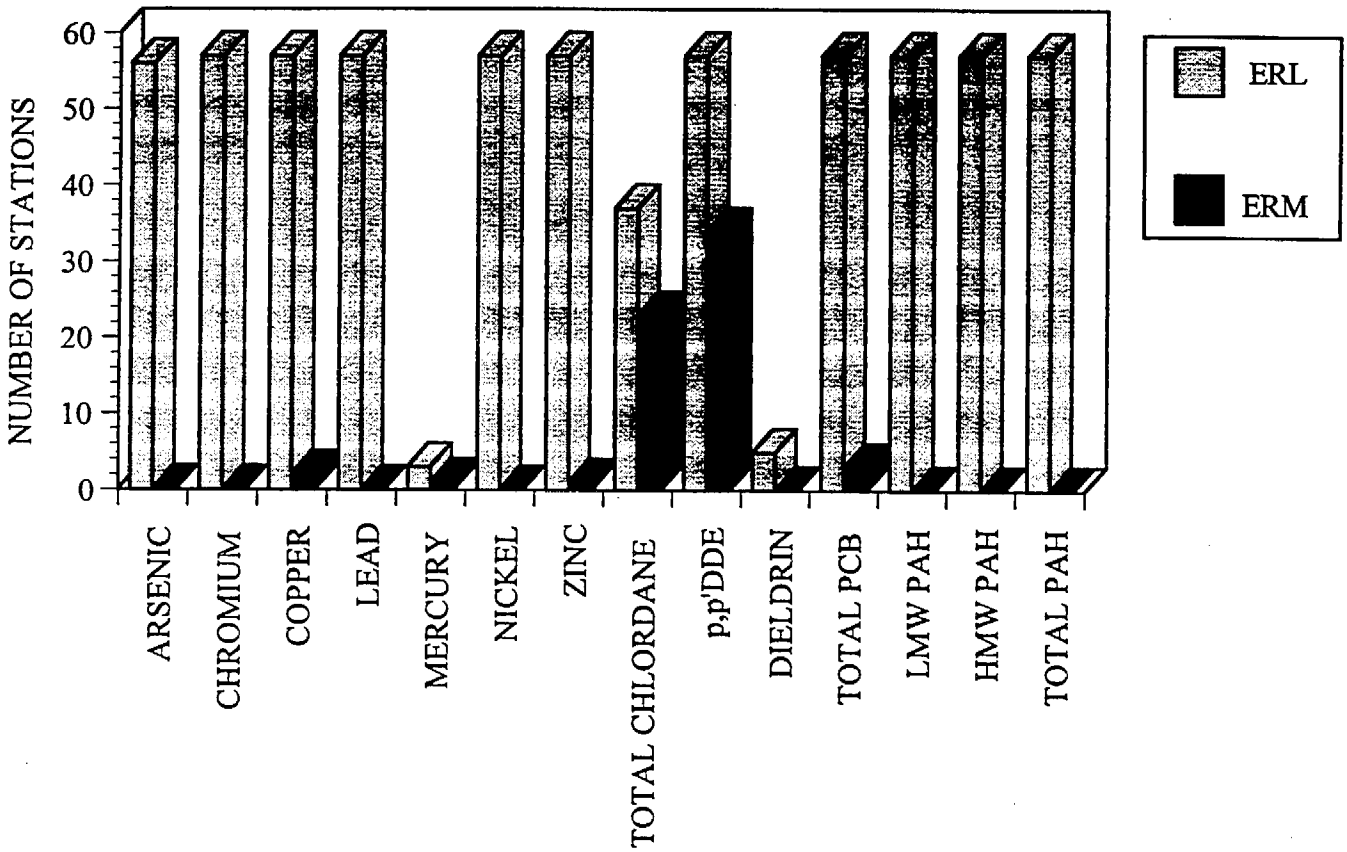


Figure 4. Frequency of stations exceeding ERL or ERM sediment quality guidelines.

Table 13. Anaheim Bay chemistry results. Stations ranked by mean ERM Quotient.

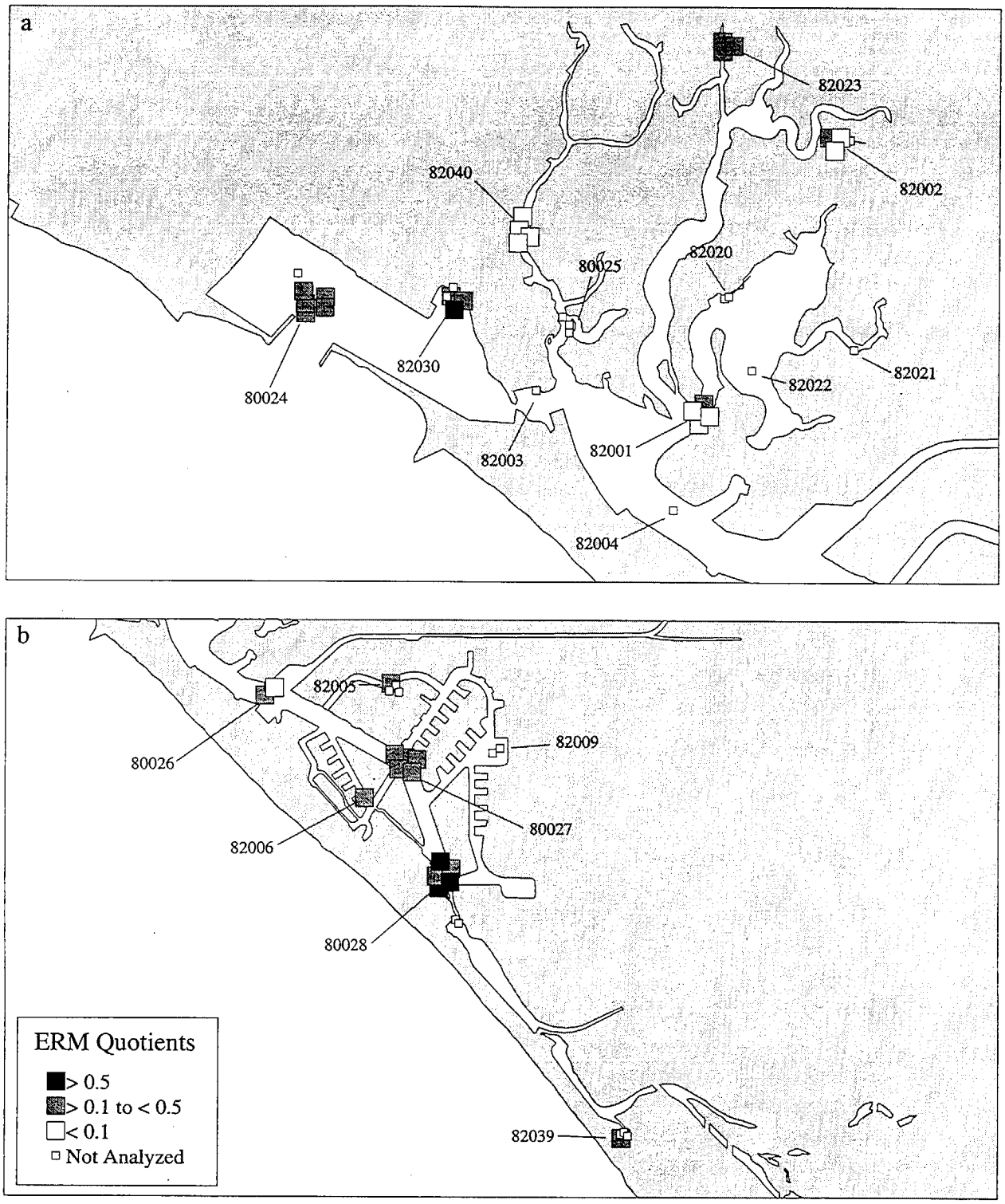
Station No.	Station Name	IDOrg	Leg	ERMQ	ERM Exceedances
82030.0	Anaheim Bay - Naval Res. - R3	1046	25	0.597	ΣChlordane, p,p'DDE
82030.0	Anaheim Bay - Naval Res. - R2	1045	25	0.183	ΣChlordane, p,p'DDE
82030.0	Anaheim Bay - Naval Res. - R1	1044	25	0.182	ΣChlordane, p,p'DDE
82030.0	Anaheim Bay - Naval Res.	430	9	n/a	n/a
82030.0	Anaheim Bay - Naval Res.	772	17	n/a	n/a
82030.0	Anaheim Bay - Naval Res. - R1	1195	30	n/a	n/a
82030.0	Anaheim Bay - Naval Res. - R2	1196	30	n/a	n/a
82030.0	Anaheim Bay - Naval Res. - R3	1197	30	n/a	n/a
82030.0	Anaheim Bay - Naval Res.	1335	32	n/a	n/a
80024.3	Outer Anaheim Bay - R1	1171	29	0.210	ΣChlordane, p,p'DDE
80024.3	Outer Anaheim Bay - R2	1172	29	0.206	ΣChlordane, p,p'DDE
80024.3	Outer Anaheim Bay - R3	1173	29	0.194	ΣChlordane, p,p'DDE
80024.3	Outer Anaheim Bay	87	4	0.141	None
80024.3	Outer Anaheim Bay	807	19	n/a	n/a
82023.0	Seal Beach NWR - Bolsa Ave - R3	1094	26	0.131	None
82023.0	Seal Beach NWR - Bolsa Ave - R2	1093	26	0.117	None
82023.0	Seal Beach NWR - Bolsa Ave - R1	1092	26	0.107	None
82023.0	Seal Beach NWR - Bolsa Ave.	423	9	n/a	n/a
82023.0	Seal Beach NWR - Bolsa Ave.	771	17	n/a	n/a
82002.0	Anaheim Bay - Navy Marsh #2 - R1	1089	26	0.108	None
82002.0	Anaheim Bay - Navy Marsh #2 - R3	1091	26	0.099	None
82002.0	Anaheim Bay - Navy Marsh #2 - R2	1090	26	0.090	None
82002.0	Anaheim Bay - Navy Marsh #2	402	9	n/a	n/a
82002.0	Anaheim Bay - Navy Marsh #2	809	19	n/a	n/a
80024.1	Outer Anaheim Bay	85	4	0.101	None
82001.0	Anaheim Bay - Navy Marsh - R3	1088	26	0.101	None
82001.0	Anaheim Bay - Navy Marsh - R1	1086	26	0.082	None
82001.0	Anaheim Bay - Navy Marsh - R2	1087	26	0.078	None
82001.0	Anaheim Bay - Navy Marsh	401	9	0.073	None
82040.0	Seal Beach NWR - R2	1096	26	0.094	None
82040.0	Seal Beach NWR - R3	1097	26	0.089	None
82040.0	Seal Beach NWR - R1	1095	26	0.086	None
82040.0	Seal Beach NWR	440	9	0.078	None
80024.2	Outer Anaheim Bay	86	4	n/a	n/a
80025.1	Anaheim Bay - Oil Island	88	5	n/a	n/a
80025.2	Anaheim Bay - Oil Island	89	5	n/a	n/a
80025.3	Anaheim Bay - Oil Island	90	5	n/a	n/a
82003.0	Anaheim Bay - Entrance	403	9	n/a	n/a
82004.0	Anaheim Bay - Fuel Dock S.	404	9	n/a	n/a
82020.0	Seal Beach NWR - Nasa Is.	420	9	n/a	n/a
82020.0	Seal Beach NWR - Nasa Is.	769	17	n/a	n/a
82021.0	Seal Beach NWR - Hog Is.	421	9	n/a	n/a
82022.0	Seal Beach NWR - Sunset AGU	422	9	n/a	n/a

Table 14. Huntington Harbor chemistry results. Stations ranked by mean ERM Quotient.

Station No.	Station Name	IDOrg	Leg	ERMQ	ERM Exceedances
80028.3	Upper Huntington Harbor - R1	1174	29	0.654	ΣChlordane, p,p'DDE
80028.3	Upper Huntington Harbor - R2	1175	29	0.626	ΣChlordane, p,p'DDE
80028.3	Upper Huntington Harbor - R3	1176	29	0.582	ΣChlordane, p,p'DDE
80028.3	Upper Huntington Harbor	99	4	0.352	ΣChlordane, p,p'DDE
80028.2	Upper Huntington Harbor	98	4	0.356	ΣChlordane, p,p'DDE
80027.3	Middle Huntington Harbor - R3	1179	29	0.332	ΣChlordane, p,p'DDE
80027.3	Middle Huntington Harbor - R1	1177	29	0.309	ΣChlordane, p,p'DDE
80027.3	Middle Huntington Harbor - R2	1178	29	0.296	ΣChlordane, p,p'DDE
80027.3	Middle Huntington Harbor	96	4	0.250	ΣChlordane, p,p'DDE
82006.0	Huntington Harbor - Peter's	406	9	0.296	ΣChlordane, p,p'DDE
80027.2	Middle Huntington Harbor	95	4	0.261	ΣChlordane, p,p'DDE
82005.0	Huntington Harbor - Launch	405	9	0.163	p,p'DDE
82005.0	Huntington Harbor - Launch - R1	1201	30	n/a	n/a
82005.0	Huntington Harbor - Launch - R2	1202	30	n/a	n/a
82005.0	Huntington Harbor - Launch - R3	1203	30	n/a	n/a
82039.0	Bolsa Chica Ecological Reserve	439	9	0.146	None
82039.0	Bolsa Chica Ecol. Reserve - R1	1204	30	n/a	n/a
82039.0	Bolsa Chica Ecol. Reserve - R2	1205	30	n/a	n/a
82039.0	Bolsa Chica Ecol. Reserve - R3	1206	30	n/a	n/a
80026.1	Lower Huntington Harbor	91	4	0.117	None
80026.2	Lower Huntington Harbor	92	4	0.076	None
80026.3	Lower Huntington Harbor	93	4	n/a	n/a
80027.1	Middle Huntington Harbor	94	4	n/a	n/a
80028.1	Upper Huntington Harbor	97	4	n/a	n/a
82009.0	Huntington Harbor - HAR. LA	409	9	n/a	n/a
82024.0	Bolsa Bay - Mouth of Eggw Flood	424	9	n/a	n/a
82024.0	Bolsa Bay - Mouth of Eggw Flood	770	17	n/a	n/a
82009.0	Huntington Harbor - HAR. LA	808	19	n/a	n/a

Table 15. Newport Bay chemistry results. Stations ranked by mean ERM Quotient.

Station No.	Station Name	IDOrg	Leg	ERMQ	ERM Exceedances
85013.0	Newport Bay (Rhine Channel)	1424	36	1.270	Cu, Hg, p'p-DDE, ΣPCB
85013.0	Newport Bay (Rhine Channel)	1633	45	1.124	Cu, Hg, p'p-DDE, ΣPCB
85014.0	Newport Bay (Newport Island)	1425	36	0.733	Hg, Zn, ΣChlordane, p,p'DDE, ΣPCB
85015.0	Newport Bay (Arches Storm Drains)	1426	36	0.668	ΣChlordane, p,p'DDE
85006.0	Newport Bay (1009)	1392	34	0.318	Hg, p,p'DDE
85017.0	Newport Bay (Unit II Basin)	1428	36	0.256	ΣChlordane, p,p'DDE
85005.0	Newport Bay (949)	1391	34	0.244	p,p'DDE
85002.0	Newport Bay (616)	1388	34	0.239	Hg, p,p'DDE
85010.0	Newport Bay (819)	1421	36	0.216	p,p'DDE
85012.0	Newport Bay (1064)	1423	36	0.212	ΣChlordane, p,p'DDE
85011.0	Newport Bay (905)	1422	36	0.200	ΣChlordane, p,p'DDE
85011.0	Newport Bay (523)	1634	45	0.089	None
85004.0	Newport Bay (877)	1390	34	0.198	p,p'DDE
85001.0	Newport Bay (523)	1387	34	0.180	p,p'DDE
85001.0	Newport Bay (523)	1788	54	n/a	n/a
85008.0	Newport Bay (670)	1419	36	0.175	ΣChlordane, p,p'DDE
85016.0	Newport Bay (Yachtmans Cove)	1427	36	0.163	None
85003.0	Newport Bay (791)	1389	34	0.147	p,p'DDE
85009.0	Newport Bay (705)	1420	36	0.131	p,p'DDE
85018.0	Newport Bay (Unit I Basin)	1429	36	0.093	None
85007.0	Newport Bay (431)	1418	36	0.070	None
86001.0	San Diego Creek - Campus	1789	54	n/a	n/a
86002.0	San Diego Creek - MacArthur	1790	54	n/a	n/a
86003.0	Santa Ana/Delhi Channel - Bridge	1791	54	n/a	n/a
86004.0	Santa Ana/Delhi Channel - Outer	1792	54	n/a	n/a



Figures 5a and 5b. Mean ERM quotients for stations in Anaheim Bay and Huntington Harbor.

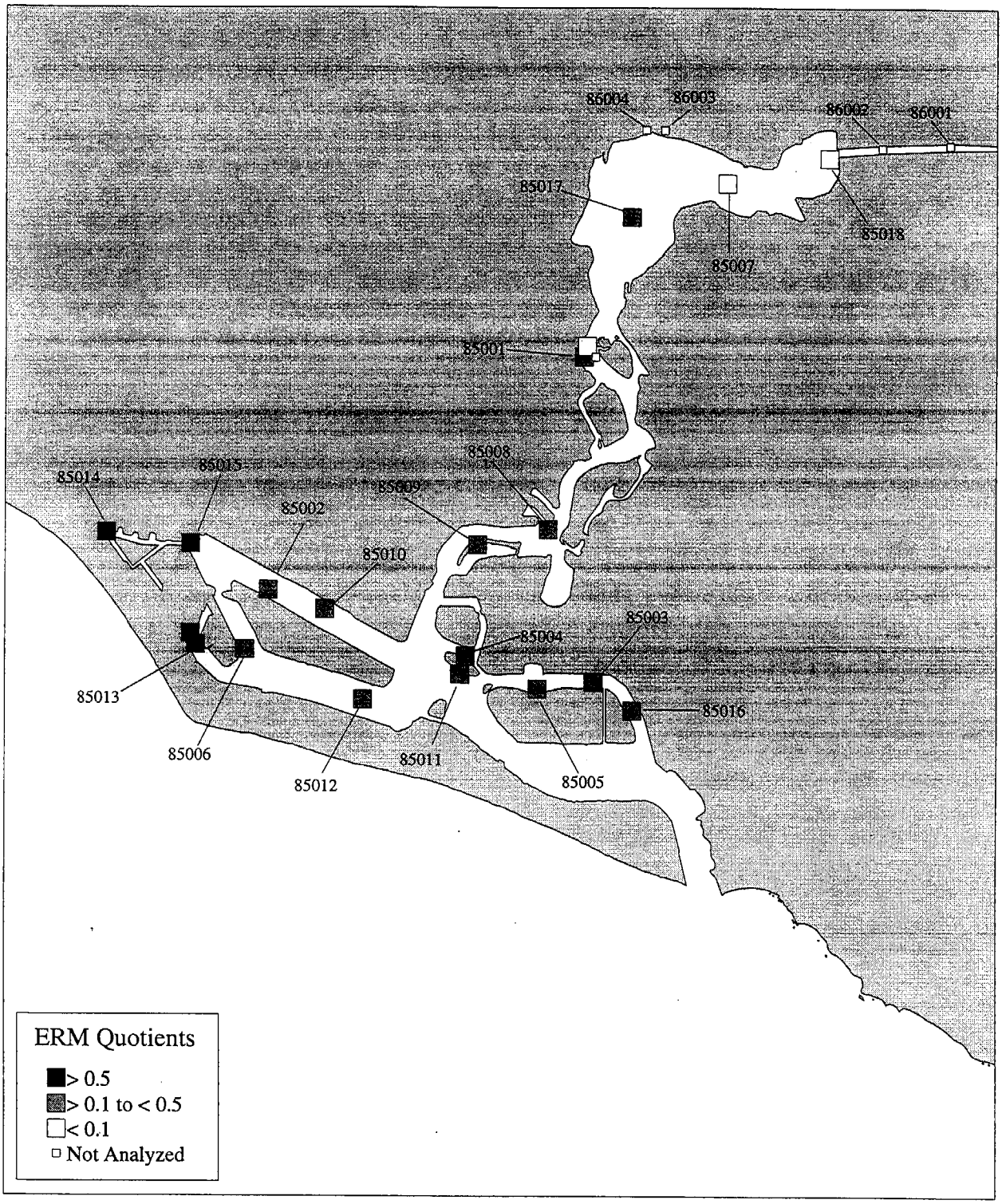


Figure 5c. Mean ERM quotients for stations in Newport Bay.

Individual Chemicals Compared to Sediment Guideline Values

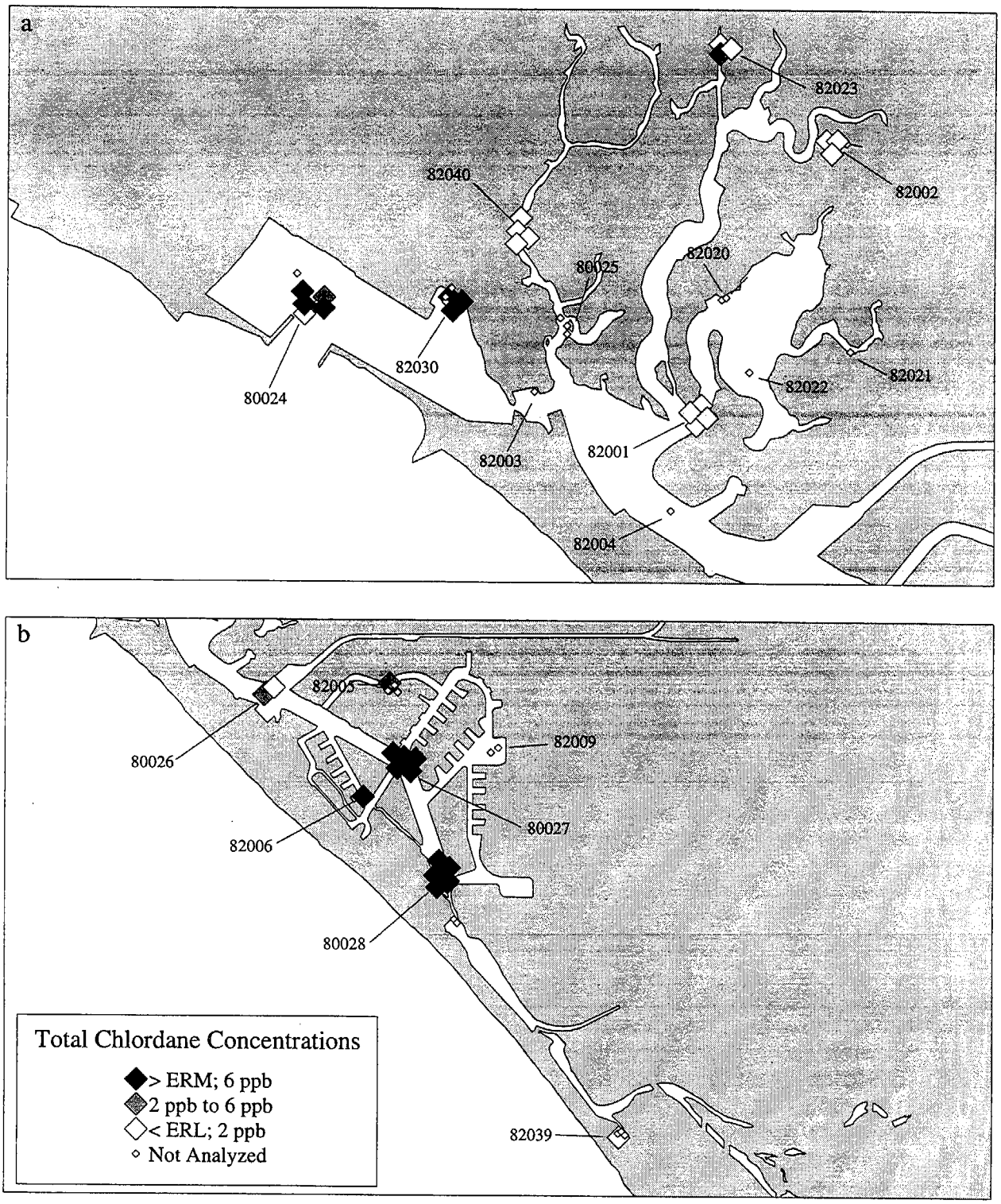
Total chlordane is the summation of the major constituents of technical grade chlordane and its metabolites and comprise a group of nonsystemic stomach and contact insecticides which until the mid 1970's had been used extensively in home and agricultural applications. Although the use of this compound was discontinued in this country due to its 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 (Wilcock et al., 1993).

DDT and its metabolites are a class of relatively water insoluble organo-chlorine compounds that 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 elevated concentrations (Hoke et al., 1994; Swartz et al., 1994). p,p'DDE is a metabolite of DDT and can also persist in the environment.

The Anaheim Bay region had 12 ERM exceedances among two stations (80024.3 and 82030.0). Six of the exceedances were for total chlordane and six were for p,p'DDE (Figures 6a and 7a). Exceedances for both chemicals were relatively low in magnitude (1.1-1.4x the ERM) except for station 82030.0, Replicate 3, which exceeded the ERM for total chlordane by 7.4 times.

Huntington Harbor had 23 exceedances among 12 stations. Eleven of the exceedances were for total chlordane and twelve were for p,p'DDE. Both of these chemicals exceeded the ERM guidelines by up to 7 times (Figures 6b and 7b).

Newport Bay had 33 exceedances among 16 stations. All 16 stations exceeded the ERM guideline for p,p'DDE (Figure 7c). Within those 16 stations, six exceeded the ERM for total chlordane, the highest concentration being at Arches Storm Drain (85015.0, 5.2x the ERM, Figure 6c). The largest overall exceedances in Newport Bay were for mercury in the Rhine Channel (85013.0, 12.3x the ERM). The Rhine Channel station also exhibited ERM exceedances for Copper and Total PCBs (Figure 8). Newport Bay had the most ERM exceedances for any individual stations, four in the Rhine Channel (85013.0), and five at Newport Island (85014.0), which included exceedances for copper, mercury, zinc and total PCBs. Anaheim Bay and Huntington Harbor had no more than two exceedances at any one station.



Figures 6a and 6b. Total chlordane concentrations for stations in Anaheim Bay and Huntington Harbor.

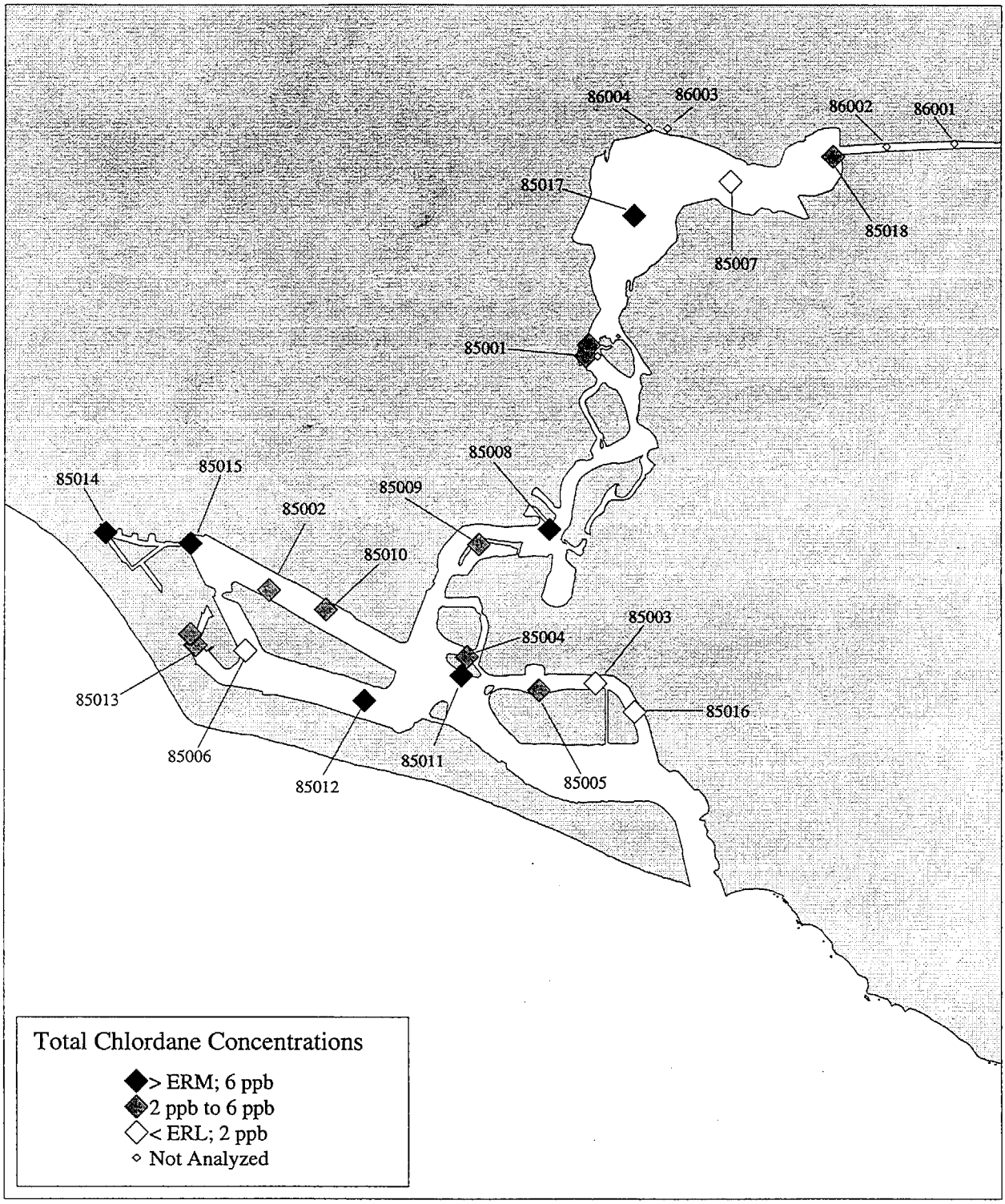
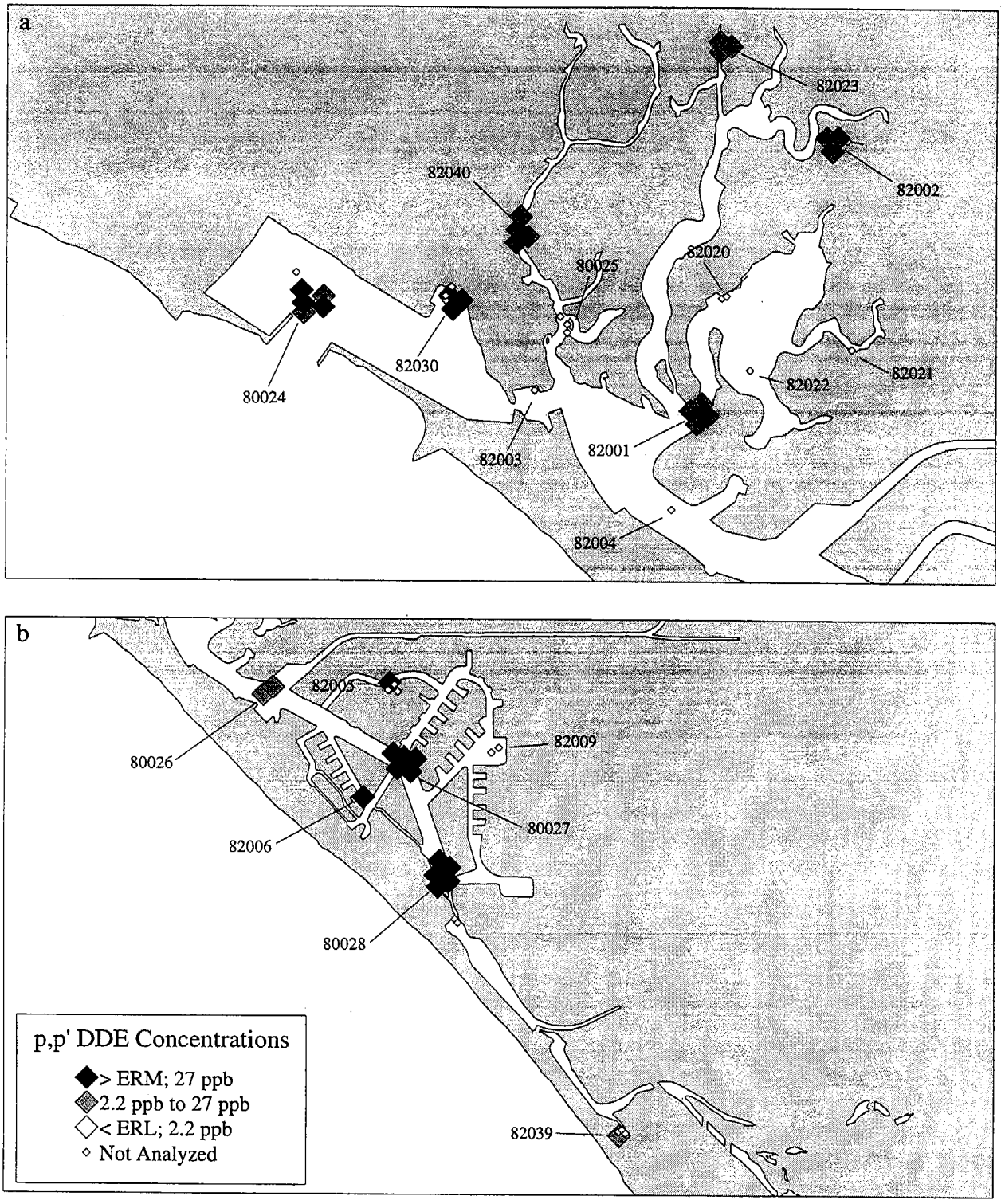


Figure 6c. Total chlordane concentrations for stations in Newport Bay.



Figures 7a and 7b. p,p' DDE concentrations for stations in Anaheim Bay and Huntington Harbor.

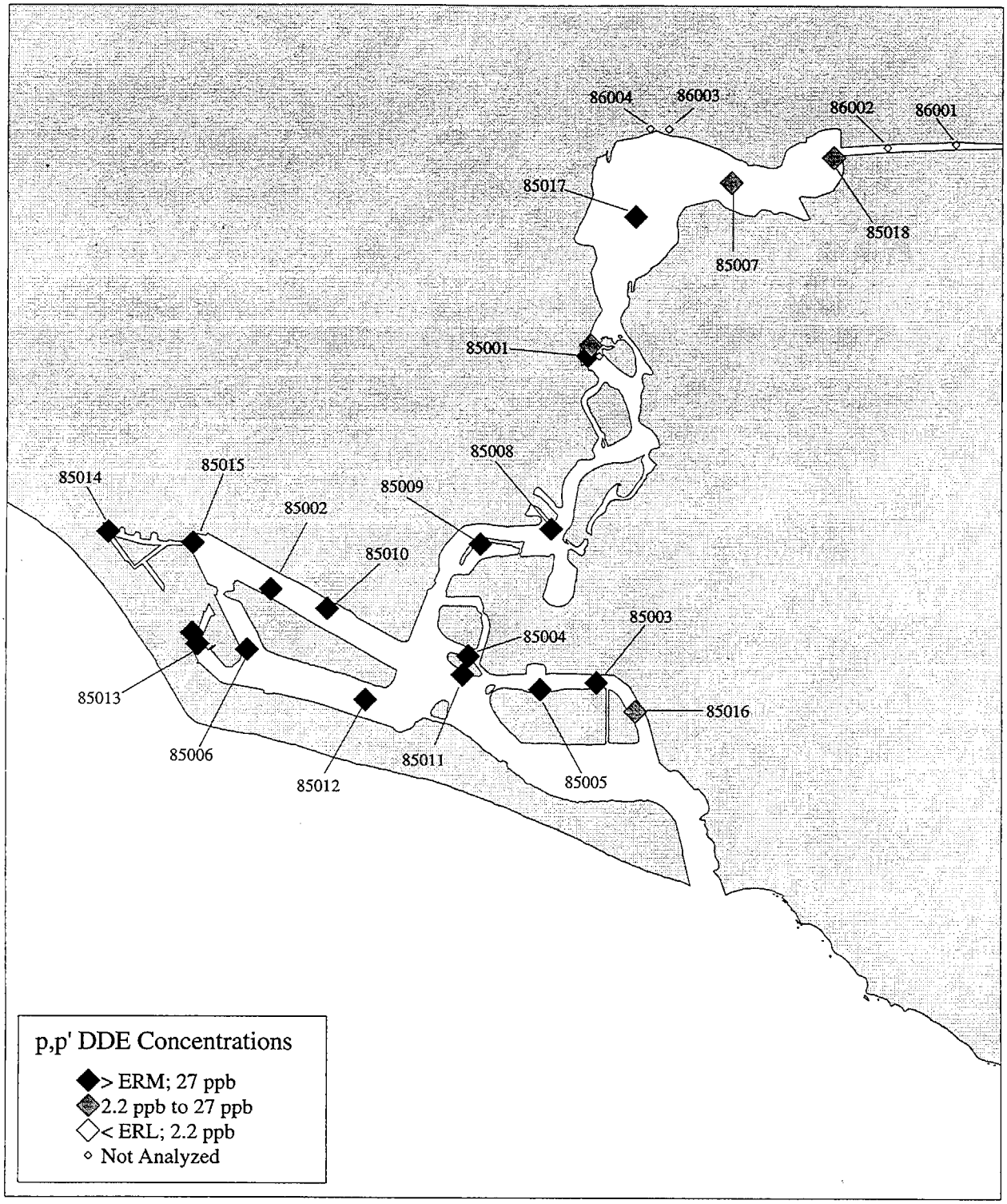


Figure 7c. p,p' DDE concentrations for stations in Newport Bay.

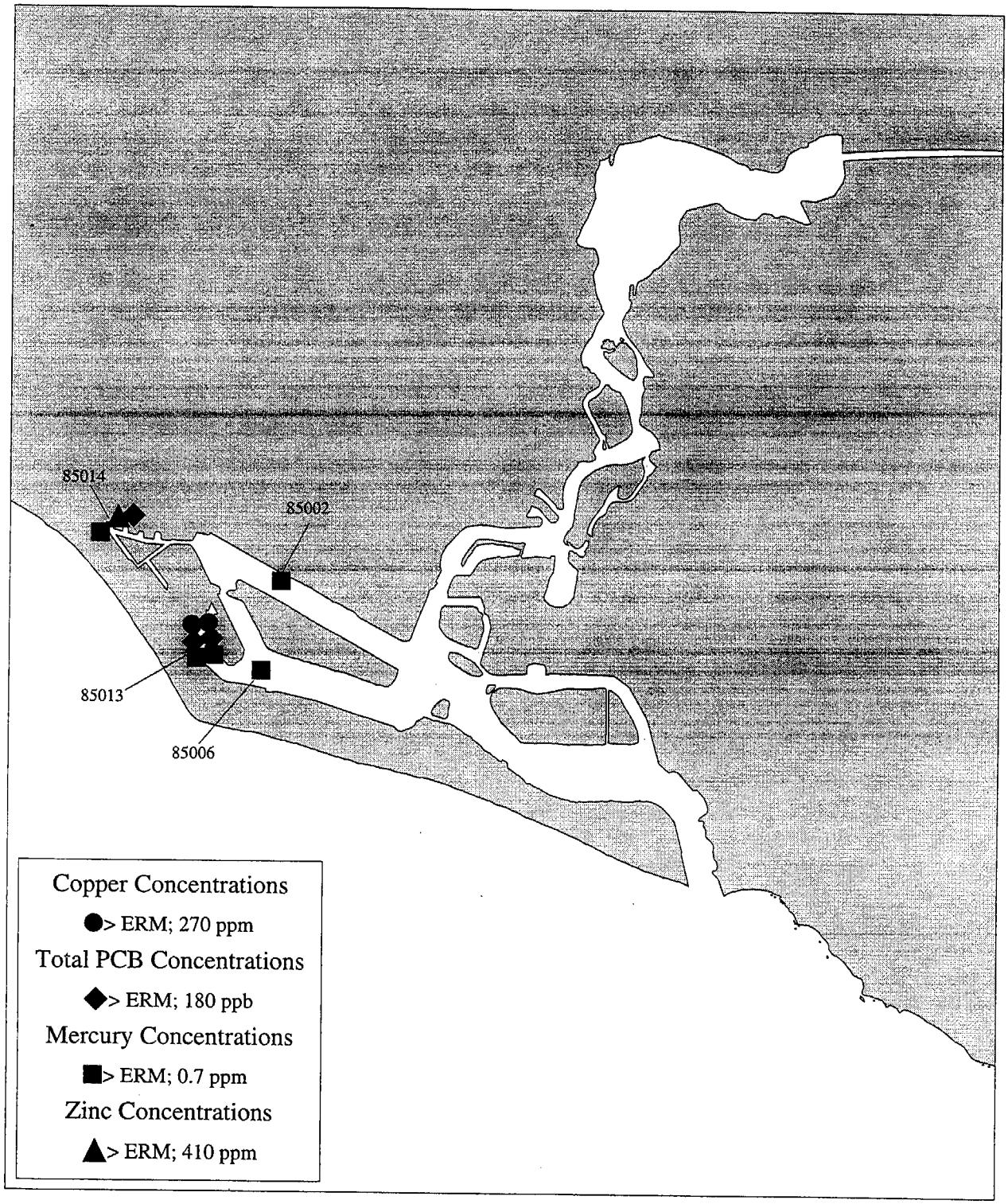


Figure 8. Copper, total PCB, Mercury, and Zinc concentrations for stations in Newport Bay.

Porewater Chemistry Results

Three stations were analyzed for porewater metals chemistry and one station was analyzed for SEM/AVS (Table 16). Middle and Upper Huntington Harbor (80027.2 and 80028.2) and Newport Bay's Rhine Channel (85013.0) had high concentrations of trace metals. SEM/AVS analysis was also conducted at the Rhine Channel station. The ratio of SEM to AVS was 4.65. SEM/AVS ratios greater than one indicate that not all metals are bound by sulfide complexes and may be bioavailable. Because this is generally true only in anoxic sediments, these data should be viewed carefully.

Table 16. Concentrations of selected trace metals in porewater, and SEM/AVS in station 85013.0.

Station Number	IDOrg	Porewater Metals Al	Cd	Cu	Fe	Pb	Mn	Ni	Ag	Zn
80027.2	95	76	0.019	2.6	7500	1.30	2300	3.00	ND	14.0
80028.2	98	45	0.025	4.5	1900	0.56	600	2.70	ND	25.0
85013.0	1633	1090	0.100	30.0	7000	3.48	1270	3.33	0.0008	15.8

		AVS	SEM Cd	SEM Cu	SEM Ni	SEM Pb	SEM Zn	SEM Sum	SEM/ AVS	DOC
85013.0	1633	1.46	0.0022	4.36	0.045	0.374	2.02	6.80	4.645	2971

Tissue Chemistry Results

Only the Rhine Channel in Newport Bay was analyzed for bioaccumulation of chemicals in fish tissue. A complete list of analyzed chemicals is contained in Appendix C. Topsmelt collected from Rhine Channel did not contain levels of mercury, total DDT, total PCB or total Chlordane that were higher than acceptable Maximum Tissue Residue Levels (SWRCB, 1993; Table 17).

Table 17. Concentrations of selected tissue contaminants from station 85013.0.

Station Number	IDOrg	Tissue	Hg	p,p'DDD	p,p'DDE	Total DDT	Total Chlordane	Total PCB
82017.0	285.0	Topsmelt	0.0022	4.36	0.045	0.374	2.02	6.80

Toxicity Testing

Discussion of Data Relative to QA Criteria

All toxicity test data were evaluated for acceptability using the Quality Assurance guidelines presented in the BPTCP Quality Assurance Project Plan (Stephenson et al., 1994). Most of the data reported here met test acceptability standards for each test protocol. Departures from acceptability standards are summarized in Appendix E. 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. Major exceedances of quality assurance criteria occurred in purple urchin fertilization and larval development tests of samples from stations 85007 and

85008, which both had excessively low dissolved oxygen concentrations. In both samples the 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.

Minor exceedances of quality assurance criteria occurred in several areas. Precision measurements are calculated by measuring a water quality standard three times throughout the water quality series. Ammonia precision exceeded the quality criterion by 8.4% during ammonia readings for the Leg 26 amphipod test. This should be taken into consideration when evaluating ammonia data from this test. Actual ammonia concentrations may differ from the measured value by up to 38.4% 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 met the 90% survival criterion (Home sediment from Wickford, RI). Studies on the effect of sediment holding times on amphipod (*Rhepoxynius*) mortality suggest that survival decreases with increasing storage time after a period of 11 weeks (Becker and Ginn, 1995). In their study no significant difference in amphipod survival was noted up to a 6-week storage time. Since storage time for samples in this study was three weeks, it is unlikely that amphipod survival was inordinately biased. Control survival in Leg 36 was 92%. This is similar to the average control survival we have obtained in other tests when using East Coast *Ampelisca*.

Leg 36 *Rhepoxynius* test organisms were acclimated at test salinity for less than 48 hours. Because the control response was greater than 90%, the short acclimation time probably had a negligible affect on the amphipods. The final minor exceedance was sample 85001 in the Leg 54 purple urchin sediment-water interface test. A low dissolved oxygen concentration of 4.57 mg/L might have contributed to reduced normal larval development.

Minor exceedances of quality assurance criteria that are coded -3 (Appendix E) have negligable effects on the results of toxicity tests. Stations are listed for exceedances of dissolved oxygen and salinity. While low DO concentrations can have a significant impact on mortality in toxicity tests, concentrations slightly higher than 100% saturation are not considered biologically important to the species and life stages used in these experiments. Salinity exceedances were not outside the tolerance range of the test organisms.

Amphipod Toxicity Testing Results

The results for the samples collected and tested concurrently on each sampling leg for Anaheim Bay, Huntington Harbor and Newport Bay are in Tables 18 through 20. These tables show the mean proportion survival of amphipods at each station and site, with significant toxicity relative to controls reported at $p<0.05$ (t-test) and toxicity reported as significant with a t-test and MSD. Anaheim Bay and Huntington Harbor were both tested with the amphipod *Rhepoxynius*.

Newport Bay was tested with a combination of *Rhepoxynius* and *Eohaustorius*. Additional tests using *Ampelisca* were conducted in Newport Bay as part of a protocol comparison study.

A total of 16 of 43 samples (37%) from twelve sites were toxic to amphipods in Anaheim Bay. Eight sites demonstrated toxicity for at least one station. The highest incidence of toxicity occurred at the Seal Beach Naval Weapons Reserve (82040.0) where three of four stations were toxic to amphipods (Figure 9a). This site had relatively low chemical concentrations at its stations and ranked seventh in terms of ERMQ in Anaheim Bay (Table 18). Three of five stations demonstrated toxicity at the Seal Beach Naval Weapons Reserve – Bolsa Ave. site (82023.0), where the ERMQ ranked third. Anaheim Bay Naval Reserve (82030.0) had the highest chemical concentrations (ERMQs from 0.182 to 0.597), and was toxic at three out of nine stations. Amphipod toxicity was evenly distributed around Anaheim Bay.

Fourteen of 28 samples from eight sites were toxic to amphipods in Huntington Harbor (Table 19). Seven sites demonstrated toxicity for at least one station (Figure 9b). Bolsa Chica Ecological Reserve (82039.0) demonstrated the most toxicity with four of four stations. This site had the seventh highest ERMQ in Huntington Harbor. Middle Huntington Harbor (80027.1-3) was toxic at five of six stations, and had the third and fifth highest mean ERMQs. The site with the highest ERMQs, Upper Huntington Harbor (80028.1-3), was toxic at two of six stations. Amphipod toxicity in Huntington Harbor was concentrated mostly along the channel from the middle harbor site to the Bolsa Chica Reserve site. Additional toxicity occurred in the marina areas.

Nine of 25 samples from 22 sites were toxic to amphipods in Newport Bay (Table 20). Toxicity was concentrated around Lido Island at the Rhine Channel and Newport Island sites (85013.0 and 85014.0), that had the highest ERMQ values in the bay. Toxicity also occurred on the north and south sides of Lido Island at sites 85002.0, 85010.0 and 85012.0 (Figure 9c). Additional toxicity occurred in the upper bay at sites 85008.0 and 85001.0. In twelve duplicate amphipod tests with *Ampelisca* conducted during Leg 36, ten results agreed with those of the *Rhepoxynius* test. Sites 85010.0 and 85012.0 were toxic to *Rhepoxynius* and not toxic to *Ampelisca*. Toxic responses with *Ampelisca* also occurred at the Rhine Channel and Newport Island sites and site 85008.0.

Table 18. Toxicity of Anaheim Bay sediments to *Rhepoxynius* amphipods (n = 5).

Station No.	IDOrg	Rhepoxynius Mean	Rhepoxynius SD	Significance	Toxicity
82030.0	1046	62.00	13.51	*	T
82030.0	1045	69.00	19.17	*	T
82030.0	1044	38.00	16.81	*	T
82030.0	430	87.00	7.60	*	NT
82030.0	772	87.00	9.70	NS	NT
82030.0	1195	82.00	24.14	NS	NT
82030.0	1196	79.00	2.24	*	NT
82030.0	1197	90.00	6.12	NS	NT
82030.0	1335	79.00	9.62	*	NT
80024.3	1171	91.00	8.94	NS	NT
80024.3	1172	88.00	5.70	*	NT
80024.3	1173	85.00	3.54	*	NT
80024.3	87	82.00	14.40	NS	NT
80024.3	807	34.00	15.20	*	T
82023.0	1094	51.00	11.94	*	T
82023.0	1093	67.00	18.23	*	NT
82023.0	1092	59.00	12.94	*	T
82023.0	423	86.00	6.50	*	NT
82023.0	771	59.00	7.40	*	T
82002.0	1089	72.00	13.04	*	NT
82002.0	1091	79.00	9.62	*	NT
82002.0	1090	76.00	4.18	*	NT
82002.0	402	72.00	17.50	*	T
82002.0	809	32.00	10.40	*	T
80024.1	85	87.00	4.50	*	NT
82001.0	1088	91.00	5.48	*	NT
82001.0	1086	64.00	36.64	NS	NT
82001.0	1087	57.00	27.75	*	T
82001.0	401	42.00	31.10	*	T
82040.0	1096	63.00	10.37	*	T
82040.0	1097	87.00	10.37	*	NT
82040.0	1095	62.00	12.04	*	T
82040.0	440	59.00	17.50	*	T
80024.2	86	84.00	8.20	*	NT
80025.1	88	65.00	11.20	*	T
80025.2	89	80.00	10.00	*	NT
80025.3	90	75.00	10.00	*	NT
82003.0	403	93.00	2.70	*	NT
82004.0	404	91.00	5.50	*	NT
82020.0	420	84.00	8.20	*	NT
82020.0	769	49.00	18.80	*	T
82021.0	421	94.00	6.50	NS	NT
82022.0	422	79.00	6.50	*	NT

Table 19. Toxicity of Huntington Harbor sediments to Rhepoxynius amphipods (n = 5).

Station No.	IDOrg	Rhepoxynius Mean	Rhepoxynius SD	Significance	Toxicity
80028.3	1174	75.00	7.91	*	T
80028.3	1175	83.00	12.04	*	NT
80028.3	1176	80.00	7.91	*	NT
80028.3	99	52.00	14.40	*	T
80028.2	98	73.00	16.00	*	NT
80027.3	1179	89.00	9.62	*	NT
80027.3	1177	93.00	5.70	*	NT
80027.3	1178	78.00	35.46	NS	NT
80027.3	96	44.00	23.80	*	T
82006.0	406	22.00	10.40	*	T
80027.2	95	67.00	13.00	*	T
82005.0	405	43.00	19.90	*	T
82005.0	1201	80.00	11.73	*	NT
82005.0	1202	87.00	9.08	*	NT
82005.0	1203	74.00	23.02	NS	NT
82039.0	439	57.00	14.80	*	T
82039.0	1204	21.00	35.95	*	T
82039.0	1205	9.00	8.94	*	T
82039.0	1206	38.00	29.07	*	T
80026.1	91	86.00	8.20	NS	NT
80026.2	92	92.00	5.70	NS	NT
80026.3	93	82.00	7.60	*	NT
80027.1	94	64.00	9.60	*	T
80028.1	97	73.00	13.00	*	NT
82009.0	409	73.00	7.60	*	T
82024.0	424	81.00	8.20	*	NT
82024.0	770	66.00	14.30	*	T
82009.0	808	20.00	7.90	*	T