Southern California Bight
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Sediment Toxicity

BIGHT'08

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Regional Monitoring Program: I. Sediment Toxicity

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Steven M. Bay[1], Darrin J. Greenstein[1], Matthew Jacobe[2], Carlita Barton[3], Ken Sakamoto[4], Diana Young[1], Kerry J. Ritter[1] and Kenneth C. Schiff[1]

[1] *Southern California Coastal Water Research Project*

[2] *City of Los Angeles, Environmental Monitoring Division*

[3] *Los Angeles County Sanitation Districts*

[4] *Orange County Sanitation District*

SEDIMENT TOXICITY TECHNICAL COMMITTEE

Joe Freas	Aquatic Bioassay and Consulting Laboratories
Michael Machuzak	Aquatic Bioassay and Consulting Laboratories
Philip Chang*	City of LA, EMD
Matthew Jacobe	City of LA, EMD
Dave Gutoff*	City of San Diego
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Lan Wiborg	City of San Diego
Carlita Barton	Los Angeles County Sanitation Districts
Misty Brown	Los Angeles County Sanitation Districts
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Darrin Greenstein	SCCWRP
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Shelly Anghera*	Weston Solutions
Liz Batliner	Weston Solutions
Amy Margolis	Weston Solutions
Chris Osuch*	Weston Solutions
Wendy Rose Hovel*	Weston Solutions
Dan Sowersby	Weston Solutions

*No longer affiliated with the agency listed.

FOREWORD

The Southern California Bight 2008 Regional Monitoring Program (Bight'08) is part of an effort to provide an integrated assessment of environmental condition through cooperative regional-scale monitoring. The Bight'08 program is a continuation of regional surveys conducted in 1994, 1998 and 2003, and represents the joint efforts of more than 90 participating organizations. The Bight'08 program consists of several elements including: Sediment Toxicity, Sediment Chemistry, Areas of Special Biological Significance (ASBS), Demersal Fishes and Megabenthic Invertebrates, Benthic Macrofauna, Offshore Water Quality, Rocky Reefs, Shoreline Microbiology, and Bioaccumulation. Bight'08 workplans, quality assurance plans, as well as the data described in this report and assessment reports for other elements are available at www.sccwrp.org.

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Toxicity testing was provided by seven laboratories: Aquatic Bioassay and Consulting Laboratories (Ventura, CA), City of Los Angeles (Playa del Rey, CA), City of San Diego, Nautilus Environmental (San Diego, CA), Orange County Sanitation District (Fountain Valley, CA), UC Davis Department of Environmental Toxicology, Marine Pollution Studies Laboratory (Monterey, CA), and Weston Solutions (Carlsbad, CA).

EXECUTIVE SUMMARY

Although more than $30 M is spent annually monitoring the effects of anthropogenic discharges to the coastal ocean of the southern California Bight (SCB), outside of the SCB region surveys that occur every five years, virtually no sediment toxicity monitoring occurs. The goal of this study was to answer three questions: 1) What is the extent and magnitude of sediment toxicity in the SCB? 2) How does the extent and magnitude of sediment toxicity compare among specific habitats of interest? and 3) How does the extent and magnitude of sediment toxicity compare to previous regional surveys?

Two hundred and twenty-two sites between Point Conception, California, and the United States-Mexico international border were sampled between July 1 and September 30, 2008. The sites were selected using a stratified random design to ensure representativeness and minimize bias. A total of five strata were sampled. One stratum was offshore and included the mainland shelf (3 - 200 m depth). Four strata were located in embayments and included estuaries, marinas, ports, and other bay areas. Van Veen grab samples were taken at each station and the surface sediment (upper 2 cm for offshore and upper 5 cm for embayments) was collected for toxicity testing.

Two types of toxicity tests were used in Bight'08. A 10-day solid phase sediment toxicity test using the amphipod *Eohaustorius estuarius* was conducted on all samples. This standardized test has been used by EPA, NOAA, and during two previous SCB regional surveys in 1998 and 2003 (Bight'98 and Bight'03). A second test, a sediment water interface (SWI) test using mussel embryos, was also conducted on all embayment samples. These two toxicity tests were included in Bight'08 in order for the data to be compatible with California's new sediment quality objectives (SQO) policy for bays and estuaries.

In addition, a large number of embayment stations throughout the SCB were screened for possible toxicity identification evaluation (TIE). TIE studies were conducted at three locations: Mugu Lagoon, Marina del Rey, and Ballona Estuary.

A rigorous quality assurance program, consisting of interlaboratory comparisons, standardized test methods and controls, laboratory audits, and analysis of split samples was used to ensure data comparability and high quality. All of the participating laboratories met the data quality objectives for the study. Of the 222 sediment samples collected, 100% were successfully tested using the amphipod test and 94% were successfully tested using the SWI test, which exceeded our data quality objective of 90% success. Control survival, holding times, and reference toxicants were all acceptable for these samples. Data for 12 SWI test samples were excluded from analysis because control performance did not meet the quality control criterion.

Four levels of test response, corresponding to categories established in the SQO policy, were used to describe sediment toxicity in this study. The first level was "Nontoxic" where the test result was within the acceptable range for controls. The second level of "Low Toxicity" corresponded to a small, but statistically significant reduction in test organism response. Two higher levels of toxicity, "Moderate" and "High", were assigned to test responses of greater magnitude that were considered reliable indicators of substantial toxicity. The results for both toxicity tests (when available) were combined into an average category describing the toxicity of the sample. Stations having sediment classified as having either Moderate or High Toxicity were considered toxic for purposes of comparison to previous surveys.

Sediment toxicity was not widespread in the SCB with no toxicity observed in an estimated 76% of the region. There were no instances of Moderate or High Toxicity among the stations collected offshore. Marinas and estuaries contained the greatest incidence of observed sediment toxicity. Substantial toxicity (Moderate or High categories) was present in 24% of marina sediments and 22% of estuary sediments. In

addition, marina and estuary strata had the greatest prevalence of High Toxicity sediments relative to the other strata.

Temporal analysis indicated that the extent of sediment toxicity in the SCB has declined over the past five years. Substantial toxicity was absent from the shelf stratum in 2008 and the extent of toxicity in embayments has declined by approximately 50%. Examination of the sediment chemistry data for these strata is needed to identify the cause for this apparent improvement in sediment quality. Additional surveys are needed to confirm that a long-term improvement in sediment quality has occurred.

Limited results were obtained from the sediment TIE portion of the study, primarily because few stations that met the TIE selection criteria were identified for evaluation. TIE studies were completed for two stations, which identified pyrethroid pesticides and ammonia as the most likely toxicants. These data are insufficient to generalize about causes of sediment toxicity in other locations, but they are consistent with recent studies that have found pyrethroid pesticides to be the dominant cause of toxicity in freshwater and marine sediments throughout California.

The Bight'08 sediment toxicity survey was a success on multiple levels. This survey attained a high level of test completion and comparability through the coordinated efforts of seven testing laboratories. In addition, the results provide a comprehensive evaluation of sediment toxicity throughout the SCB and represent the largest application of the new SQO test methodology in California. Besides providing a current assessment of sediment toxicity, the Bight'08 survey results will be provide a valuable reference for future monitoring programs that employ the new SQO methods.

Toxicity is just one of several lines of evidence needed to make an accurate assessment of sediment quality. Caution should therefore be exercised in using the toxicity results reported here as the sole basis for describing sediment quality in the SCB. Each of the stations tested for sediment toxicity was also analyzed for sediment chemistry and benthic macrofauna community composition, and the results for these parameters will be reported in other Bight'08 documents. The integrated results from all three lines of evidence should be used to make the most reliable assessment of sediment quality for the SCB.

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I. INTRODUCTION

Tremendous effort is spent monitoring marine benthos for sediment quality and biological integrity in the southern California Bight (SCB). More than $30M annually is expended on monitoring of the SCB, two-thirds of which is used to assess impacts near the outfalls of treated waste discharges of publicly owned treatment works (POTWs) that are released to the ocean environment in southern California (Schiff *et al*. 2002). The majority of the effort on sediment monitoring is spent on chemical measurements to assess sediment contamination. Most of the remaining effort is spent on monitoring soft-bottom biological communities. Virtually no effort is spent on sediment toxicity testing as part of these regulatory-based monitoring programs even though sediment chemistry and biological assemblage indicators provide only partial information on sediment quality. Sediment chemistry provides unambiguous measurements of contaminant levels, but provides inadequate information to predict potential biological impact. Biological assemblages provide a direct measure of community impacts, but are also prone to perturbations that are not contaminant driven.

Sediment toxicity plays a vital role in the assessment of sediment quality. Unlike sediment chemistry that measures one chemical at a time and may leave many chemicals unmeasured, toxicity tests integrate the effect(s) of contaminant mixtures. Laboratory toxicity testing directly measures biological impacts similar to benthic assemblage measurements, but the effects of natural variation are minimized and only the effect of sediment toxicants are measured. Finally, new techniques are being developed that allow scientists to isolate the specific toxicant(s) in a contaminant mixture that are responsible for the observed effects through the use of sediment toxicity identification evaluations (TIEs). However, measurement of sediment toxicity is not without its own drawbacks including limited number of approved sediment toxicity test species and species-specific responses.

Sediment toxicity tests are most effective for assessing sediment quality when used in combination with sediment chemistry and biological assemblage information. The so-called "sediment quality triad" of measurements has been used since the mid-1980's and is the basis of many large-scale monitoring programs such as the United States Environmental Protection Agency (USEPA) environmental monitoring and assessment program (EMAP) and National Oceanic and Atmospheric administration (NOAA) National Status and Trends program (NS&T). In 2009, the State of California promulgated regulatory criteria for sediment quality in bays and estuaries using a multiple lines of evidence approach. Sediment toxicity, sediment chemistry and biological assemblage information are used for determining sediment impairments within the State because reliance on any one indicator of sediment quality can lead to false conclusions.

The Southern California Bight 2008 Regional Monitoring Project (Bight'08) has a goal of assessing sediment quality from Point Conception to the US/Mexico International Border. Sediment toxicity, sediment chemistry and biological assemblage information will be used to make these assessments. This report focuses specifically on the sediment toxicity component.

The sediment toxicity portion of Bight'08 was designed to answer the following three questions: 1) What is the extent and magnitude of sediment toxicity in the SCB? 2) How does the extent and magnitude of sediment toxicity compare among specific habitats of interest? and 3) How does the extent and magnitude of sediment toxicity compare to previous regional surveys?

Extent and magnitude of sediment toxicity is defined as the square kilometers or percent of total soft-bottom habitat with toxic responses in laboratory assays relative to control exposures. There are six habitats of interest evaluated using sediment toxicity in Bight'08, ranging from the shallowest (3 m) SCB habitats in estuaries and embayments to the deeper oceanic water (200 m) located on the SCB continental

shelf. Unlike previous regional surveys, but consistent with the State of California's new sediment quality objectives, Bight'08 uses multiple test species to assess toxicity. Finally, because similar designs were used in previous regional surveys from 1998 and 2003, Bight'08 will examine temporal changes in the extent and magnitude of sediment toxicity over time.

This report is structured in eight chapters. Chapter II of this report describes the methods used to prepare the samples and measure toxicity. A quality assurance evaluation of the test results is provided in Chapter III, which addresses issues of data comparability and laboratory performance during the study. Chapter IV describes the test results and illustrates patterns in the prevalence and severity of toxicity among the sampled subpopulations. A regional assessment of the percent area affected and a description of temporal patterns is included in Chapter V. Discussion and interpretation of the results is contained in Chapter VI. Conclusions from the study are presented in Chapter VII and recommendations for future studies are presented in Chapter VIII. Evaluation of the relationships between sediment toxicity, chemistry, and benthic community responses is not included in this report. These comparisons will be incorporated into a future integrative report, scheduled for completion in 2011.

II. METHODS

A. Sampling Design

There were 223 sites on the continental shelf between Point Conception, California and the United States-Mexico international border (Figure II-1) that were targeted for toxicity sampling between July 1 and September 30, 2008. The study used a Generalized Random Tessellated Stratified (Stevens 1997) sampling design for site selection, which creates a spatially balanced random sampling of resources. Toxicity samples came from five strata: shelf, marinas, ports, bays, and estuaries. Enhancement of the sampling design was achieved through intensified sampling in targeted areas and by resampling of stations from previous surveys. Intensified sampling was performed in the San Diego Bay region by increasing inclusion probabilities in that area. In order to assess temporal trends, 50% of the Bight'08 samples were new sites while 25% of the sample sites were from Bight'98 and 25 % from Bight'03.

Two toxicity tests were used for the regional survey. Whole sediment toxicity testing was performed on stations from all strata using the amphipod (*Eohaustorius estuarius*) 10-day survival test. In addition, a sediment-water interface test was conducted using mussel (*Mytilus galloprovincialis*) embryos on all but the offshore strata stations.

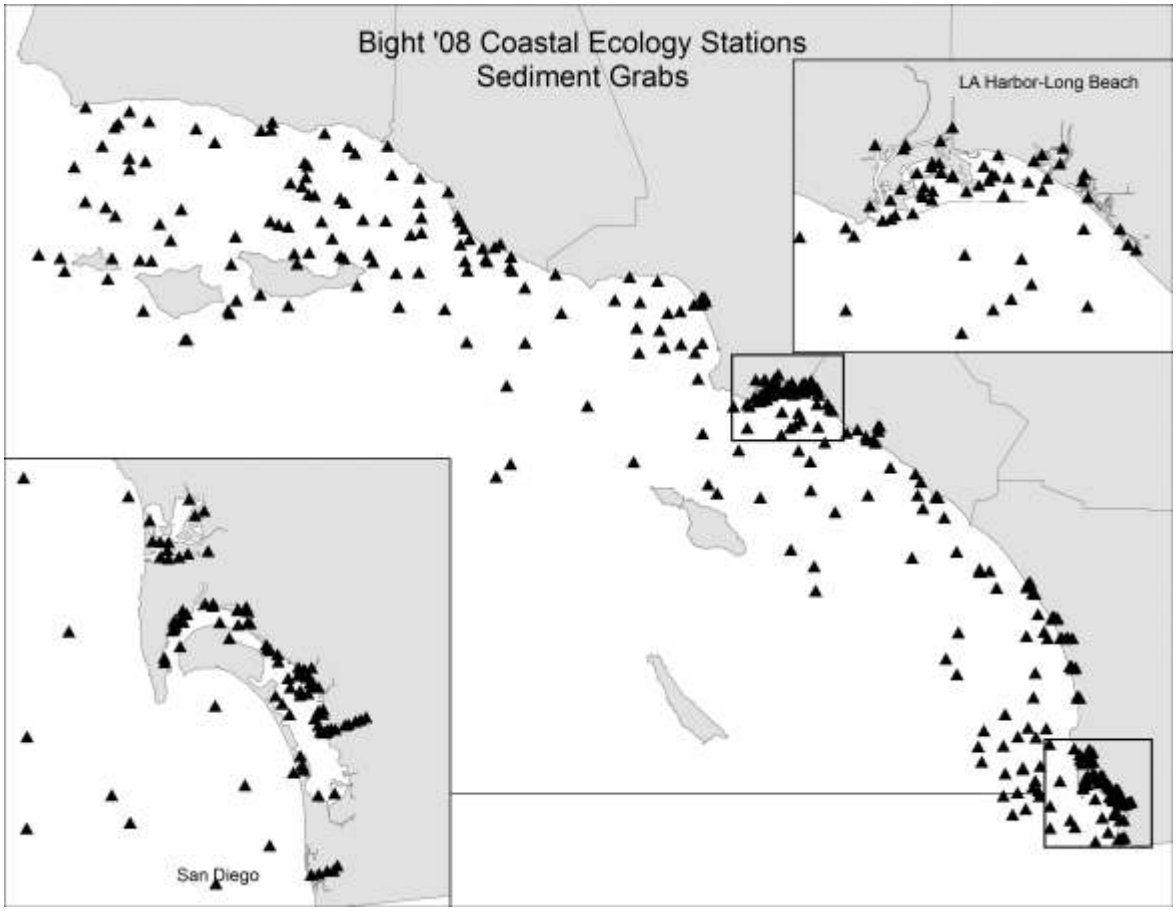

Figure II-1. Locations of all stations targeted for sediment collection in the Bight'08 project.

B. Field Methods

Sediment samples were collected with a 0.1 m^2 modified Van Veen grab. Up to 5.0 L of sediment were collected for measurement of sediment toxicity using the amphipod survival test and the sediment-water interface exposure test. Additional sediment was collected at selected stations for possible toxicity identification evaluations (See Appendix B) and laboratory intercalibration testing. A plastic (high-density polyethylene [HDPE], polycarbonate, or Teflon) scoop was used to collect sediment from the top 2 cm from offshore stations and top 5 cm for embayment stations of the undisturbed surface material in the grab. Contact with sediment within 1 cm of the sides of the grab was avoided in order to minimize cross-contamination. The sediment was placed in clean HDPE containers and distributed to the testing laboratories. In most cases, multiple grabs were required to obtain enough sediment for toxicity testing. If more than one grab was required, sediment was distributed to each of the containers so that approximately equal amounts were aliquoted into each jar from each grab, thereby maximizing comparability among containers. Once collected, the samples were stored in the dark at 4° C in the laboratory for no longer than four weeks prior to testing.

C. Laboratory Methods

Whole Sediment Toxicity

The toxicity of whole sediment to amphipods was determined using a 10-d survival test (USEPA 1994, ASTM 2002) with *E. estuarius* (EE) under static conditions. Amphipods and negative control sediment were collected from a non-contaminated estuarine site (Beaver Creek, OR) by Northwestern Aquatic Sciences (Newport, OR). The amphipods were held under laboratory conditions for at least 2 d, but not longer than 10 d, prior to the initial test date. Feeding of the amphipods during the acclimation period was left to the discretion of the individual testing laboratories. Testing was conducted in 1 L glass containers. Sediment samples were sieved through a 2 mm mesh screen and homogenized in the laboratory before testing. Sediment samples were added to the test containers to form a sediment layer approximately 2 cm deep. Filtered ($\leq$ 20 µm) seawater (32 g/kg salinity) was added slowly until a final volume of 800 ml was reached. Pipettes connected to an air source provided continuous aeration. Sediments were allowed to equilibrate overnight under these conditions before addition of the amphipods. Each sample consisted of five randomly arranged replicates, along with two surrogate containers for water quality (dissolved oxygen, pH, total ammonia and salinity) measurements of overlying water and pore water. A negative control (amphipod collection site sediment) was included with each batch of samples tested

Overlying water quality measurements were made at time zero and at the end of the exposure. Pore water measurements were made at sample receipt and at time zero. The measurement at sample receipt was used to determine if adjustments to testing procedure were necessary due to high ammonia or low salinity (see below). Temperature of overlying water was measured daily throughout the test. At the start of the test, amphipods were added randomly until a total of 20 animals per container were present. Tests were conducted at 15 ± 2 °C under constant illumination. Test animals were exposed to the sediment samples for 10 d. Each test chamber was examined daily to verify that adequate aeration was present and to record observations of emergence of the animals or changes in sediment appearance. Any floating animals were submerged by gently pushing them beneath the surface with a probe. At the end of the exposure period, the sediment was screened through a 0.5 mm mesh screen and the number of surviving amphipods was recorded. For the data from any given test batch to be considered acceptable, the mean control survival must be greater than 90% and the between replicate coefficient of variation must be less than 11.9% in the control.

A concurrent reference toxicant test was performed with each sample testing batch. The reference toxicant exposure consisted of four replicates of five concentrations of ammonia dissolved in seawater,

plus a control. No sediment was included in the reference toxicant tests. Ten amphipods were added to each replicate and exposed to the reference toxicant for 4 days. If the pore water measurement performed at the receipt of samples indicated that any station within the batch had a pore water un-ionized ammonia concentration exceeding 0.8 mg/L, the reference toxicant was extended to 10 days for comparison to the whole sediment results. Water quality of the reference toxicant tests was measured using a similar methodology to the sediment phase of the test. At the end of 4 days, the total number of surviving animals was recorded and median lethal concentration (LC_{50}) was calculated. The Trimmed Spearman Karber, probit, or linear interpretation methods (USEPA 1995) were used to calculate the LC_{50}, which was then compared to a control chart of past reference toxicant tests conducted by the laboratory. A test result within two standard deviations of the mean control chart LC_{50} for each individual laboratory was considered acceptable.

Previous studies have suggested that finer grained sediments may be toxic to *E. estuarius*, independent of any contaminants that might be present (DeWitt *et al*. 1989, Tay *et al*. 1998). To account for this possibility, a sediment grain size control was also included with each batch of tests. This sample consisted of a fine grained sediment collected from a relatively clean site prior to the start of the survey. Twenty gallons of sediment was collected from Channel Islands Marina site 2131 by Aquatic Bioassay Consultants. The sample was put on ice and taken to the lab where it was homogenized with a power drill in a large stainless steel tub. The sediment was then placed into 1L HDPE wide mouth containers, put into coolers with ice, and shipped overnight to the testing laboratories where it was held under normal conditions until use.

Sediment-water Interface Toxicity

For the sediment-water interface test, embryos of the mussel, *M. galloprovincialis* (MG), were used following the methodology of USEPA (1995) and Anderson *et al.* (1996). Sediment was added to a glass chamber having a similar diameter to a sediment core tube (7.5 x 14 cm), 600 ml tall form beakers were the recommended chamber. Sediment was passed through a 2 cm sieve, homogenized and added to the chamber to a depth of 5 cm. Approximately 300 ml of filtered (≤1 µm) seawater (32 g/kg salinity) was carefully added over the sediment. The overlying water was gently aerated and exposure chambers placed at 15 ºC with a 16 light: 8 hour dark cycle. The sediment was allowed to equilibrate overnight before addition of a screen tube (Figure II-2). The screen tubes were made of polycarbonate tubing with a 25 to 30 µm mesh polyethylene screen. A negative control consisting of the exposure container and screen tube, but no sediment, was tested with each batch to verify the test system was not causing toxicity. In addition, a second control with laboratory water in glass shell vials was tested to verify organism health. The controls from the concurrent reference toxicant were often used for this purpose.

Approximately 250 fertilized mussel eggs from a stock solution were added to the screen tube to initiate the bioassay. The same volume of embryo stock was also added to five replicate glass vials for determination of the initial number of embryos. Water quality parameters (dissolved oxygen, salinity, pH, and ammonia) were measured on the overlying water at the beginning and end of the exposure period. After 48 hours, the embryos were washed from the screen tube into another vessel for fixing and storage. The embryos were then counted and examined for normal development under a microscope. The number of normal embryos divided by the initial number of embryos determines the endpoint which is termed percent normal-alive (PNA). For the data from any given test batch to be considered acceptable, the mean control PNA must be ≥70%.

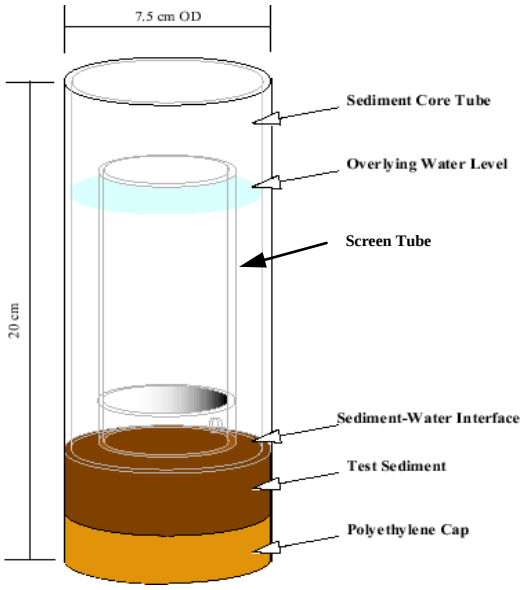

Figure II-2. Schematic diagram of sediment-water interface exposure system (Anderson *et al*. 1996).

A concurrent reference toxicant test was performed with each sample testing batch. The reference toxicant exposure consisted of five replicates of five concentrations of ammonia dissolved in seawater, plus a control. Embryos were added to glass shell vials and exposed for 48 hours. Water quality for the reference toxicant tests was measured using a similar methodology to the sediment phase of the test. Samples were examined microscopically as described above to determine the PNA. The median effective concentration for abnormality-mortality (EC_{50}) was then calculated using the Trimmed Spearman Karber, probit, or linear interpretation methods (USEPA 1995). The EC_{50} was then compared to a control chart of past reference toxicant tests conducted by the laboratory. A test result within two standard deviations of the mean control chart EC_{50} for each individual laboratory was considered acceptable.

D. Data analysis

Data were analyzed using three methods: 1) calculation of the mean response relative to the control for each batch; 2) determination of the level of toxicity using California Sediment Quality Objectives (SQO) methodology; and 3) assessment of the percent area within each stratum that was classified into each of the SQO toxicity categories.

Calculation of the response relative to the control is simply the mean test response at a given station divided by the mean response of the associated control for that batch multiplied by 100. Control normalized data is more amenable to comparisons across time and between laboratories.

The level of toxicity associated with each station was calculated using thresholds established for the SQO program (Bay *et al*. 2009). The thresholds are specific to each of the toxicity test methods (Table II-1). Using the thresholds, each sample was classified as Nontoxic, Low Toxicity, Moderate Toxicity, or High Toxicity. Each of these toxicity categories reflects both severity of toxicity and the confidence that the effects are real.

- **Nontoxic**: Response is not substantially different from that expected in sediments that are uncontaminated and have optimum characteristics for the test species (e.g., control sediments).
- **Low Toxicity**: A response that is of relatively low magnitude; the response may not be greater than test variability.
- **Moderate Toxicity**: High confidence that a statistically significant toxic effect is present.
- **High Toxicity**: High confidence that a toxic effect is present and the magnitude of response includes the strongest effects observed for the test.

For stations where both test methods where used, the overall toxicity assessment was calculated by averaging the category score (e.g., Nontoxic equals one, Low Toxicity equals two) for each method and rounding up if the average fell between two categories.

Table II-1. Thresholds for calculating toxicity categories.

Test Species/Endpoint	Nontoxic (Percent)	Low Toxicity (Percent of Control)	Moderate Toxicity (Percent of Control)	High Toxicity (Percent of Control)
Eohaustorius estuarius Survival	90 to 100	82 to 89[a]	59 to 81[b]	< 59
Mytilus galloprovincialis Normal	80 to 100	77 to 79[a]	42 to 76[b]	< 42

[a] If the response is not significantly different from the negative control, then the category becomes Nontoxic.

[b] If the response is not significantly different from the negative control, then the category becomes Low toxicity.

For descriptive purposes in the results and discussion, the simple terms not toxic and toxic are often used in the results sections of this report. The term not toxic refers to stations or areas classified as either Nontoxic or Low Toxicity using the SQO thresholds. The Low Toxicity category was grouped with the Nontoxic category because the biological significance and reliability of this category is uncertain. The term toxic refers to samples classified as either Moderate Toxicity or High Toxicity. Use of the terms toxic and not toxic facilitates comparisons with previous studies. Results for all four SQO categories are also presented so that the results may be compared to other studies using the SQO assessment method.

Analysis of the field toxicity data relied on the design-based inference procedures to provide unbiased estimates of area weighted proportions and areal extent (e.g., the number of square kilometers of a subpopulation satisfying some toxicity criterion or response level). Using information provided by the sample design, these probability-based areal estimates take into account the relative area each sample site represents. Specifically, the estimates are a weighted average where the weights are determined by the size of each disjoint sampling area divided by the number of samples falling into that area. These "area weights" are the same as the inverse of the inclusion probabilities for that particular sample. The area weighted proportions were computed as a ratio of the sum of the area weights for all sites which fell within a particular toxicity category and the sum of the area weights for the entire subpopulation or stratum. The areal extent was computed by multiplying the area-weighted proportion by the size of the subpopulation. The local neighborhood variance estimator, which takes advantage of any spatial

proximity with the data set, was used to compute standard errors for constructing 95% confidence limits (Stevens and Olsen 2003). Prior to any statistical computation, area weights were adjusted to account for missing data (See section III B), which were due to inability to access sites, failure to meet quality control criteria, or minor inaccuracies in the initial sample frame. The study design included oversampling of stations in an attempt to account for sampling failures in the field. However, this system could not account for laboratory failures. Area weights for stations within a stratum having missing data were not included in the analysis, resulting in reduced area being evaluated for toxicity. For a complete description of the statistical tools used in this analysis as well as a download of scripts for probability-based estimation go to http://www.epa.gov/nheerl/arm website.

III. QUALITY ASSURANCE EVALUATION

A. Introduction

In order to ensure good data quality, the Toxicology Technical Committee instituted a quality assurance (QA) plan for the Bight'08 survey. This QA plan was devised by the committee and disseminated among its members through the Toxicology Laboratory Manual. The QA plan describes five elements that were used to ensure data quality. First, sampling success was to meet the measurement quality objective and samples had to be tested before the determined holding time had elapsed. Second, requirements for obtaining and holding test organisms were established. In addition, the participating laboratories conducted reference toxicant tests on each batch of test organisms to determine whether test organism response and test procedures were comparable among different testing periods within a laboratory. Third, an interlaboratory study prior to the survey and split samples tested during the survey would provide information regarding the comparability of data among the participating laboratories. Fourth, criteria for test performance and parameters for water quality were established. Deviations from the QA plan would be examined by the Toxicology Technical Committee. Those deemed as minor deviations would be flagged in the database, while major deviations would be expunged from the database. Evaluations of the effects of ammonia and grain size were also examined. Fifth, a laboratory audit was conducted during the survey in order to identify and correct deviations, and as an informative tool for increasing laboratory comparability in future surveys.

B. Sampling Success

Sampling success was assessed by comparing the number of stations that were sampled to the number that had been targeted for toxicity testing (Table III-1). The measurement quality objective for sampling success was 90%. The success rate for stations targeting samples for the amphipod test or both the amphipod and SWI tests was greater than 99%. Only one targeted station was not collected, which was in the estuary stratum.

Table III-1. Toxicity sample collection effort and success.

	Amphipod Test			SWI Test		
Stratum	Targeted	Collected	Percent of Target Sampled	Targeted	Collected	Percent of Target Sampled
Bay	38	38	100	38	38	100
Marina	44	44	100	44	44	100
Port	46	46	100	46	46	100
Estuary	55	54	98	55	54	98
Shelf	30	30	100	0	0	
TOTAL	223	222	99.6	193	192	99.5

C. Sample Storage

The ideal holding time for sediment toxicity samples was 14 days or less, while the maximum allowable holding time was 28 days. Samples held between 15 and 28 days were flagged in the database. Of the EE samples successfully tested, 96% were stored for 14 days or less at the time the test organisms were added to the test chambers (Table III-2). Of the MG samples successfully tested, 78% were stored for 14 days or less at the time the test organisms were added to the test chambers. Problems with successful spawning of the MG adults contributed to the testing of samples beyond the ideal holding time. No MG samples were held for more than 28 days at the time of testing. Two EE samples were held for 29 days, counting from the day of collection to addition of the test animals. However, it was noted that the holding time could either be calculated from the date of collection to the date the sample was introduced into the test chambers or to the date the test organisms were added to the test chambers. It was not specified in the planning documents as to which of these methods would be used. Therefore, the data garnered from these two samples were retained.

Table III-2. Toxicity sample holding time (from sample collection to animal addition).

	EE		MG	
Time Interval (days)	Number of Samples	Percent of Total	Number of Samples	Percent of Total
0-14	213	96	149	78
15-28	7	3	43	22
>28	2	1	0	0

D. Organism Holding

The amphipods were acclimated for at least 2 days under laboratory test conditions, but no more than 10 days before addition to the test chambers. All organisms used for sediment testing were held within this range and holding time did not have a significant effect on control survival (Figure III-1).

Of the 28 batches of amphipods used in the survey, 8 were fed ground Tetramin during holding. This did not have a significant effect on the control survival. For the unfed and fed batches, the mean control survival was 95% and 94%, respectively (Figure III-2).

Mytilus galloprovincialis adults were procured from Mission Bay (San Diego, CA). Although the Bight'08 Toxicology Laboratory Manual specified that the mussels should be held in the laboratory under test conditions for at least 2 days prior to spawning, 22 of the 34 SWI test batches used animals that had been held for less than 2 days. Each laboratory participating in SWI testing also held a back-up batch throughout the study period. One laboratory used a single batch of animals for all SWI testing.

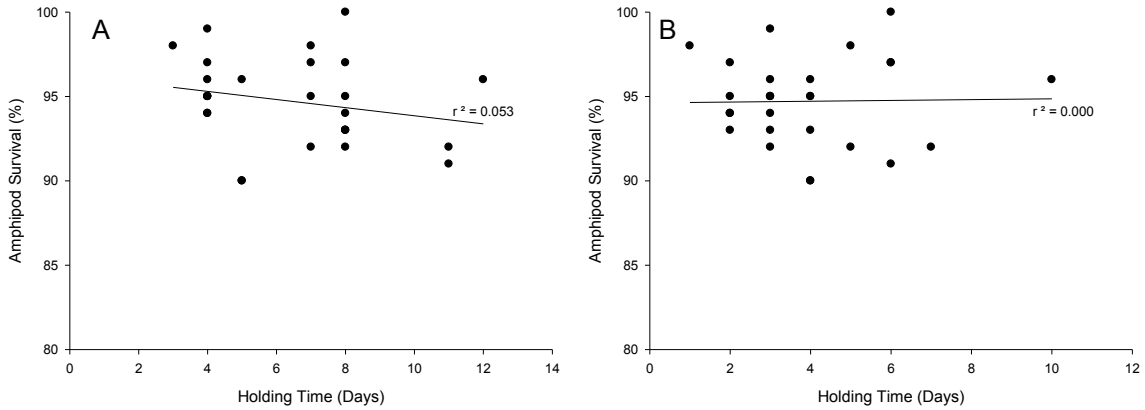

Figure III-1. The effect of holding time on the control survival of *Eohaustorius estuarius,* calculated in days. Holding time calculated from animal collection to testing (A); holding time calculated from animal delivery to testing (B).

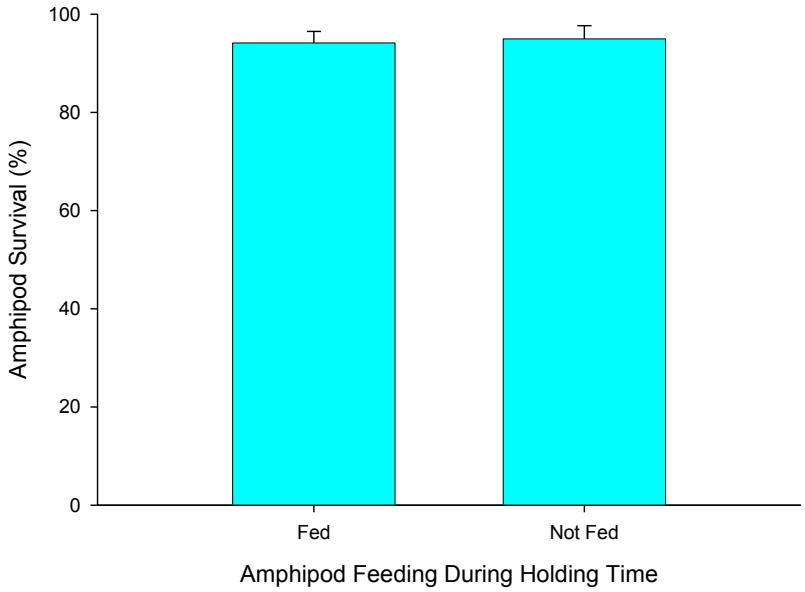

Figure III-2. Comparison of the control survival of fed and unfed batches of *Eohaustorius estuarius,* during the laboratory acclimation period.

E. Reference Toxicant Testing

Reference toxicant tests were conducted by each participating laboratory in order to determine whether test organism response and test procedures remain consistent within a laboratory. A total of 30 EE and 16 MG reference toxicant tests were conducted. For the amphipod test, all samples with a pre-test pore water un-ionized ammonia concentration that exceeded 0.8 mg/L would require an extension of the concurrent reference toxicant test from 4 to 10 days. Only 1 of the 30 EE reference toxicant tests required an extension. With the exception of one EE reference toxicant test, all reference toxicant tests were within two standard deviations of the mean EC_{50}/LC_{50} for each laboratory. The reference toxicant test in question was repeated with the same batch of test organisms and the results were deemed acceptable.

To evaluate the comparability of the Bight'08 reference toxicant testing with previous studies, each laboratory submitted ammonia EC_{50}/LC_{50} values for all tests conducted in the previous 18 months for both test species. A mean and standard deviation of the historical data was calculated. Compared to the historical control limits (±2 SD) the grand mean of Bight'08 reference toxicant results for each test species, showed good comparability among the participating laboratories (Figures III-3 and III-4). Only 5 of 30 EE reference toxicant tests fell outside of two historical standard deviations of the grand mean LC_{50}, one of those being the test that failed. All 16 MG reference toxicant tests fell within two historical standard deviations of the grand mean EC_{50}.

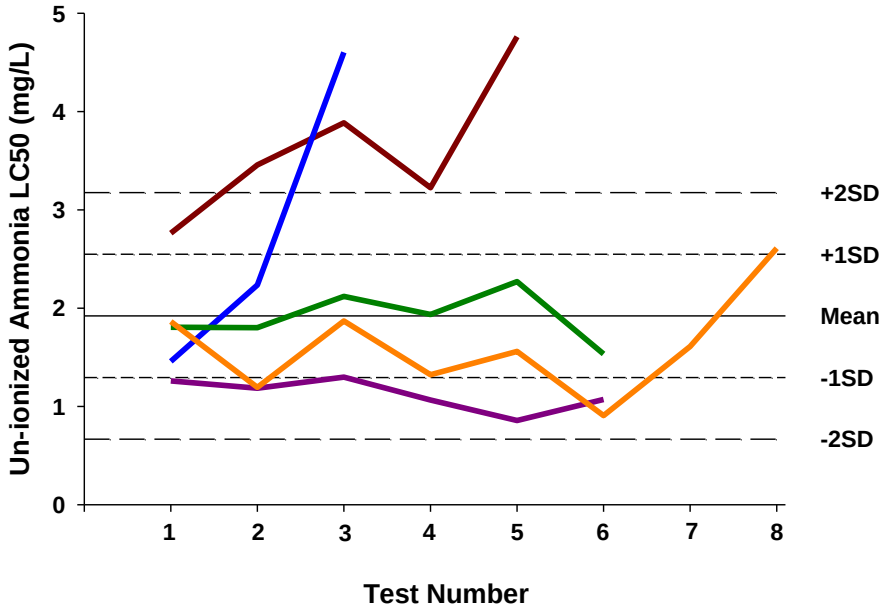

Figure III-3. Bight'08 *Eohaustorius estuarius* un-ionized ammonia reference toxicant tests compared using the grand mean and the historical standard deviation. Each line represents a different laboratory.

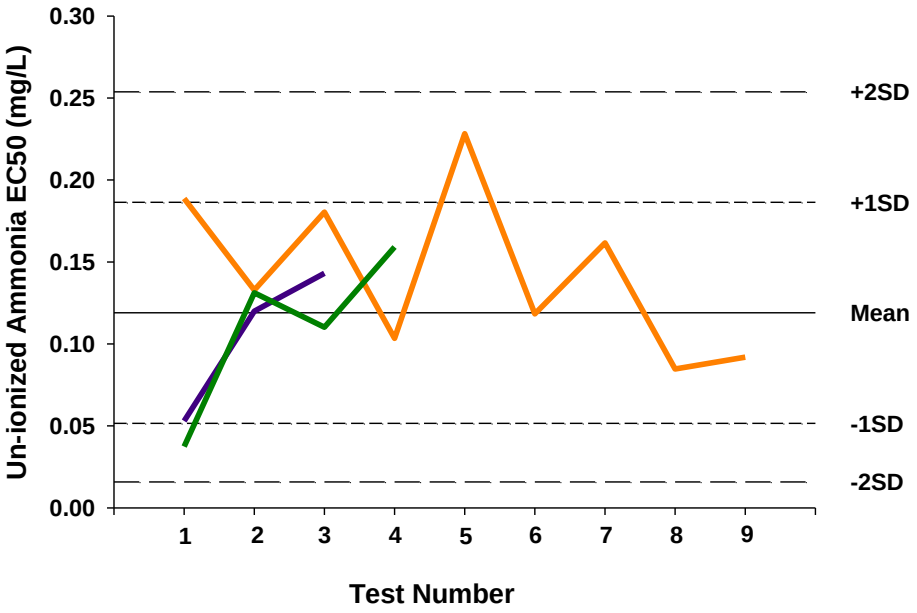

Figure III-4. Bight'08 *Mytilus galloprovincialis* un-ionized ammonia reference toxicant tests compared using the grand mean and the historical standard deviation. Each line represents a different laboratory.

F. Test Performance

Control Criteria

All collected EE samples were successfully tested; all EE test batches met the mean control survival criteria of ≥90% and the coefficient of variation criteria of ≤11.9% (Tables III-3 and III-4). For the MG samples, 94% of those collected were successfully tested, (i.e., m*et al*l test acceptability criteria; Table III-3). Of the 31 MG batches tested, 3 did not meet the control % normal-alive criterion of ≥70% (Table III-4). Control replicate outliers were found using the Dixon's Test (USEPA 2000) in two of these batches and there was a complete control failure in the third batch. Since there was no corroborating evidence collected that would warrant the exclusion of just the outlier control replicates, the entire batch was deemed invalid. This resulted in MG data from all affected batches (i.e., 12 samples) being excluded from the database and further analysis.

As a result of stations not being successfully tested, the total area of the Bight for which an assessment could be made was reduced. For the EE testing, the total area possible was 3887 km^2, of which 3884 km^2 was successfully sampled and tested, thereby achieving an assessment of >99.9% of the targeted area. For the MG testing, the total area was 129 km^2, of which 125 km^2 were successfully sampled and tested. This resulted in an assessment of 96% of the targeted area.

Table III-3. Toxicity sample testing success.

	EE			MG		
	Tested	Successful	Testing Success Percentage	Tested	Successful	Testing Success Percentage
Bay	38	38	100	38	38	100
Marina	44	44	100	44	39	89
Port	46	46	100	46	44	96
Estuary	64	64	100	64	59	92
Shelf	30	30	100	0	0	
TOTAL	222	222	100	192	180	94

Table III-4. Toxicity test batch success in meeting acceptability criteria.

	EE			MG			
	Number of Batches		Percentage		Number of Batches		Percentage
Criterion	Met	Not Met	Meeting Criterion	Criterion	Met	Not Met	Meeting Criterion
Mean Control Survival ≥90%	38	0	100	Mean Control %Normal-alive ≥70%	31	3	90
Coefficient of Variation ≤11.9	38	0	100				

Outliers

Two SWI test replicates were noted to be outliers and had corroborating evidence of an abnormal occurrence. In both cases, the samples had been double inoculated with embryos. Both replicates were deemed invalid and removed from the database.

G. Water Quality

Temperature

After rounding, almost all EE temperature readings were in the prescribed range of 13 to 17°C. There were five exceedances that had temperature readings of 18°C: four from the same batch on day 8, and the other from a different batch on day 5. These deviations were flagged as minor. All MG temperature readings were within the prescribed range of 13 to 17°C.

Dissolved Oxygen

Low dissolved oxygen (DO) readings were observed for both EE and MG tests. While the prescribed DO for the EE test was ≥8 mg/L, DO readings were reported as low as 3.8 mg/L. This reading was taken on Day 1 of a single test, and all other readings associated with the same test were no lower than 6.0 mg/L

(Note: DO was only required to be measured on Day 0 and 10, but some laboratories made daily measurements). Low DO in the EE test was not associated with reduced survival (Figure III-5). The prescribed DO for the MG test was ≥ 4 mg/L. Only one DO reading from the MG tests fell below this threshold, which occurred on the last day of testing for one sample. The DO deviations for both test types were flagged as minor.

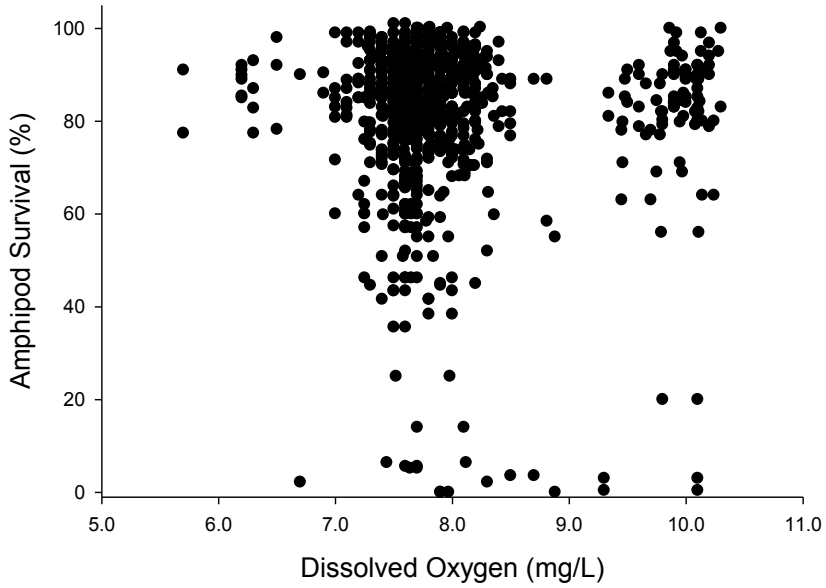

Figure III-5. Survival of *Eohaustorius estuarius* relative to overlying water dissolved oxygen concentration at Day 0 and Day 10.

pH

For the EE test, the range of pH of the overlying water in the test chambers was prescribed as 7.7 to 8.3. During the study, pH readings were recorded from 7.5 to 8.8. However, the pH exceedances were not significantly associated with EE survival (Figure III-6). For the MG tests, pH readings ranged from 7.5 to 8.4 while the prescribed pH range for this test was 7.6 to 8.3. Only 4 pH readings for the MG tests fell outside of this range (Figure III-7). There was insufficient data to draw any conclusions regarding the effects of these outlying pH readings on the MG percent normal alive result. Therefore, the pH deviations for both test types were flagged as minor.

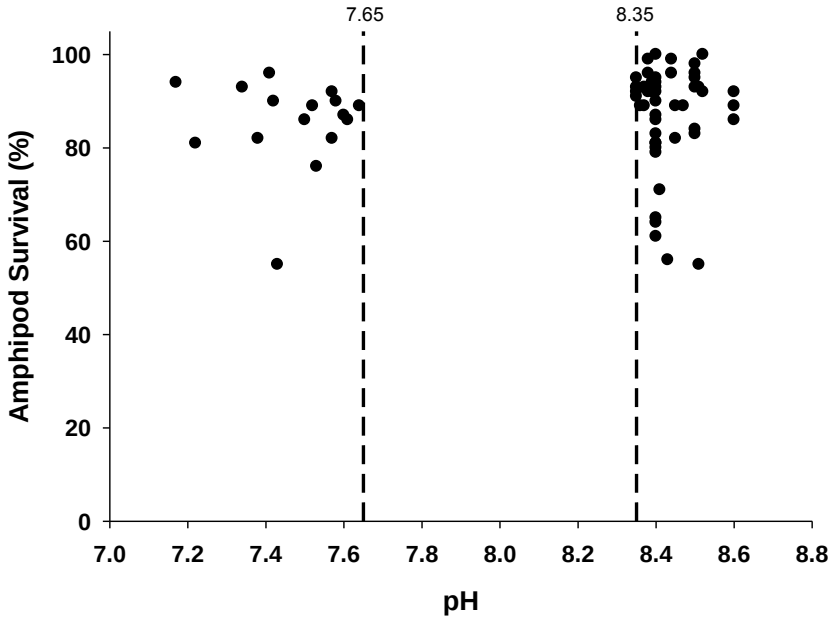

Figure III-6. Survival of *Eohaustorius estuarius* at pH values below and above the QA limits of 7.7 and 8.3 (after rounding), respectively. The reference lines indicate the levels that were used to flag data as a minor deviation.

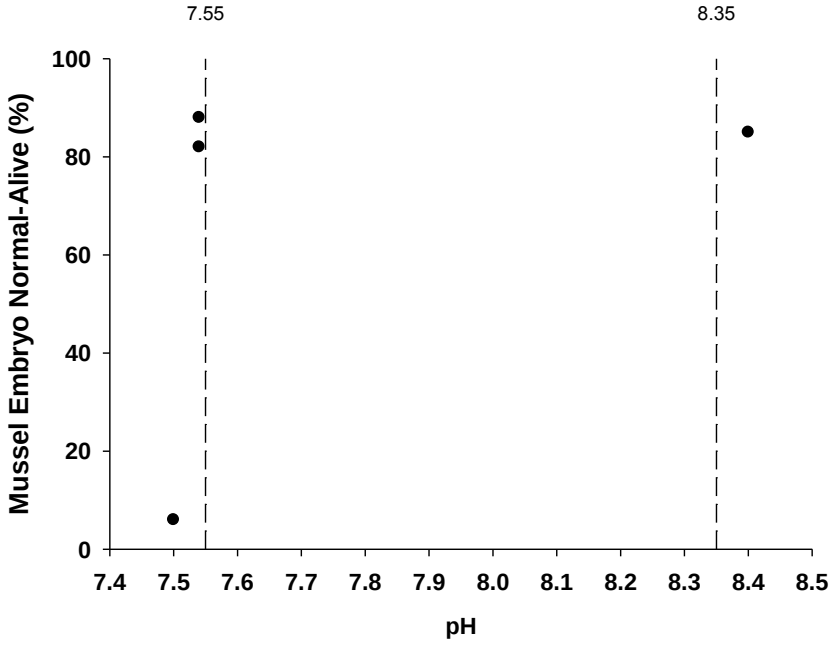

Figure III-7. Development and survival of *Mytilus galloprovincialis* embryos below and above the QA limits of 7.6 and 8.3, respectively. The reference lines indicate the levels that were used to flag data as a minor deviation.

Salinity

Depending on the pore water salinity upon receipt of the samples to the testing laboratory, the EE tests were conducted at 1 of 3 salinities: 22 if the pore water salinity was 20 to 24 g/kg, 27 if the pore water was 25 to 29 g/kg, or 32 if the pore water was 30 to 34 g/kg. It was expected that, based on sample collection protocols, the salinity would be ≥20 g/kg in all samples. Measured salinity for the amphipod tests was as high as 35 g/kg. The MG SWI tests were to be conducted at 30 to 34 g/kg. Salinities in the range of 29 to >35 g/kg were measured for these tests. Elevated salinity did not seem to affect the test organism responses (Figure III-8), and therefore was flagged as a minor deviation. One collected sample, from station 6242 had an initial water salinity of 9 g/kg, which is below the 20 g/kg cutoff for the study. The sample was tested at 12 g/kg, concurrently with a salinity control of 12 g/kg. Results from this sample were retained in the database, but flagged.

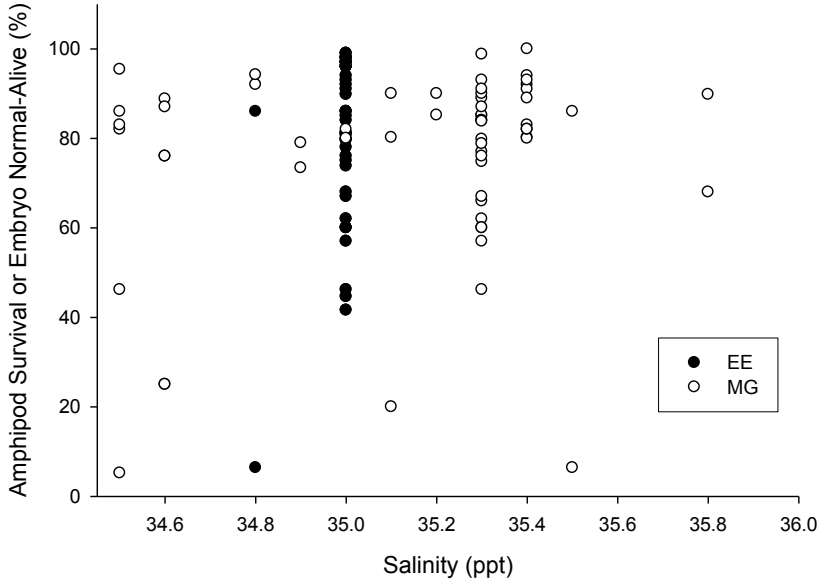

Figure III-8. Effect of elevated salinity (≥34.5 g/kg) on test organism response.

Ammonia

No EE samples exceeded the ammonia pore water ammonia concentration criterion of 0.8 mg/L un-ionized ammonia (UNH3) at time point 0. Total ammonia (TNH3) measurements ranged from 0.026 to 35 mg/L. Un-ionized ammonia measurements ranged from 0 to 0.7 mg/L.

During review of the Bight‘08 un-ionized ammonia data for the SWI test, it was noted that some stations had concentrations in the overlying water that exceeded SCCWRP’s historical EC_{50} for *M. galloprovincialis* embryos (0.12 mg/L). Additional analyses described below were performed to determine if the ammonia concentrations might be contributing to observed toxicity.

A polynomial regression analysis was performed on pooled data from all Bight'08 *M. galloprovincialis* ammonia reference toxicant tests (Figure III-9). The equation for this regression was used to predict the concentration of UNH3 which would be expected to cause toxicity (i.e., PNA ≤70%). The regression predicted this concentration to be 0.057 µg/L UNH3. Stations with un-ionized ammonia concentrations greater than or equal to this value were subjected to further analysis. Any of these stations that had observed PNA that were greater than 70%, and therefore were not toxic, were assumed to be unaffected by ammonia and not subjected to further analysis. Of the 44 stations identified by the regression, 14 had observed PNA ≤ 70% (Table III-5). An additional two stations were found to be Nontoxic due to a lack of statistical significance coupled with a borderline PNA. In light of the poor correlation (r^2=0.49) and low predictive value of the polynomial regression equation, the pooled data approach was deemed undesirable.

Instead of pooling all of the data, individual polynomial regressions were performed on the PNA and UNH3 data from each concurrent reference toxicant exposure in order to account for batch-specific organism sensitivity. For the one station that did not have valid concurrent reference toxicant data, the pooled regression was used. This method generates an expected PNA, assuming ammonia is the only toxic constituent in the sample. Each site's observed PNA was compared to its expected PNA.

The individual regressions showed good fit (r^2 values ranging from 0.755 to 0.975). Of the 12 toxic stations, 10 had un-ionized ammonia concentrations that were predicted to reduce the PNA endpoint below 70% (Figure III-10) which suggested that the toxicity from those 10 stations were likely to have been influenced by ammonia in the overlying water. Some of the stations had observed toxicity that was less than predicted, indicating that ammonia might account for all of the toxicity (e.g., 6157, 6138). The remainder of the stations had toxicity that was greater (i.e., lower PNA) than that predicted by ammonia alone, indicating that there were likely contributions both from ammonia and other toxic substances in the sediment (e.g., 6242).

Table III-5 provides a summary of potential ammonia toxicity. In addition, the samples in which ammonia likely contributed to the observed toxicity are flagged as such in the Toxicity Summary Results Table in the Bight'08 database. For stations where both the observed and predicted PNA were zero, it is not possible to distinguish between the effects of ammonia and other toxicants.

There were three stations (6242, 6442, and 6520) that appeared to be jointly affected by ammonia and other toxicants. For these three stations, the ammonia toxicity prediction equation was used to factor out the effect of ammonia on the test response. To accomplish this, the observed response value was subtracted from the response predicted by the ammonia equation. This quantity was then subtracted from 100 to give the adjusted PNA (Table III-5). This adjusted value was used for analysis within this report, but the original data remain in the database.

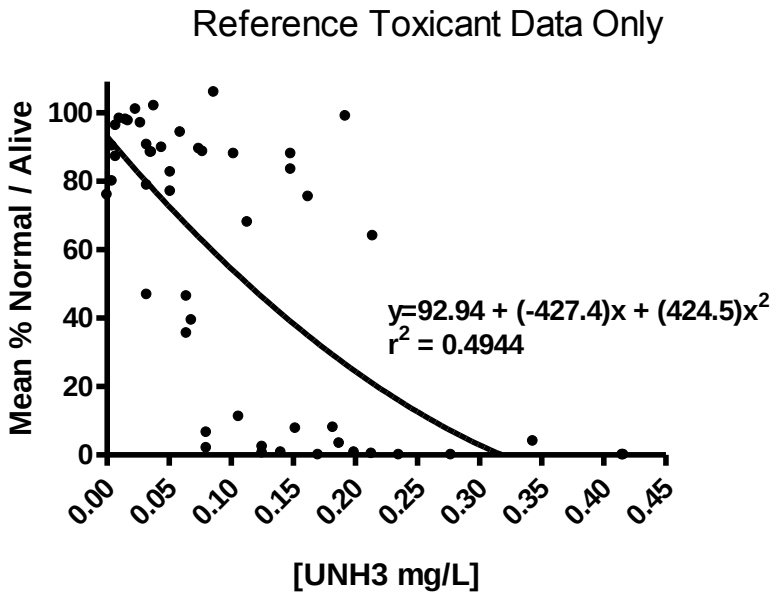

Figure III-9. Polynomial regression of all Bight'08 ammonia reference toxicant data for *Mytilus galloprovincialis*.

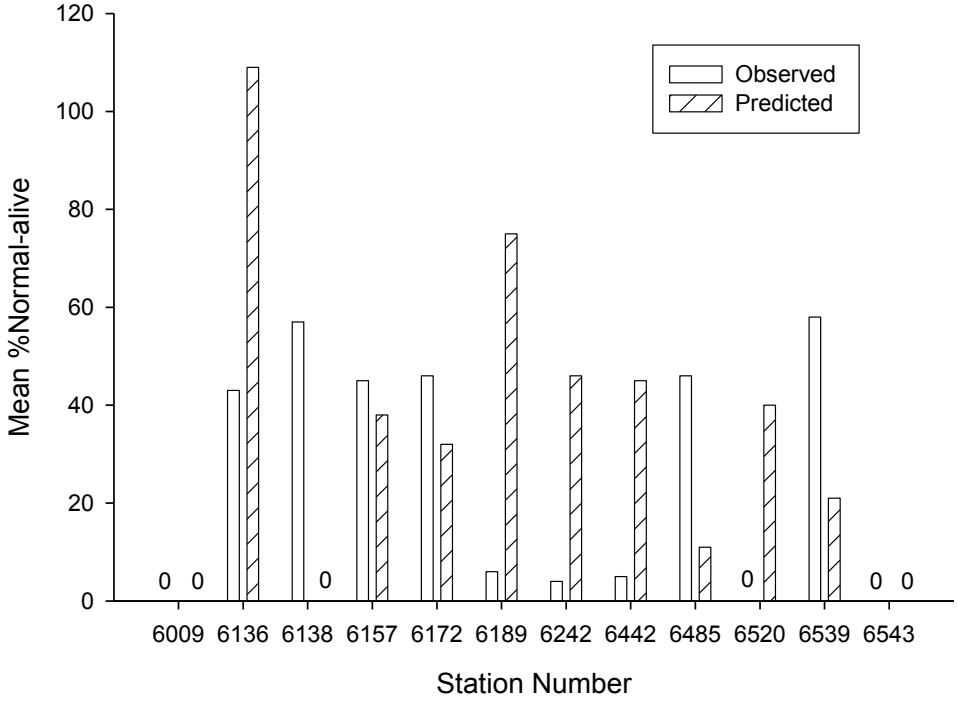

Figure III-10. Predicted and observed *Mytilus galloprovincialis* percentage normal-alive based on a polynomial equations from Bight'08 reference toxicant tests.

Table III-5. Potential contribution of ammonia to toxicity response of *Mytilus galloprovicialis* in Bight'08 sediment-water interface tests.

Station ID	Un-ionized Ammonia	Observed %Normal -Alive	Predicted %Normal-Alive	Sample Toxic	Ammonia Affected	Additional Toxicants Likely	Adjusted %Normal-Alive
6009	0.370	0	0	Yes	Yes	Unknown	NA
6136	0.081	43	109	Yes	No	Yes	NA
6138	0.126	57	0	Yes	Yes	No	NA
6157	0.152	45	38	Yes	Yes	No	NA
6172	0.179	46	32	Yes	Yes	No	NA
6189	0.097	6	75	Yes	No	Yes	NA
6242	0.130	4	46	Yes	Yes	Yes	50
6442	0.131	5	45	Yes	Yes	Yes	32
6485	0.259	46	11	Yes	Yes	No	NA
6520	0.148	0	40	Yes	Yes	Yes	34
6539	0.098	58	21	Yes	Yes	No	NA
6543	0.379	0	0	Yes	Yes	Unknown	NA

NA=Not adjusted; sample not toxic, ammonia affected and affected by additional toxicants.

H. Interlaboratory Study and Split Samples

Three types of interlaboratory studies were conducted to determine the comparability of toxicity data between all of the survey participating laboratories. The first was an interlaboratory study that was performed prior to the Bight'08 index period to determine the comparability between the multiple laboratories. The second was the testing of two split samples by each of the laboratories during the index period. The third was the testing of split samples between pairs of laboratories to verify the comparability of an additional laboratory that joined the survey after the interlaboratory study.

Seven laboratories participated in the interlaboratory study for the EE 10-day toxicity test. Five laboratories participated in the interlaboratory study for the MG SWI two-day toxicity test. Three sediment samples were collected for this study, which were distributed to the laboratories to be tested blindly and within the same time frame. The sediment samples were selected to represent one Nontoxic sample (from offshore Orange County (OC)), one sample of Moderate Toxicity (Cabrillo Marina), and one High Toxicity sample from LA Harbor (East Basin). Unbeknownst to the testing laboratories, a duplicate of the East Basin sample was included to assess within laboratory variability. Therefore, four sediment samples were tested for each of the toxicity methods. In addition, each laboratory performed a reference toxicant test using ammonia, for each of the species tested. Interlaboratory comparability was evaluated using four criteria.

Comparability Criteria

- The first criterion was based on the difference from the grand mean of results for each sample. Mean percent survival or mean percent normal-alive were calculated and data were normalized to the control for each laboratory. The grand mean was calculated by pooling results for each sample for all of the laboratories. The absolute difference of each laboratory's mean percent survival or mean percent normal-alive from the grand mean was then used to assign points (Table III-6).
- The second criterion was based on the agreement in toxicity category. For each sample, the grand mean percent survival or percent normal-alive was used to determine the toxicity category using SQO thresholds. Each laboratory's results were then used to characterize the toxicity of each individual sediment sample. The toxicity categories were then compared. The number of categories difference was then used to assign a score for evaluation (Table III-6).
- The third criterion was based on the reproducibility of the results for the two duplicate samples. The first step was to calculate the relative percent difference (RPD) between the duplicates for each laboratory. The RPD was then compared to values in Table III-6 and points were assigned.

$$RPD=\frac{Abs(Dup1-Dup2)\times 100}{Avg\ of\ Dups} \quad Abs=Absolute\ Value$$

- The fourth criterion was based on the reference toxicant data. This evaluation involved collecting all of the historical ammonia reference toxicant data from all laboratories conducting the *E. estuarius* and *M. galloprovincialis* tests. The standard deviation (SD) was calculated for the historical EC_{50}/LC_{50} data for each species. In addition, the interlaboratory comparison reference toxicant EC_{50}/LC_{50} data from each laboratory were pooled, and the grand mean was calculated. The mean EC_{50}/LC_{50} for each laboratory was compared to the grand mean. This difference was then compared to the historical standard deviation; points were assigned based on deviation (Table III-6).

The total points from each laboratory were combined to determine the laboratory's comparability grade, which was based on the percentage of the total points available (Table III-7). To participate in B'08 testing, laboratories had to be graded as moderately comparable or higher for each of the test methods that they intended to employ.

Table III-6. Summary of the scoring system for evaluation of interlaboratory comparability.

Toxicity Result Agreement (percentage deviation from grand mean)		Toxicity Category Agreement		Duplicate Sample (RPD)		Reference Toxicant (deviation from grand mean)	
Result	Points	Result	Points	Result	Points	Result	Points
0 – 10 %	3	Same category	1.5	0 – 10 %	3	Within 1 historical SD	3
>10 – 20 %	2	1 category difference	1.0	>10 – 20 %	2	Within 2 historical SD	2
>20 – 30 %	1	2 category difference	0.5	>20 – 30 %	1	Within 3 historical SD	1
> 30 %	0	3 category difference	0	>30 %	0	>3 historical SD	0

Table III-7. Comparability grade for laboratory intercalibration based on the scoring system shown in Table III-6.

Description	% of Maximum Possible Score	Number of Points
Very High comparability	90-100	24.0-21.5
High comparability	80-89	21.0-19.0
Moderate comparability	70-79	18.5-16.5
Low comparability	<70	<16.0

Eohaustorius estuarius Interlaboratory Comparison

Seven laboratories participated in the interlaboratory comparison exercise using *E. estuarius*. Each of the participating laboratories met the test acceptability criteria for mean percent survival and between replicate variability in the control sediment. The mean survival for the Cabrillo Marina sample indicated that it was a Moderate Toxicity sample, but three of the seven laboratory results placed this sediment in the High Toxicity category (Figure III-11). All laboratories were in agreement with the toxicity category of the other sediment samples (OC and East Basin). The results of the relative percent difference of the duplicate samples indicated that two laboratories had >30% difference in survival between the duplicate samples. In addition, three laboratories showed a deviation of 10 to 20% for the duplicates. For the reference toxicant results, all of the laboratories were within a factor of two of the historical standard deviation. The results of the laboratories participating in the *E. estuarius* sediment toxicity test indicated that almost all the laboratories had either a very high or high comparability; one laboratory exhibited moderate comparability (Table III-8).

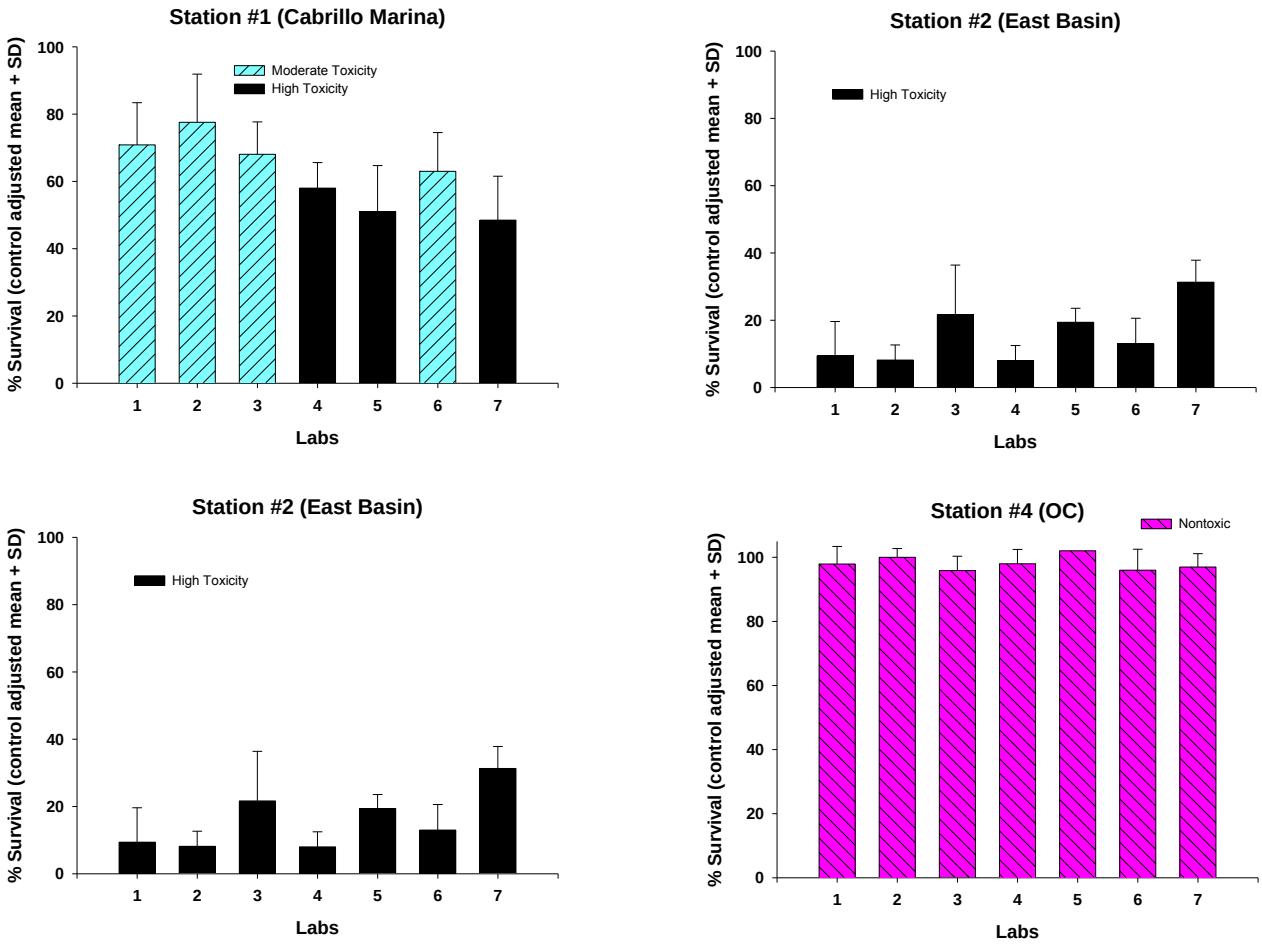

Figure III-11. Comparison of toxicity results from each laboratory using *Eohaustorius estuarius*.

Table III-8. Assessment of each laboratory's performance of the *Eohaustorius estuarius* sediment toxicity test.

Laboratory	Toxicity Result (Criterion #1)	Toxicity Category (Criterion #2)	RPD (Criterion #3)	Reference Toxicant (Criterion #4)	Total Points	Assessment
1	12	6	0	3	21	High
2	11	6	2	2	21	High
3	12	6	2	3	23	Very High
4	12	5.5	1	3	21.5	Very High
5	11	5.5	0	3	19.5	High
6	12	6	1	3	22	Very High
7	9	5.5	2	2	18.5	Moderate

Mytilus galloprovincialis Interlaboratory Comparison

Four laboratories participated in the testing using *M. galloprovincialis*. When the total points were calculated, results indicated that all the laboratories were classified as moderate or low comparability (Table III-9). Additionally, 1 laboratory did not pass the test acceptability criteria for the MG SWI test (control % normal-alive must be $\geq$ 70%). Based on these results, a second set of sediments were collected and the comparisons repeated. The new sediments were expected to represent one Nontoxic sample from Terminal Island (TI), one sample of Moderate Toxicity (Cabrillo Marina), and one High Toxicity sample from LA Harbor (East Basin). The East Basin sample also served as a duplicate for a total of four samples to test.

Table III-9. Assessment of each laboratory's performance in first round of the *Mytilus galloprovincialis* sediment toxicity test interlaboratory comparison.

Laboratory	Toxicity Result (Criterion #1)	Toxicity Category (Criterion #2)	RPD (Criterion #3)	Reference Toxicant (Criterion #4)	Total Points	Assessment
1	9	4.5	1	2	16.5	Moderate
2	3	5	0	1	9	Low
4	2	3	2	3	10	Low
7*	11	5.5	0	0	16.5	Moderate

* Did not pass test acceptability criteria

An additional laboratory (Laboratory 8) participated in the second MG SWI interlaboratory study. Each laboratory met the test acceptability criterion for the controls. The largest discrepancy between the laboratories was found in the toxicity characterization of the East Basin sediment sample (Figure III-12). Two of the laboratories found this sample to be Nontoxic and one laboratory classified this sample as Low Toxicity, while the remaining three laboratories identified it as Moderate Toxicity. The average classification for this sample was Moderate Toxicity. There was good agreement among the laboratories for the Cabrillo Marina and TI samples. All laboratories classified the Cabrillo Marina sample to be Nontoxic and all but one of the laboratories classified the TI sample as Nontoxic, with the one classifying the sample as Moderate Toxicity. Similar results were found for the duplicate East Basin sample, where the majority of the laboratories classified the sample as Nontoxic and one laboratory classified the sample as Moderate Toxicity. The average toxicity category for the Cabrillo Marina, TI and East Basin duplicate samples was Nontoxic.

For the reference toxicant results, all laboratories were within 1 standard deviation from the grand mean. The results of the second interlaboratory comparison indicated one laboratory had very high comparability, three laboratories had moderate comparability, and one laboratory had low (Table III-9). The laboratory with low comparability did not conduct SWI testing for Bight'08.

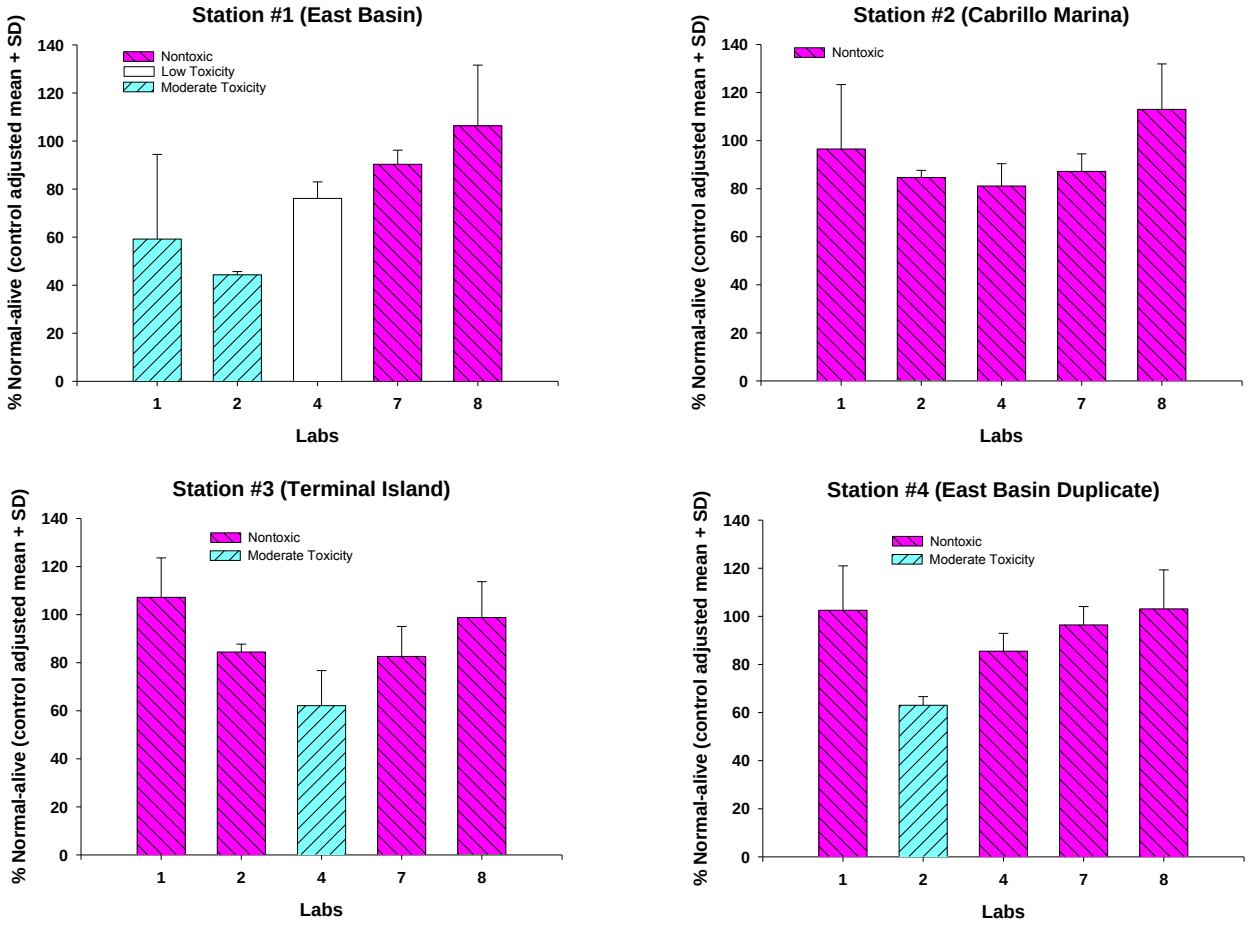

Figure III-12. Results for the second interlaboratory comparison exercise using the *Mytilus galloprovincialis* sediment-water interface test.

Table III-9. Assessment of each laboratory's performance in the second round of the *Mytilus galloprovincialis* sediment toxicity test interlaboratory comparison.

Laboratory	Toxicity Result (Criterion #1)	Toxicity Category (Criterion #2)	RPD (Criterion #3)	Reference Toxicant (Criterion #4)	Total Points	Assessment
1	8	6	0	3	17	Moderate
2	7	5	0	3	15	Low
4	9	4.5	2	3	18.5	Moderate
7	11	5	3	3	22	Very High
8	6	5	3	3	17	Moderate

Split Samples

Split samples from two stations were tested by laboratories which were qualified (i.e., demonstrated comparability) to conduct one or both of the Bight'08 toxicity test methods. The analysis of these split samples was used to monitor interlaboratory variability, but the results were purely informational; there were no consequences if a laboratory's comparability was low for this exercise. The sediments used for the split sample analysis were actual Bight'08 samples and were tested by all laboratories within two weeks of collection. The comparison criteria used to evaluate this laboratory performance were similar to those used for the pre-survey interlaboratory comparison; however, the reproducibility comparison was not used due to the absence of duplicate samples. In addition, the mean reference toxicant EC_{50} value was calculated from all of the reference toxicant tests that were conducted during the survey, since some laboratories did not conduct a concurrent reference toxicant test with the split samples. The maximum point score for comparability was also reduced to 12 in order to adjust for the reduced number of samples and exclusion of the reproducibility criterion. The ranges used for assessment were: 11.0 to 12.0 points, very high comparability; 10.5 to 9.5 points, high comparability; 9.0-8.0 points, moderate comparability; and <8.0 points, low comparability.

The laboratories which tested *E. estuarius* showed good agreement for the split samples (Table III-10). The largest discrepancies between laboratory results were found in the reference toxicant comparisons. All laboratories were classified as moderately comparable or higher.

Table III-10. Split sample assessment of each laboratory's comparability using the *Eohaustorius estuarius* sediment toxicity test.

Laboratory	Toxicity Result (Criterion #1) (max=6)	Toxicity Category (Criterion #2) (max=3)	Reference Toxicant (Criterion #4) (max=3)	Total Points (max=12)	Assessment
1	6	3	2	11	Very High
2	5	3	2	10	High
3	5	2	2	9	Moderate
5	5	3	2	10	High
6	6	2.5	1	9.5	High
7	6	3	2	12	Very High
9	5	2.5	3	10.5	High

Comparison of the MG results for the split samples was limited since one of the laboratories was unable to achieve acceptable control performance. Therefore, only two laboratories successfully tested the split samples which precluded the application of the comparison criteria used for the amphipod results. Both laboratories agreed well on station 6406, but showed different results for station 6485 (Figure III-13). Laboratory 7 found station 6485 to be Nontoxic, while Laboratory 9 found it to be Moderate Toxicity.

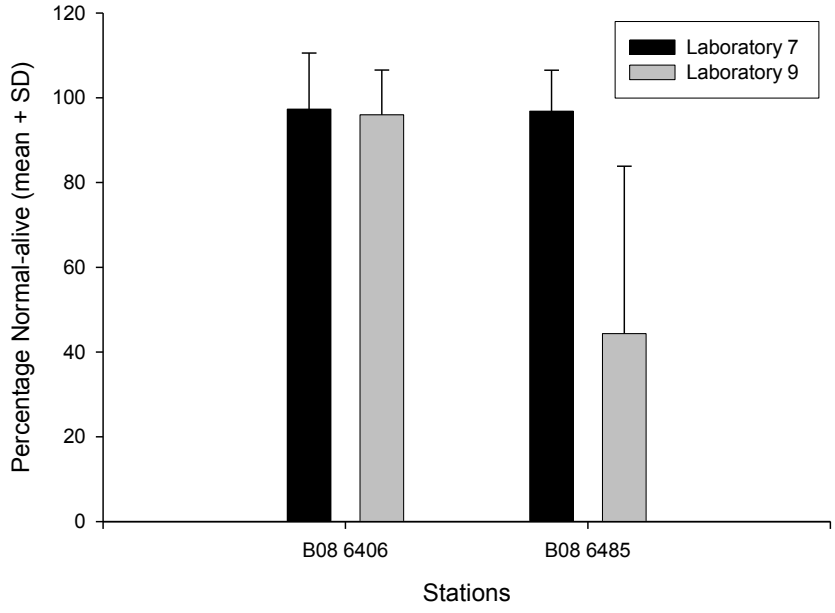

Figure III-13. Results of split sampling testing using the mussel embryo sediment-water interface method.

Single Laboratory Split Comparisons

Since Laboratory 9 joined the Bight'08 toxicity effort after the completion of the pre-survey intercalibration exercise, an additional evaluation of that agencies comparability was conducted. Split samples from four Bight'08 stations were tested in a pairwise fashion between Laboratory 9 and other Bight'08 participating laboratories using the amphipod test, and three split samples were analyzed for the SWI test. The split samples included two discussed in the previous section (Table III-11).

For one of the stations, comparisons were made between Laboratory 9 and two other laboratories to give a grand total of five comparisons for the amphipod test. The comparison criteria used to evaluate this laboratories performance was similar to the earlier split sample comparison, but each combination of laboratory and station was assessed individually. This led to there being five comparisons worth three points each for the toxicity result and toxicity category evaluations for the amphipod test. For the reference toxicant comparison, the data from Laboratory 9 was only compared to the grand mean of the other laboratories, so only three points were possible. The maximum total possible points that Laboratory 9 could achieve was 33 for EE. The total points achieved were compared to possible points using the same percentage grading scale as for previous assessments. Of 33 possible points, Laboratory 9 achieved a total of 25 placing it in the moderate comparability category (Table III-12).

For the SWI test, there were only three combinations of sample and laboratory to compare, so only nine points were possible for the toxicity result and toxicity category evaluations. The reference toxicant comparisons were done the same way for the SWI tests as for the amphipod tests. The maximum total possible points that Laboratory 9 could achieve was 21 for MG. Of this, Laboratory 9 achieved a total of 15 points, again placing it in the moderate comparability category (Table III-13).

Table III-11. Stations and number laboratories used for the comparability assessment of Laboratory 9 for the *Eohaustorius estuarius* and *Mytilus galloprovincialis* toxicity tests.

E. estuarius Stations	# of Laboratories Compared to Laboratory 9	*M. galloprovincialis* Stations	# of Laboratories Compared to Laboratory 9
6138	1	6406	1
6171	1	6485	1
6489	2	6489	1
6527	1		

Table III-12. Assessment of Laboratory 9 performance using the *Eohaustorius estuarius* sediment toxicity test.

	Toxicity Result	Toxicity Category	Reference Toxicant	Total Points	Assessment
Possible Points	15	15	3	33	
Laboratory 9	11	11	3	25	Moderate

Table III-13. Assessment of Laboratory 9 performance using the *Mytilus galloprovincialis* sediment-water interface toxicity test.

	Toxicity Result	Toxicity Category	Reference Toxicant	Total Points	Assessment
Possible Points	9	9	3	21	
Laboratory 9	5	7	3	15	Moderate

I. Laboratory Audit

In addition to the pre-survey interlaboratory studies and split sample analyses, the Toxicology Technical Committee also for the first time conducted laboratory audits during the survey to verify that the methods and QA procedures were also comparable. The Toxicology Technical Committee also decided *a priori* that the goal of the audits would be informational and the findings would not result in punitive consequences. Instead, the audit would identify any deviations from the laboratory manual and provide an opportunity for the affected laboratories to implement necessary corrections. Furthermore, the findings will be used to improve the test protocols and enhance comparability among laboratories for future Bight sediment toxicity studies.

The auditors performed site visits to each laboratory while toxicity tests were actively underway. The laboratories were assessed for proper sample handling, testing procedures, and record keeping (Appendix C). Overall, the audits found that differences in sample handling or test chambers were the most common variations encountered.

Sieving method

Prior to testing, all field-collected sample and control sediments were homogenized and sieved through a 2.0 mm mesh screen in order to remove organisms and debris from the sediment that might affect the testing. The types of sieves used included: brass, manufactured plastic, stainless steel, and homemade plastic. Though a gloved hand was most commonly used to push the sediment through the sieve, other devices used included a plastic spoon, a homemade Teflon device, a stainless steel spatula, and a silicon spatula. Other than the use of the brass sieve, the variations in the devices used to sieve the sediment were deemed acceptable. Brass is not a recommended material for use in toxicity studies since it, could contaminate the samples (i.e., copper and zinc).

Test Chambers

Though the type of test chamber used for each test was specified prior to the initiation of the study, the test chambers used by each laboratory were variable. For the EE tests, 1L glass beakers were specified for use in testing. The test chambers actually used were all glass, but included 1L canning jars, large I-Chem jars, unspecified large jars, and 1L beakers. All jars were capable of holding the prescribed depth of sediment and volume of water, but the surface area was variable. Variations in the surface area of the sediment in the EE tests may affect the stress level of the amphipods, since their population density in the sediment would vary.

The recommended chambers for the MG tests were 600 ml tall form beakers. No two laboratories that conducted the MG tests used the same type of chambers. Because the amount of sediment that was collected had been calculated based on the volume of sediment required to conduct the test in the recommended test chambers, the variation in test chambers used was problematic when a test needed repeating. Some chambers used had larger diameters than those of the recommended test chambers, thus necessitating a greater volume of sediment. In addition, variations in the dimensions of the test chambers used altered the area of the sediment-water interface, thus potentially affecting the exchange of constituents between the overlying water and the sediment.

Aeration

The use of 1 ml glass pipettes was recommended to provide aeration in the EE and MG test chambers. Again, there was variation among the participating laboratories. For the EE tests, two laboratories used larger Pasteur pipettes, one used plastic pipette tips, while those who used 1 ml pipettes either used glass or plastic pipettes. In addition to the 1 ml pipettes, Pasteur pipettes and plastic capillary tubing were also used to deliver aeration in the MG tests. These variations were deemed inconsequential, though the amount of air flow delivered may have contributed to the low DO readings that were observed in one laboratory.

Other

Two laboratories did no randomization of their exposure chambers. Another laboratory randomized, but labeled all containers with sample information so that it was not blind. One laboratory did not do daily checks for animal emergence and sediment condition for the EE test. One laboratory used a single batch of mussels throughout the duration of the study. One laboratory did not measure pore water quality parameters at the beginning of their amphipod tests until it was pointed out at the audit.

Overall, the audit process served its purpose well. The laboratories complied well with most of the key aspects of the test methods and laboratory manual guidance. The variations that were noted did not appear to have any significant effect on the test results. In a few cases, deviations that were found were quickly corrected following the audit.

IV. DESCRIPTIVE RESULTS

A. Frequency of Toxicity

Sediment toxicity for Bight'08 was defined as stations falling into in the Moderate or High SQO toxicity categories. With the exception of the offshore stratum, toxicity was observed for at least one station from each stratum in both the *E. estuarius* survival and the mussel embryo sediment-water interface tests (Tables IV-1 and IV-2). Of the 222 stations tested with the amphipod survival test, 24 (11%) were identified as having toxicity (i.e., Moderate or High toxicity categories). Another 56 stations (25%) fell into the more uncertain Low Toxicity category (defined as not toxic). Of the 180 stations tested with the sediment-water interface test, 32 (18%) were identified as toxic, while another 10 (6%) stations were in the Low Toxicity category.

Toxicity was more prevalent for embayment stations than for the offshore stations. For the amphipod test, none of the 30 offshore stations were found to be toxic. However, seven stations were identified as being in the Low Toxicity category (Figure IV-1). For the embayment stations, 24 of the 192 stations (12%) were found to be toxic, with another 49 stations (26%) in the Low Toxicity category. Within embayments, the estuary stratum had the greatest prevalence of toxicity to amphipods (20% of stations). The bay stratum had the second highest percentage of toxic stations (13%). For the SWI test, the marina and estuary strata had the highest percentage of toxic stations (31% and 17%, respectively). In contrast to the EE test, fewer SWI test samples were classified in the Low Toxicity category than were identified as toxic (Figure IV-2).

Some localized results are worth noting within the embayment strata. Within the estuary stratum, four of the five stations from the Sweetwater River Estuary were in the Moderate Toxicity category with the fifth being Nontoxic for the amphipod test, but were all Nontoxic in the SWI test (Appendix D). All five stations from the San Diego River Estuary were Nontoxic to the amphipods, but showed a mixed response for the SWI test, with three stations classified as Nontoxic, one as Low Toxicity, and one as High Toxicity. Amphipod test results for the Tijuana Estuary indicated one Nontoxic and four Low Toxicity stations, while the SWI test results indicated four Nontoxic and one High Toxicity stations. All three Upper Newport Bay stations were classified as Nontoxic using the SWI test, but had a mixed response for the amphipod test. The five Santa Margarita Estuary stations were all Nontoxic to the amphipods. All stations within the Santa Margarita, Aqua Hedionda and Los Penasquitos Estuaries were Nontoxic using the SWI test. The San Elijo Estuary had four Nontoxic and one High Toxicity stations based on the SWI test. Two stations were tested in the Ballona Creek Estuary, one of which was Nontoxic for both toxicity tests and one that was High Toxicity for each test. The two stations in the San Gabriel River Estuary were classified as Nontoxic and Low Toxicity for the amphipod test, but as Moderate and High Toxicity for the SWI test. The remaining estuary regions showed either a mixed response or had a too few stations to discern trends.

The bay stratum encompassed the areas of San Diego Bay, Mission Bay and Los Angeles/Long Beach that are not either harbors or marinas. All four stations within Mission Bay were found to be Nontoxic to both the amphipod and SWI tests (Appendix D). The San Diego and Los Angeles/Long Beach bay regions each had a large number of stations and a wide range of toxicity.

The individual water bodies that made up the marina stratum showed a wide range of toxicity results. All four marina stations in Mission Bay were Nontoxic for both toxicity tests (Appendix D). The four stations in Marina Del Rey were Nontoxic using the SWI test, but ranged from Nontoxic to High Toxicity for the amphipod test. All Oceanside Harbor stations were also Nontoxic using the SWI test, while two of the three stations were Nontoxic to the amphipods with the remaining station being Low Toxicity. Dana Point Harbor had three Nontoxic stations for each test, with one Low Toxicity station for the amphipods and one High Toxicity station for the SWI.

The port stratum consisted primarily of stations in the Los Angeles/Long Beach Harbors and San Diego Bay. Of the 15 stations in the Los Angeles/Long Beach region, 14 were not toxic (Nontoxic and Low Toxicity categories) to the amphipods and 14 were not toxic for the SWI test (Appendix D). In the San Diego region, 28 of 30 stations were not toxic to the amphipods and 23 of 28 were not toxic for the SWI test.

When the data from the two toxicity tests are combined (integrated), the distribution of toxicity categories changes somewhat due to the moderating effects of averaging. A smaller percentage of stations were found to be in the Nontoxic category than for either test alone. A smaller number of stations were also classified in the High Toxicity category than for the SWI test results alone (Table IV-3 and Figure IV-3). Since the SWI test was not conducted on shelf stations, there are no integrated results for the offshore stratum.

B. Magnitude of Toxicity

The magnitude of toxicity within each stratum is further described as the mean of control normalized results for each test method. Only two samples were identified as having High Toxicity within any stratum for the amphipod tests. Of these High Toxicity samples, station 6520 from Ballona Creek in the estuary stratum had the lowest survival at 3% (Table IV-4). The mean survival for the two stations in the High Toxicity category was 12%. The mean survival for samples classified as Moderate Toxicity was similar among strata.

For the SWI test, the highest magnitude of toxicity was found in the estuary stratum (Table IV-5). Estuary stations 6009 (Tijuana River) and 6543 from Mugu Lagoon each had 0% normal-alive. Stations 6189 (San Diego River) and 6382 (Bolsa Chica) each had 6% normal-alive. Station 6520 from Ballona Creek had 0% normal-alive. This station was the only one in the entire survey to be classified as High Toxicity for both the amphipod and SWI tests. Also in the estuary stratum, station 6242 (San Elijo) had 4 percent normal-alive and station 6442 (San Gabriel River) had a percent normal-alive of 5. These last three stations (6520, 6242 and 6442) all likely were affected by a combination of ammonia and other stressors. The estimate of what the response would have been without the effect of ammonia is 34, 50 and 32% normal-alive, respectively (see section III-ammonia). The remaining station having a percent normal-alive result of less than 10% was station 6327 (Dana Point Harbor), which is in the marina stratum and had a result of 2%. The mean % normal-alive for all SWI test stations exhibiting High Toxicity was 10%.

Table IV-1. *Eohaustorius estuarius* toxicity category by stratum, expressed as number of stations.

Stratum	Nontoxic	Low Toxicity	Moderate Toxicity	High Toxicity	Total
Bay	18	15	5	0	38
Marina	32	9	2	1	44
Port	34	9	3	0	46
Estuary	35	16	12	1	64
Shelf	23	7	0	0	30
Total	142	56	22	2	222

Table IV-2. Sediment-water interface toxicity category by stratum, expressed as number of stations.

Stratum	Nontoxic	Low Toxicity	Moderate Toxicity	High Toxicity	Total
Bay	31	3	4	0	38
Marina	27	0	9	3	39
Port	35	3	5	1	44
Estuary	45	4	3	7	59
Total	138	10	21	11	180

Table IV-3. Integrated *Eohaustorius estuarius* and sediment-water interface toxicity category by stratum, expressed as number of stations.

Stratum	Nontoxic	Low Toxicity	Moderate Toxicity	High Toxicity	Total
Bay	16	19	3	0	38
Marina	19	13	6	1	39
Port	27	13	4	0	44
Estuary	25	25	7	2	59
Total	87	70	20	3	180

Table IV-4. Mean control-normalized survival of amphipods in each stratum and for stations categorized as Moderate or High Toxicity.

	Moderate Toxicity		High Toxicity	
Stratum	Mean	Range	Mean	Range
Bay	77	73-81	na	na
Marina	75	69-81	21	na
Port	69	59-78	na	na
Estuary	71	59-81	3	na
Shelf	na	na	na	na
All Strata	73	59-82	28	3-59

na=not applicable

Table IV-5. Mean control-normalized %normal-alive of mussel embryos in each stratum and toxicity category.

	Moderate Toxicity		High Toxicity	
Stratum	Mean	Range	Mean	Range
Bay	65	50-76	na	na
Marina	58	49-74	16	3-27
Port	68	60-74	41	na
Estuary	60	44-72	3	0-8
All Strata	62	44-76	10	0-41

na=not applicable

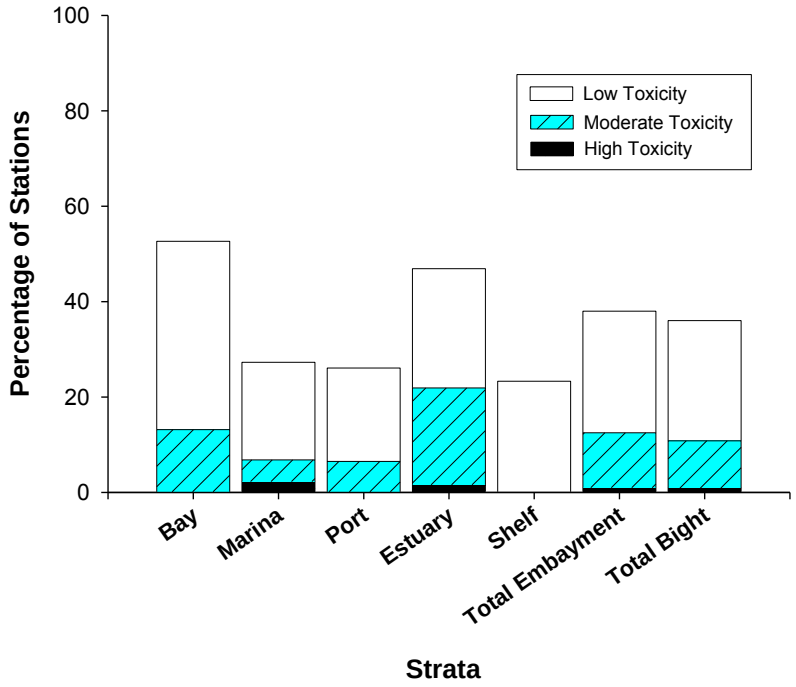

Figure IV-1. The percentage of stations in each toxicity category by stratum for *Eohaustorius estuarius* survival.

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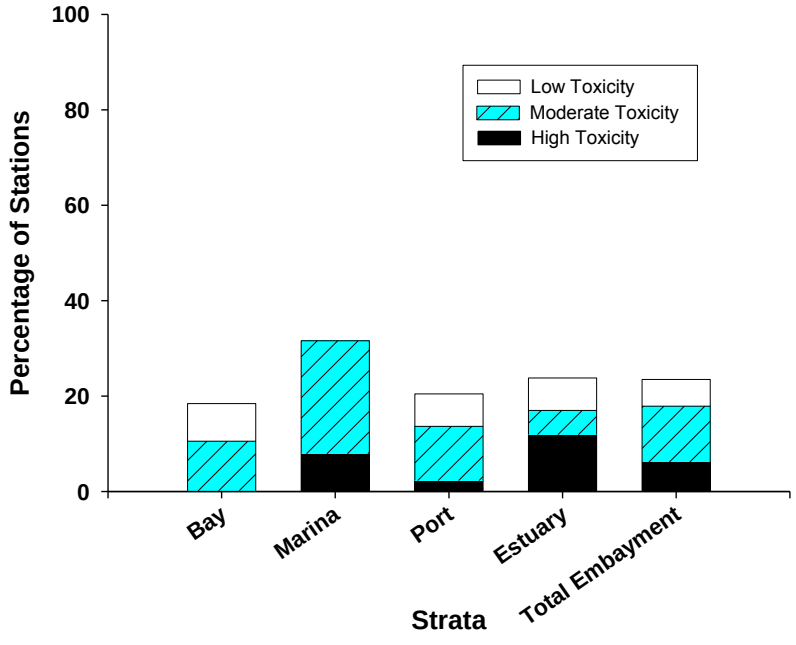

Figure IV-2. The percentage of stations in each toxicity category by stratum for *Mytilus galloprovincialis* embryo sediment-water interface test.

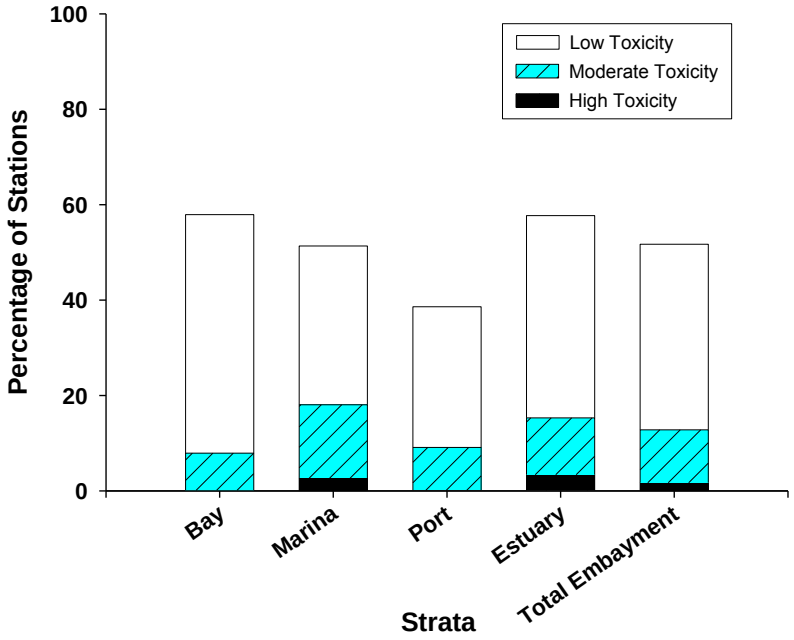

Figure IV-3. The percentage of stations in each toxicity category by stratum for the integration between the amphipod survival and sediment-water interface tests.

C. Toxicity Characterization in Estuaries

Embayment stations from throughout the Bight were targeted for possible toxicity identification evaluation (TIE) during the 2008 survey. The criterion for triggering a TIE on any given station was 60% or less survival in the initial testing using *E. estuarius*. Two strategies for collection of sediment to conduct the TIEs were employed. For stations in San Diego County and the Los Angeles/Long Beach (LA/LB) Harbors, stations meeting the criterion would be resampled to provide sediment for the TIEs. For other embayments, 15 stations were targeted based on prior knowledge of potential toxicity. At those 15 stations, an additional 4 L of sediment was collected along with the initial samples and held for possible TIEs. No stations in the San Diego or LA/LB Harbors met the <60% survival criterion. Three of the 15 remaining sites met the selection criterion and underwent whole sediment and pore water TIE testing using the amphipod 10 day survival test (Station 6520: Ballona Creek; Station 6527: Marina Del Rey, and Station 6543: Mugu Lagoon; Appendix B).

Whole sediment TIE results for Ballona Creek (Station 6520) indicated that the likely toxicant(s) were organic chemicals, with additional evidence suggesting pyrethroid pesticides as the primary toxicant. Similar results were found for the pore water TIE. These results are comparable with those found during Bight'03 for the Ballona Creek Estuary and other recent studies (Bay *et al*. 2010). For Mugu Lagoon (Station 6543), none of the TIE treatments were any more effective than simple dilution with clean sediment. This indicated that a toxicant for which there was not a TIE treatment may have been responsible (e.g., ammonia or sulfides). The pore water for the Mugu Lagoon sample was very toxic and unresponsive to TIE treatments. However, the ammonia concentration in the pore water was sufficient to account for the observed toxicity. Baseline testing of the Marina del Rey sample (Station 6527), found that the sample was no longer toxic. The control-adjusted survival increased from the initial 21% to 79%

at re-test. This reduction in toxicity during storage suggested that a labile substance may have been a source of toxicity observed at baseline.

D. Grain Size Controls

The grain size control (Channel Islands Marina sediment) was tested along with most amphipod batches throughout the survey period. In some cases, the grain size control was tested after four months in storage. However, the prolonged holding time did not materially affect the ammonia concentration or amphipod survival when compared to aliquots of the same sample which were tested earlier. There was a fairly wide range of response throughout the survey with control normalized percent survival ranging from 100 to 72%. Of the 28 tests of the grain size control, 16 exhibited some degree of response, with 5 falling into the Moderate Toxicity category (Figure IV-4). However, there was no apparent pattern either amongst the laboratories or over time. The grain size control was 89% fines (silt + clay). There were 24 stations in the Bight'08 toxicity survey with greater than or equal to 89% fines. Of these, 19 were not toxic (Nontoxic or Low Toxicity categories) to amphipods (Figure IV-5). These results indicate that grain size on its own was unlikely to be a major source of toxicity in the tests with *E. estuarius*.

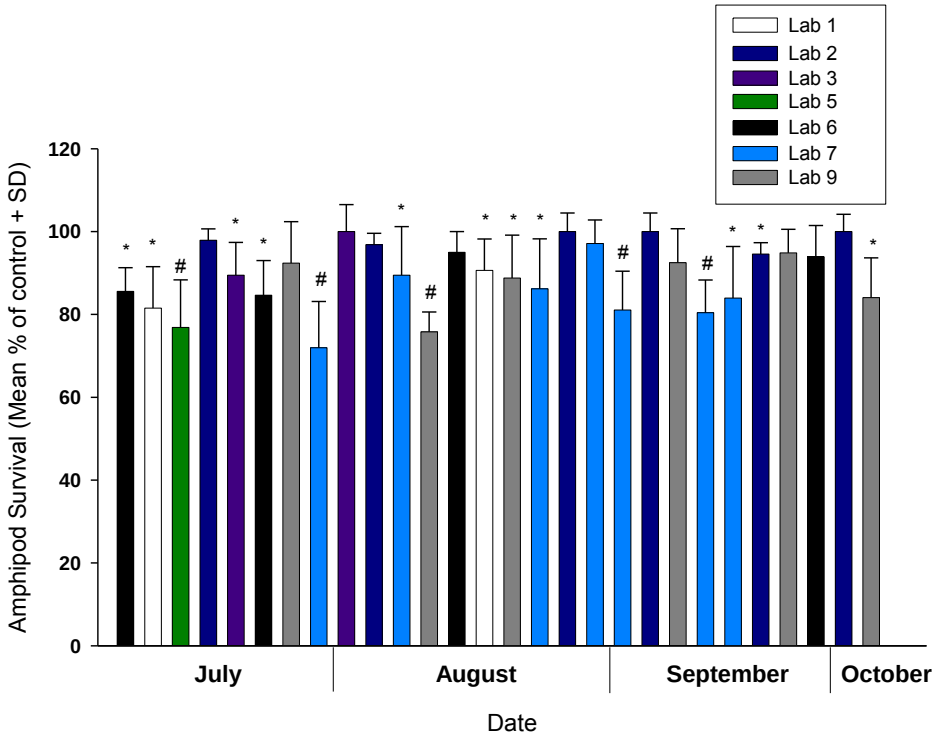

Figure IV-4. Results of grain size control testing using the amphipod, *Eohaustorius estuarius*. Bars marked with * are in the Low Toxicity category; those marked # are in the Moderate Toxicity category.

E. Comparison of Toxicity to Sediment Fines and Total Organic Carbon

Amphipod survival was significantly correlated with both sediment fines and total organic carbon (TOC) content of the sediments (Figures IV-5 and IV-6). While both of these relationships were significant, the association was not as strong as has been observed in previous Bight surveys. This may partially be due to the fact that there were fewer samples with low amphipod survival than in previous surveys. Both greater sediment fines and higher concentrations of TOC generally co-occur with elevated concentrations of contaminants in the sediment. Therefore, the correlations reported in this survey may be more associated with the effects of chemicals in the sediment than those of sediment grain size or amount of TOC. This was corroborated by the results of the grain size control testing that found that grain size was not an independent predictor of toxicity to EE (Section IV-D).

The percentage normal-alive endpoint for the SWI test did not correlate with sediment fines or with total organic carbon (Figures IV-7 and IV-8). Since the exposure method for this test does not include direct contact with sediment, physical characteristics of the sample may have less effect on the test results. Samples with high TOC concentrations would also be expected to have higher ammonia concentrations, which were shown to contribute to toxicity in some of the samples.

There were Nontoxic EE and MG test results for samples at the upper end of both the TOC and sediment fines ranges, indicating that these factors alone were unlikely a consistent cause of toxicity.

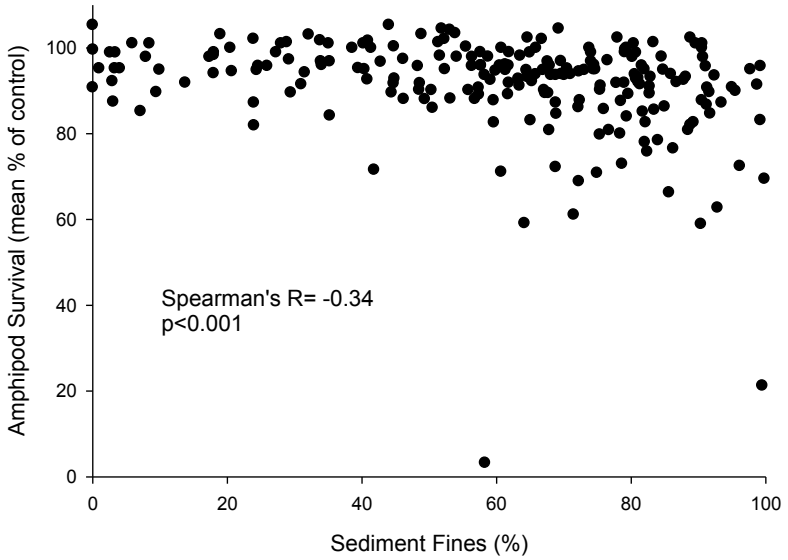

Figure IV-5. Relationship between amphipod survival and sediment percent fines content.

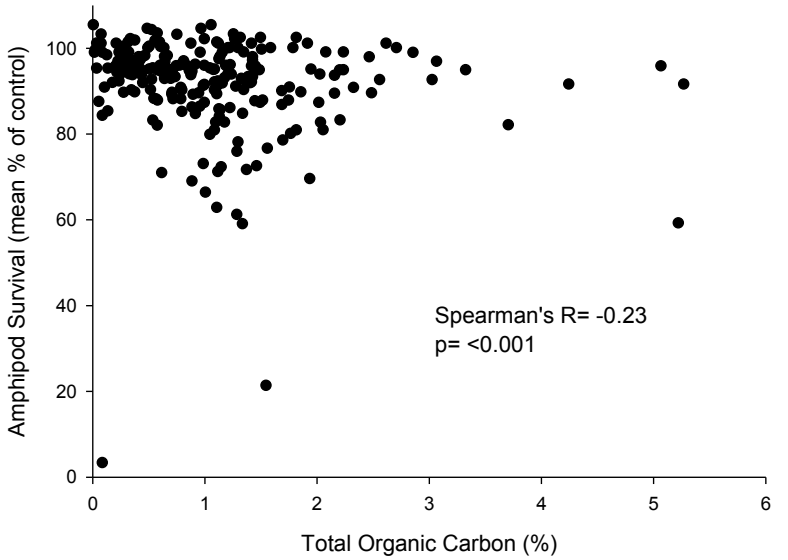

Figure IV-6. Relationship between amphipod survival and sediment total organic carbon content.

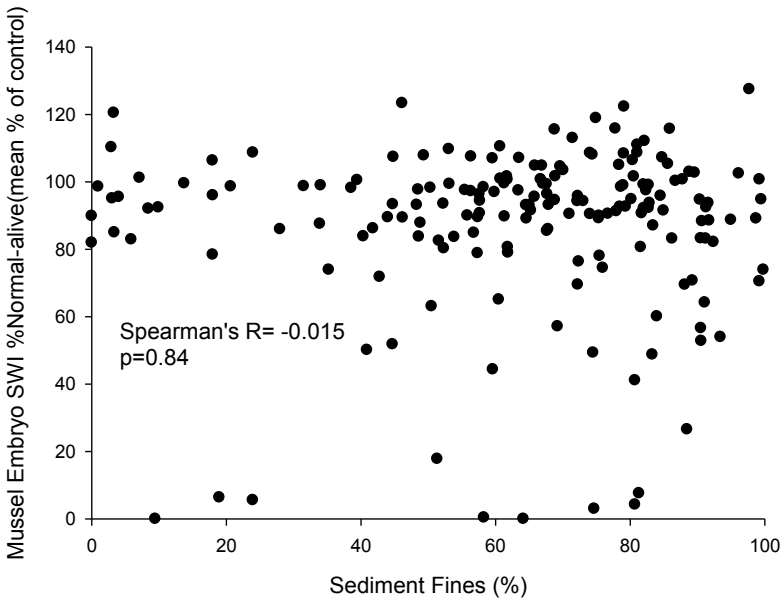

Figure IV-7. Relationship between mussel embryo sediment-water interface percent normal-alive and sediment percent fines content.

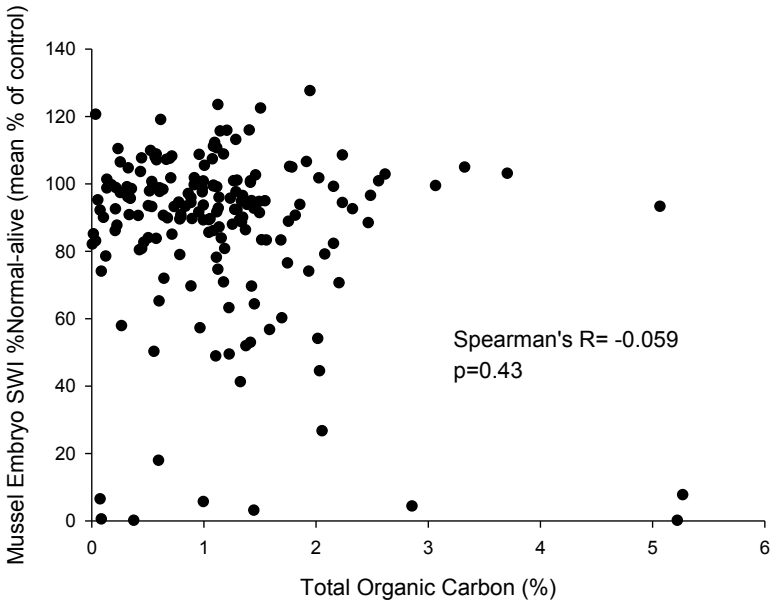

Figure IV-8. Relationship between mussel embryo sediment-water interface percent normal-alive and total organic carbon content.

V. REGIONAL ASSESSMENT OF TOXICITY

A. Extent

The majority of the SCB was found to be Nontoxic when the CA SQO toxicity thresholds were applied to the amphipod survival test results. Out of a total area of 3884 km^2 that was tested, 2963 km^2 or 76% of SCB, were in the Nontoxic category (Table V-1). The area that was found to be toxic (i.e., Moderate or High SQO toxicity categories) was 0.6% (17 km^2) of the total area.

The total area examined using the SWI test was much smaller at 124 km^2 than for the amphipods, since it only included the embayment strata (Table V-2). For all of the area where the SWI test was used, 101 km^2 or 81% of the area tested was classified as Nontoxic. The area found to be toxic was 13% (16 km^2). Integration of the two toxicity test results for the embayment strata results in a reduction in the area classified as Nontoxic to 62 km^2 (Table V-3) or 49% of the total embayment area. The area identified as toxic changed little (11%), relative to the SWI test results.

For the amphipod test data, the assessment of the SCB is driven by the shelf stratum since it accounts for approximately 97% of the area that was evaluated. None of the shelf stations were toxic. Among the embayment strata, the percent area toxic was greater than elsewhere in the SCB, ranging from 21% in the estuary stratum to 5% for ports (Figure V-1). When combined, the percentage of toxic area for all embayment strata was 14%.

For the SWI test data, marina and estuary strata had the greatest percentages of area that were toxic, 28 and 29% respectively (Figure V-2). The percentages of toxic area for the bay and port strata were similar to one another, both being 9% of their total areas. The SWI test assessment results differed from those for the amphipod test in that the area in the Low Toxicity category for each stratum was less than or equal to the combination of Moderate and High Toxicity areas.

The integrated assessment also identified the marina and estuary strata as having the greatest area classified as toxic (24 and 22% respectively, Figure V-3). There were relatively small differences in the percentage of area toxic between the individual test results and the integrated values. The largest difference for the amphipod test was in the marina stratum, which changed from 13 to 24% toxic. The differences relative to the SWI test were smaller, with the largest being for the estuary stratum which dropped from 29 to 22% toxic area when integrated. Larger differences between the individual test and integrated assessments were observed for the Low Toxicity category. This was especially true for the SWI test where there was a greater than 10% increase at every stratum. The largest change was in the bay stratum which increased from 9 to 51% with integration. This is due to the effect of averaging the two test results for a station, which results in a higher toxicity classification when the results between the two tests differ.

B. Temporal

Two previous toxicity surveys of the SCB have been conducted using a similar probabilistic sampling design and *E. estuarius* as the toxicity test organism: the 1998 and 2003 Southern California Bight regional surveys (Bay *et al*. 2000, Bay *et al*. 2005). The examination of temporal trends is complicated by differences in the criteria used to classify the toxic response among surveys. To make the datasets more comparable for assessment of temporal trends, toxicity data from the previous surveys were reevaluated using the same SQO thresholds used for the Bight'08 survey. Another complication in making temporal comparisons among surveys is that the areas within each of the strata have changed. The temporal comparisons were made on a percent of area basis in order to minimize the influence of the difference in areas.

Comparison of the results by stratum show a considerable decrease in the percent area classified as toxic (Moderate and High categories) from 2003 to 2008 (Figure V-4). A similar decrease in the percentage of

toxic area is also evident between the Bight'98 and Bight'08 surveys. The offshore stratum had similar percentages of toxic area in '98 and '03, while none of the area was classified as toxic in 2008. The percent of area that results from a combining of all SQO toxicity categories (Low + Moderate + High) is similar among all surveys for each stratum. This pattern indicates that the temporal changes are a transitioning of stations between the certain toxicity categories (Moderate and High) and the uncertain toxicity (Low) category.

Temporal trends were also examined by making direct comparisons between stations that were sampled in Bight'08 and a previous Bight survey. This analysis included 36 stations sampled in Bight'98 and 55 stations sampled in Bight'03. Examination of the Bight'08 toxicity data for the subset of stations that had been previously sampled indicated that they are representative of the Bight as a whole. Comparisons to previous surveys indicate that the majority of the stations did not change categories (83% for 1998 and 67% for 2003; Figures V-5 and V-6). Of those stations that did change, the majority changed from toxic to not toxic (4 of 6 for 1998 and 14 of 18 for 2003). Comparisons of the percent area toxic between surveys and the toxicity at resampled stations suggest a reduction in sediment toxicity over time.

Table V-1. Estimated area of SCB sediment classified by toxicity category using the amphipod survival test. All area measurements are in square kilometers.

	Nontoxic		Low Toxicity		Moderate Toxicity		High Toxicity	
Stratum	Estimate	95%CI	Estimate	95%CI	Estimate	95%CI	Estimate	95%CI
Bay	30.4	14.4	28.3	10.7	11.3	8.3	0	-
Marina	10.4	2.9	4.2	2.6	1.5	1.7	0.7	1.2
Port	22.6	3.7	4.0	3.2	1.5	2.3	0	-
Estuary	6.1	1.8	2.6	1.6	2.1	1.5	0.3	0.5
Shelf	2893.3	685.7	865.1	566.5	0	-	0	-
Total	2962.7	685.9	904.2	566.6	16.4	8.9	1.0	1.3

Table V-2. Estimated area of SCB sediment classified by toxicity category using the sediment-water interface test with mussel embryos. All area measurements are in square kilometers.

	Nontoxic		Low Toxicity		Moderate Toxicity		High Toxicity	
Stratum	Estimate	95%CI	Estimate	95%CI	Estimate	95%CI	Estimate	95%CI
Bay	57.8	14.1	6.1	5.8	6.1	5.5	0	-
Marina	11.5	2.2	0	-	3.4	1.9	1.0	1.3
Port	24.5	2.8	1.0	1.0	2.4	2.5	0.1	0.2
Estuary	6.8	1.6	0.4	0.5	1.3	1.2	1.7	1.3
Total	100.6	14.6	7.5	5.9	13.3	6.5	2.8	1.8

Table V-3. Estimated area of SCB sediment classified by toxicity category using the SQO integrated results. All area measurements are in square kilometers.

	Nontoxic		Low Toxicity		Moderate Toxicity		High Toxicity	
Stratum	Estimate	95%CI	Estimate	95%CI	Estimate	95%CI	Estimate	95%CI
Bay	28.4	14.4	35.6	10.4	6.1	5.5	0	-
Marina	8.0	2.9	4.0	2.2	3.1	2.2	0.7	1.2
Port	20.9	3.6	5.5	3.3	1.6	2.2	0	-
Estuary	4.2	1.6	3.7	1.6	1.4	1.2	0.8	1.0
Total	61.5	15.2	48.8	11.2	12.3	6.5	1.5	1.6

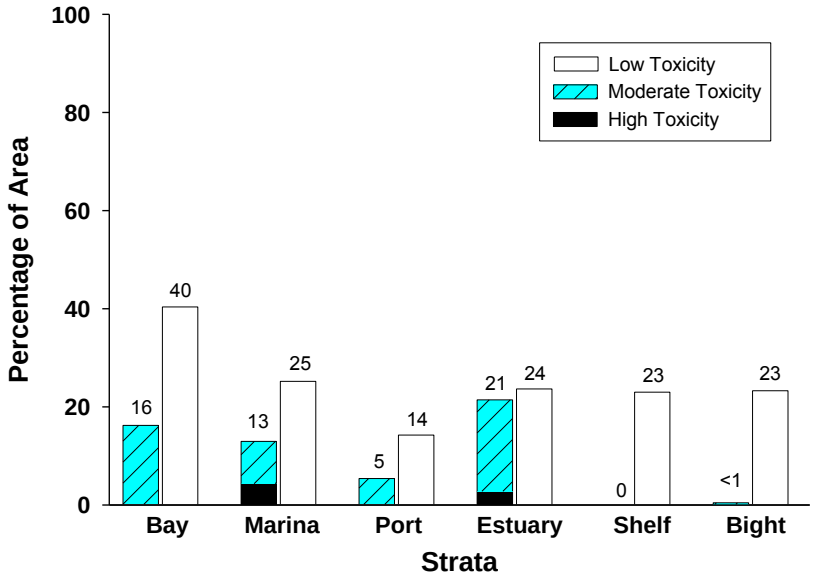

Figure V-1. Percent of area identified to be toxic using the amphipod survival test. Values above each bar indicate the total percent area toxic (Moderate + High Toxicity) or in the Low Toxicity category within each stratum.

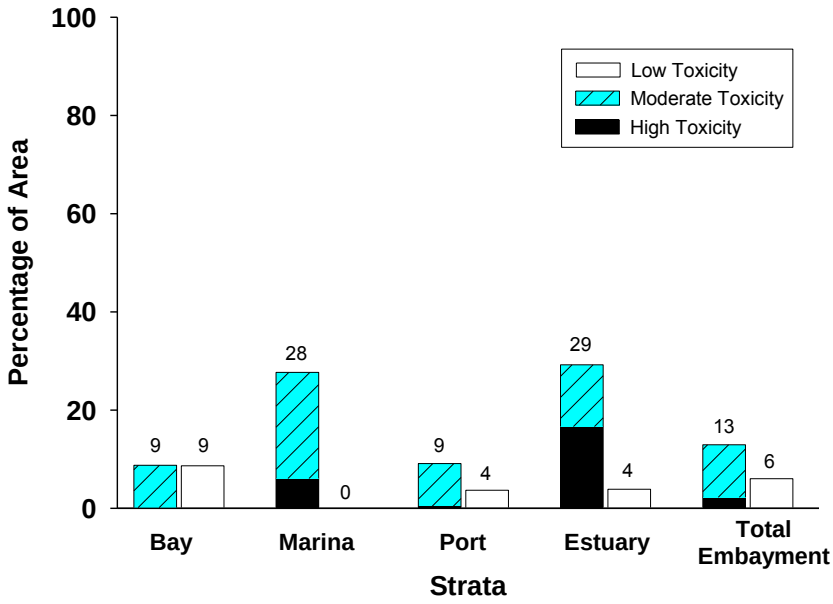

Figure V-2. Percent of area identified to be toxic using the mussel embryo sediment-water interface test. Values above each bar indicate the total percent area toxic (Moderate + High Toxicity) or in the Low Toxicity category within each stratum.

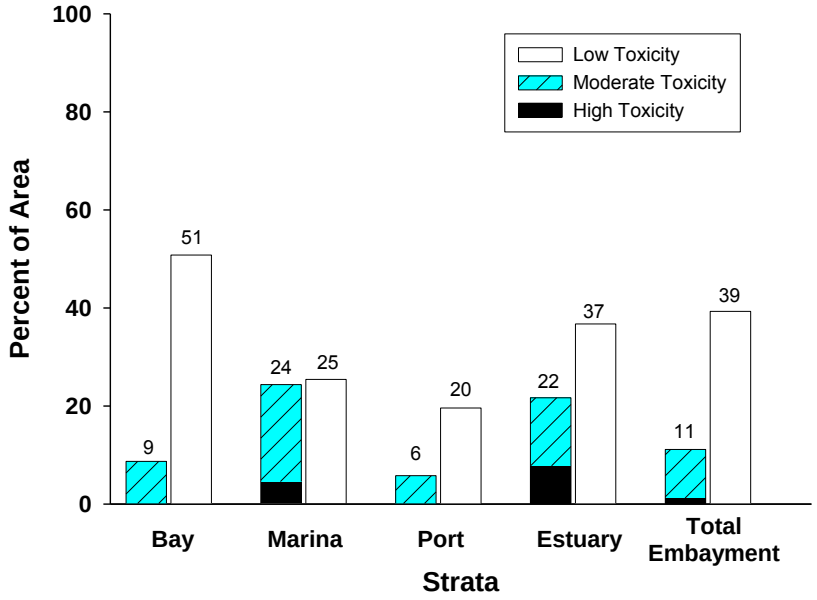

Figure V-3. Percent of area identified to be toxic integrating between both the amphipod and sediment-water interface results. Values above each bar indicate the total percent area in Low or Moderate + High Toxicity categories.

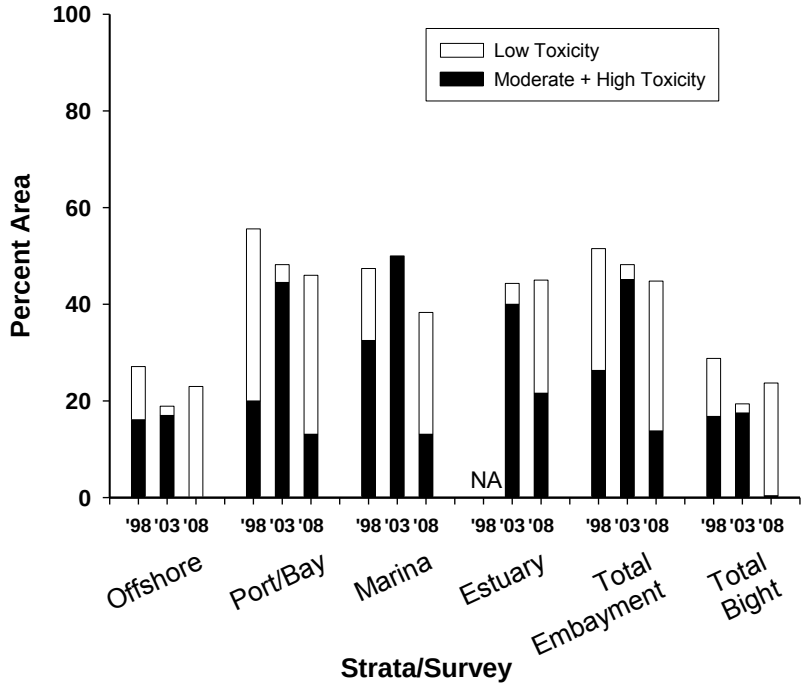

Figure V-4. Comparison of percentage areas found to be toxic with amphipod survival testing, shown by stratum over multiple surveys.

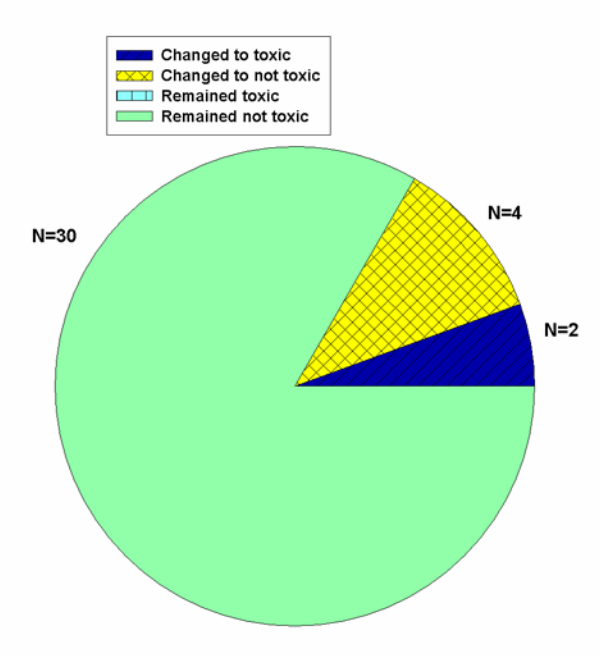

Figure V-5. Change in amphipod toxicity test results for stations sampled both in 1998 and 2008.

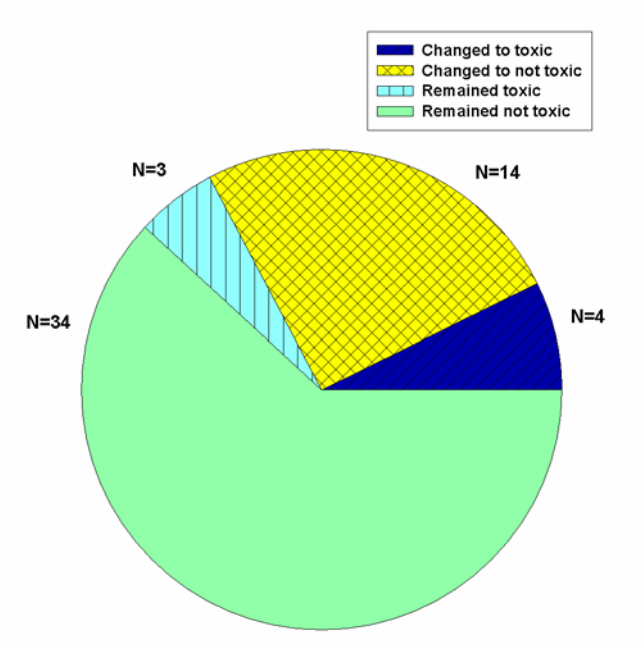

Figure V-5. Change in amphipod toxicity test results for stations sampled both in 2003 and 2008.

VI. DISCUSSION

The Bight'08 sediment toxicity study provides a confirmation of results from previous Bight surveys and advances our understanding of sediment toxicity in the SCB. Similar to the results of previous regional monitoring surveys, the 2008 survey detected the greatest prevalence and highest magnitude of acute sediment toxicity to amphipods in embayments, especially marinas and estuaries, where up to 20% of the area contained toxic sediment (i.e., Moderate or High SQO toxicity categories). For the first time, no sediment toxicity to amphipods was detected in offshore shelf sediments of the SCB. Previous surveys using the same test methods detected toxicity in approximately 16% of the shelf (Bay *et al*. 2000, 2005, 2009), suggesting an improvement in offshore sediment quality has occurred.

The use of similar test methods in previous Bight surveys provided an opportunity to examine temporal changes in sediment toxicity on a quantitative basis for the first time. These analyses showed a decline in the extent of sediment toxicity to amphipods in embayments of approximately 50%, with the greatest reductions in marinas and estuaries. Much of this decline was due to reductions in the magnitude of toxicity, as substantial areas in the Low Toxicity category remain. Whether this improvement in sediment quality is related to reduced chemical contamination of the sediments cannot be determined at this time, as sediment chemistry data for the 2008 samples are not yet available for analysis. While it is possible that part of this temporal trend may be due to interlaboratory variation, the influence of such variation on the results is expected to be negligible because all participating laboratories met the performance criteria and the quality control data indicated a high degree of comparability among the laboratories. Additional sediment toxicity surveys are needed to confirm that the reduction in sediment toxicity observed in 2008 represents a stable improvement in sediment quality.

The Bight'08 sediment toxicity survey was enhanced in several respects relative to previous surveys. Foremost, the sediment testing and data analysis methods were modified to comply with California's new SQO policy for enclosed bays and estuaries (SWRCB 2008). These modifications included the use of a second toxicity test, the mussel embryo SWI test, at all embayment stations and the use of revised thresholds to classify the test results into four categories of response. The integration of the two test methods provided a more complete and robust assessment of sediment toxicity in embayments. The integrated results confirmed that marinas and estuaries had the greatest spatial extent and magnitude of sediment toxicity. Use of the two tests also identified a substantial area of low, but uncertain, toxicity that comprised 38% of embayment sediments.

Sediment toxicity in the SCB currently appears to be less prevalent than that in San Francisco Bay, California's largest enclosed estuary. The same toxicity test methods are used in San Francisco's Regional Monitoring Program (RMP), which enables a comparison of the two regions. RMP monitoring in 2008 indicated that 70% of the stations had substantial toxicity to either amphipods or mussel embryos (SWI test), compared to 28% of SCB embayment stations (SFEI 2010). The relative responsiveness of the two test methods also differed between regions. The SWI test detected toxicity more frequently than the amphipod test in San Francisco Bay, while the amphipod test was more responsive in Bight'08. Regional differences in the relative response between test methods may indicate that the causes of sediment toxicity differ between the SCB and San Francisco Bay.

Few recent data are available to compare SCB sediment toxicity with that in northern California bays and estuaries. A synthesis of 1999 and 2005 regional monitoring data from EPA's Environmental Monitoring and Assessment Program (EMAP) indicates that northern bays and estuaries have a similar spatial extent of sediment toxicity to those in the Bight'08 survey (Barnett *et al*. 2007). EMAP amphipod toxicity tests identified 17% of northern bays and estuaries as having Moderate or High Toxicity, compared to 14% of Bight'08 embayments.

Bight'08 represents the first large-scale application of the SQO methods and demonstrated that the methods can be successfully applied in a variety of habitats and by multiple laboratories. The results from the Bight'08 survey provides a valuable reference for other monitoring agencies, as the SQO policy becomes incorporated into other monitoring and assessment programs. Because of the use of different test methods and interpretation thresholds in Bight'08, caution is needed when comparing these results to those of previous studies. These differences were addressed in our temporal comparison by recalculating the toxicity results for all surveys using a standard method and limiting the comparison to data derived from the same test method.

The regional application of the SWI test in combination with the amphipod survival test provided new insights into the use of these methods. Although the SWI test measures a sublethal endpoint (mussel embryo development), this test was not consistently more sensitive than the amphipod survival test. In fact, the amphipod test detected toxicity more frequently than did the SWI test, but the SWI test generally reported a higher magnitude of response. The two tests often yielded different results for the same sediment sample, indicating that no single test can provide a complete measurement of sediment toxicity.

The quality assurance procedures for the sediment toxicity tests were enhanced in several respects for Bight'08, resulting in improved confidence in the results. First a numeric scoring system was used to evaluate the results of the interlaboratory comparisons, and a minimum threshold of comparability was established to allow participation in the survey. The scoring system provides a more informative and objective assessment of each laboratory's performance. The scoring system also demonstrated a need for additional interlaboratory studies prior to qualifying laboratories for conducting the SWI test on Bight'08 samples. Second, all laboratories participated in an external audit of laboratory practices during the survey. The audit identified several areas of inconsistency in methods that were readily corrected during the survey. A final enhancement to the quality assurance program was the inclusion of a sediment grain size reference sample in most test batches. This sample was composed predominantly of fine sediments with low toxicity and was used to evaluate the potential influence of fine sediments on the amphipod test results. Use of this reference sample confirmed the results of other analyses indicating that the presence of fine sediments was unlikely to influence the outcome of toxicity tests using *E. estuarius*.

A final enhancement of the Bight'08 sediment toxicity survey was an expanded effort to identify the cause of sediment toxicity in SCB embayments. The study participants developed a standardized method for conducting toxicity identification evaluations (TIEs) that included a greater number of treatment types and tracked 160 embayment stations for possible TIE. However, only three stations had a sufficient magnitude of sediment toxicity to support TIE studies, thereby limiting our ability to evaluate causes of sediment toxicity. TIE analysis of these stations using the amphipods suggested pyrethroid pesticides and ammonia as the most likely contributors to the observed toxicity.

The finding of pyrethroids as a likely cause of sediment toxicity is consistent with other studies in the SCB. Previous research at Ballona Estuary, the Bight'08 site identified as having pyrethroid toxicity, has detected concentrations of multiple pyrethroids in the sediment at concentrations that were sufficient to cause toxicity (Lao *et al*. 2010). Previous TIE studies in San Diego Bay have also attributed sediment toxicity to pyrethroids (Anderson *et al*. 2010). Moreover, pyrethroid toxicity in sediments has been established as an issue of statewide concern for both estuarine and freshwater systems. A 2006 investigation of 30 urban creek sites located throughout California detected pyrethroid-associated toxicity at all sites (Holmes *et al*. 2008). Similarly, a statewide review of freshwater and estuary sediment TIE results found that pesticides were a likely cause of toxicity at every site, with most cases attributed to pyrethroids (Hunt *et al*. 2010).

Ammonia is also a frequent toxicant of concern in sediment quality studies because concentrations can be elevated to toxic levels as a result of both natural increases in organic carbon loading to sediments and waste discharge. The contribution of ammonia to the toxicity reported in Bight'08 was evaluated through measurements of pore water and overlying water during the toxicity tests and found to be a minor concern. No amphipod test samples exceeded toxic effect levels and less than 10% of the SWI results were influenced by ammonia.

The sediment toxicity results reported herein provide only part of the information needed to assess sediment quality in the SCB. As described in California's SQO policy, information on chemical exposure and benthic community condition is also needed to provide an accurate assessment of sediment quality (SWRCB 2008). Toxicity tests are valuable because they provide an integrated biological response to the sediment characteristics. Measurements of sediment chemical concentrations are needed to verify that the observed toxic responses are associated with chemical exposure. In addition, the two toxicity tests used in Bight'08 measured biological responses under controlled laboratory conditions, which may not fully represent the chemical exposure and biological sensitivity of resident sediment-dwelling organisms. Concurrent measurement of benthic community condition is needed to provide confirmation that the laboratory measurements of effects are ecologically relevant. Integrating the results of these three measures to assess sediment quality, known as the sediment quality triad, utilizes the strengths and minimizes the weaknesses of the individual components (Chapman *et al.* 1997). Data on sediment chemistry and benthic community condition are expected to be available for all of the stations evaluated for sediment toxicity. The results from all three lines of evidence will be used to make an assessment of sediment quality in the SCB for Bight'08.

VII. CONCLUSIONS

The Bight'08 sediment toxicity study provided a comprehensive regional assessment of sediment toxicity in the SCB. Analysis of the results by the Toxicology Technical Committee, representing the participating laboratories and other partners, has produced the following conclusions:

- **Most of the SCB was Nontoxic.**
  Less than 1% of the SCB exhibited toxicity (Moderate or High categories) and 76% of the area was classified in the SQO Nontoxic category. The remaining area of the SCB was classified as Low Toxicity with uncertain biological significance.

- **Embayment sediments had the greatest extent and magnitude of toxicity.**
  Sediments exhibiting toxicity were estimated to occupy 24% of marinas and 22% of estuaries. The greatest extent of sediments in the High Toxicity category was also present in marinas and estuaries. None of the sediments from offshore, shelf locations were found to be toxic.

- **Sediment toxicity has decreased relative to previous surveys.**
  The extent of toxicity in Bight'08 was less than measured in the 2003 and 1998 surveys. Reductions in toxicity occurred in both offshore and embayment areas. Comparison of these results to other indicators, such as sediment chemistry, as well as additional surveys are needed to determine whether this trend represents long-term improvement in sediment quality.

- **Incorporation of the SQO methodology was successful and informative.**
  The amphipod and mussel embryo sediment-water interface tests were successfully incorporated into the Bight'08 study design. Each method provided unique information. Integration of the findings from these methods for the embayment strata sometimes resulted in modification of toxicity classifications compared to using just a single test.

- **The enhanced QA program was a valuable component of the study.**
  The combination of increased rigor in interlaboratory comparison test evaluation, inclusion of additional evaluation samples, and laboratory audits increased the validity of the sediment toxicity results. These QA methods are also applicable to other programs seeking to achieve high quality and comparability in toxicity testing. Results from the Bight'08 sediment toxicity studies also identified the need to improve the comparability among laboratories which perform sediment-water interface tests.

- **Sediment toxicity identification studies identified pyrethroid pesticides as a potential pollutant of concern.**
  Only four of 222 embayment stations contained sufficient toxicity to meet the threshold for application of the sediment toxicity identification evaluation (TIE) protocol developed for Bight'08. TIEs conducted at three of these stations indicated that pyrethroid pesticides and ammonia were likely causes of toxicity. However, these limited studies do not provide sufficient information to describe regional patterns in the cause of sediment toxicity for SCB embayments.

VIII. RECOMMENDATIONS

Sediment toxicity testing is an essential element of the sediment quality triad upon which California's sediment quality assessment policy is based. Additional surveys of sediment toxicity are needed to determine whether the temporal changes identified in Bight'08 represent a long-term improvement in sediment quality. Based on the experiences from Bight'08 survey, the Toxicology Technical Committee recommends the following actions to improve the understanding of sediment toxicity in the SCB:

- **Conduct special studies to confirm the temporal trend of reduced toxicity.**
  Studies at selected locations should be conducted to determine whether the reduction in sediment toxicity observed in Bight'08 is due to improved sediment quality or is the result of other factors, such as short-term variability in test response. The five-year cycle of the Bight survey program may not be short enough to understand the influence of various factors. It is recommended that focused and repeated studies of sediment toxicity and chemistry be conducted at selected locations where substantial changes in sediment toxicity were observed in order to determine whether the temporal trend in toxicity is a consistent finding. The results of these studies may identify important factors that should be measured in future Bight surveys in order to improve our ability to measure and interpret sediment toxicity trends.

- **Modify TIE study designs to improve effectiveness.**
  Alternative strategies for sample identification, collection and testing are needed so that TIEs can be conducted on toxic samples with greater efficiency. These strategies should include more sensitive and reliable TIE methods so that samples with Low or Moderate Toxicity can be evaluated with greater success. A TIE workshop should be held prior to the next regional survey in order to develop an improved TIE workplan for use in future regional surveys. Workshop topics should include: sample selection criteria, methods, quality assurance, laboratory comparability, and data interpretation.

- **Measure embayment sediment toxicity to additional species.**
  The greater extent and magnitude of toxicity in embayments should be the focus of additional study in future surveys. It is recommended that toxicity tests using the other species identified in the SQO policy (i.e., *Rhepoxynius abronius*, *Leptocheirus plumulosus*, and *Neanthes arenaceodentata*) be included at a limited number of stations. Use of these additional species at selected sites will provide a more complete measure of sediment toxicity and will also provide information on the comparability of the different test methods recommended in the SQO policy. The resulting data would provide an improved understanding of the relative responsiveness of the test methods to SCB sediments and therefore assist multiple agencies in selecting test methods for use in future monitoring programs.

- **Formalize a toxicity quality assurance program for the region.**
  The Bight'08 sediment toxicity QA activities were important to the survey's success and provided multiple benefits to the participants. These benefits can be increased and made more lasting through the establishment of an ongoing and expanded toxicity QA program, similar to what has been accomplished through the Southern California Association of Marine Invertebrate Taxonomists (SCAMIT). Many of the QA issues addressed in Bight'08, such as interlaboratory comparability, method standardization and data interpretation, are also relevant to other types of toxicity testing, including evaluation of effluent, stormwater, and ambient water samples. Therefore, establishment of a formal toxicity QA program that is conducted and coordinated by local scientists would benefit multiple types of monitoring programs and likely reduce the burden of periodically reestablishing such a program for each Bight survey. This toxicity QA group

would work toward standardizing and improving toxicity testing in southern California on an ongoing basis and provide qualification of participants in advance of the Bight program. In addition, this QA group could also provide an opportunity for laboratories not involved with the Bight program to participate, thus helping to improve data quality and comparability in other monitoring programs.

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APPENDIX A. PARTICIPANTS IN THE BIGHT'08 REGIONAL MONITORING PROGRAM

Organization	Coastal Ecology	Microbiology	Water Quality	Rocky Reefs	Areas of Special Biological Significance	Coastal Wetlands and Estuaries	Bioaccumulation
AMEC Incorporated					X		
Aquatic Bioassay and Consulting Laboratories	X		X		X		
California Polytechnic University			X				
California State Parks					X	X	
California State University Channel Islands						X	
California Department of Fish and Game	X					X	X
California Department of Public Health			X				
Camp Pendleton Marine Corps Base						X	
Channel Islands National Marine Sanctuary	X						
Chevron USA Products Company	X						
City of Carlsbad						X	
City of Coronado						X	
City of Del Mar						X	
City of El Cajon						X	
City of Encinitas		X				X	
City of Escondido						X	
City of Imperial Beach						X	
City of La Mesa						X	
City of Laguna Beach					X		
City of Lemon Grove						X	
City of Long Beach			X				
City of Los Angeles Environmental Monitoring Division	X	X	X			X	
City of Poway						X	
City of San Marcos						X	
City of Santee						X	

Organization	Coastal Ecology	Microbiology	Water Quality	Rocky Reefs	Areas of Special Biological Significance	Coastal Wetlands and Estuaries	Bioaccumulation
City of Solana Beach						X	
City of Vista						X	
City of Chula Vista						X	
City of Malibu					X		
City of Newport Beach					X	X	
City of Oceanside			X			X	
City of Oxnard	X		X				X
City of San Diego	X	X	X		X		X
City of Ventura			X			X	
Coastal Conservancy			X			X	
CRG Marine Laboratories	X		X		X		X
Encina Wastewater Authority	X		X				
Jet Propulsion Laboratory			X				
Los Angeles County Department of Beaches & Harbors	X						
Los Angeles County Dept. of Health Services		X					
Los Angeles County Department of Public Works					X		
Los Angeles County Sanitation Districts	X	X	X	X			X
Los Angeles Department of Water and Power	X						
Los Angeles Regional Water Quality Control Board					X	X	X
Loyola Marymount University		X					X
Marine Pollution Studies Laboratory - Granite Canyon	X						
Marine Pollution Studies Laboratory – Rancho Cordova	X						X
Marine Biological Consultants	X						
Monterey Bay Aquarium Research Institute			X				
Natural History Museum of Los Angeles County	X						

Organization	Coastal Ecology	Microbiology	Water Quality	Rocky Reefs	Areas of Special Biological Significance	Coastal Wetlands and Estuaries	Bioaccumulation
National City						X	
National Parks Service				X			
Nautilus Environmental	X				X		
NES Energy, Inc.	X						
NOAA	X	X	X			X	
NRG Energy, Inc.	X						
Orange County Environmental Health Division		X					
Orange County Public Facilities and Resources					X	X	
Orange County Sanitation District	X	X	X				X
Port of Long Beach	X						
Port of Los Angeles	X		X	X			
Port of San Diego	X					X	X
Reliant Corporation	X						
Resource Conservation District						X	
Riverside County Flood Control District			X				
San Bernardino Flood Control District			X				
San Diego County						X	
San Diego County Department of Environmental Health						X	
San Diego Regional Water Quality Control Board					X	X	
San Diego State University				X			
San Elijo Joint Powers Authority	X						
San Elijo Lagoon Conservancy						X	
San Francisco Estuary Institute							X
Santa Ana Regional Water Quality Control Board			X			X	
Santa Ana River Watershed Management Authority						X	
Santa Monica Bay Restoration Commission						X	

Organization	Coastal Ecology	Microbiology	Water Quality	Rocky Reefs	Areas of Special Biological Significance	Coastal Wetlands and Estuaries	Bioaccumulation
Scripps Institution of Oceanography			X				
Sea Ventures							
South Orange County Water Authority							
Southern California Coastal Water Research Project	X	X	X	X	X	X	X
Stanford University		X					
State Water Resources Control Board		X	X	X	X	X	X
Tijuana Estuary National Estuarine Research Reserve						X	
University of California, Los Angeles		X	X				
University of California, San Diego				X	X		
University of California, Santa Barbara		X	X	X		X	
University of California, Santa Cruz					X		
University of South Carolina						X	
University of Southern California			X		X		
USEPA Region IX						X	X
USEPA Office of Research and Development	X						
US Fish and Wildlife Service						X	
US Geological Survey	X						
US Navy					X		
Vantuna Research Group, Occidental College	X			X	X		
Ventura County Watershed Protection Division			X			X	
Weston Solutions	X	X	X		X	X	

APPENDIX B. TOXICITY IDENTIFICATION EVALUATION OF EMBAYMENT SEDIMENTS

Monica A. Mays and Diana Young
Southern California Coastal Water Research Project

INTRODUCTION

The Bight'08 survey measured sediment toxicity at 222 marine and estuarine sites using standardized tests with two species. These tests provide a measure of the overall toxicity of the sediment but are not able to determine the specific cause of toxicity, information that is often needed to determine potential management actions. Routine chemical analysis of the test sample is frequently unable to determine the cause of toxicity with confidence because contaminants are often present in complex mixtures and the portion of contaminants that is bioavailable is typically unknown. Toxicity identification evaluation (TIE) studies are often used to investigate the causes of toxicity in sediment and pore water. TIEs use a series of physical and chemical manipulations of the sample to isolate the effects of specific contaminant classes. Changes in toxicity before and after the manipulations indicate the types of contaminants affecting the test organism.

The objective of the Bight'08 TIE study was to build upon previous Bight survey TIE studies by examining additional sites and increasing the specificity of TIE treatments. In the Bight'03 survey, TIEs were conducted on sediments from only two Los Angeles County estuaries. These studies focused primarily on phase I TIE methods intended to characterize the general type of contaminant and provided limited information on the effects of pesticides. In the Bight'08 survey, additional phase II TIE methods were included to provide more specific information on the contribution of pesticides to sediment toxicity. In addition, the Bight'08 TIE study expanded its area of focus to include marine and estuarine embayments throughout the Southern California Bight.

METHODS

Study Design

The Bight'08 TIE study was conducted by three organizations: SCCWRP, Weston Environmental, and Nautilus. The laboratories established a common TIE experimental design that standardized test methods, TIE treatments, and data interpretation. Each organization was responsible for tracking the Bight'08 survey toxicity test results for specific focus areas and conducting TIEs on a subset of sites, provided sufficient toxicity was detected. Three TIE focus areas were established, with each area tracked by one of the study participants: San Diego embayments (Weston), Long Beach/Los Angeles Harbors (Nautilus), other embayments in Ventura, LA, and Orange Counties (SCCWRP). The Bight'08 survey initial amphipod toxicity test results for each of these focus areas were monitored and stations meeting a criterion of less than 60% amphipod survival were selected for TIE. Approximately 160 stations were tracked for possible TIE evaluation.

Initial Testing and Station Selection

Initial toxicity tests were performed as part of the Bight'08 survey using the amphipod, *E. estuarius*, 10-day survival test by standard EPA methods (USEPA 1994). An additional 4 L of sediment was collected at the time of initial survey sampling at each of the stations tracked by SCCWRP for potential use in TIEs. This sediment was stored at 4 °C. The initial toxicity test results indicated that only three of the focus area stations met the TIE selection criteria of less than 60% amphipod survival (Table B-1). Two of the stations, Ballona Creek (6520) and Marina del Rey (6527) were located in Los Angeles County (Figure D-1). The third station, Mugu Lagoon (6543) was located in Ventura County (Figure D-2).

Whole Sediment TIE

Whole sediment TIEs were performed in 250 ml beakers containing approximately 40 ml of sediment and 200 ml of 32 ‰ seawater. The sediment was press sieved through a 2 mm stainless steel screen prior to homogenization and TIE treatment. Sediment, water and aeration were added to the beakers 24 hours prior to the addition of animals. Each beaker contained 10 *E. estuarius* that were purchased from Northwestern Aquatic Sciences and acclimated for 4 to 7 days at SCCWRP without feeding prior to the test. The experiment was conducted under constant light at a temperature of 15 °C (or 10ºC for the temperature reduction TIE treatment) . Five replicates were tested for the controls. Four replicates were tested for the baseline toxicity measurement (no sample treatment) and three replicates were tested for each of the treatments. Dissolved oxygen, pH, salinity and ammonia samples from overlaying water were taken at the start and end of the exposure from representative beakers for each treatment. At the end of the test, surviving amphipods were counted to determine percentage survival.

Whole sediment TIE methods were based on a combination of published manuals (USEPA 1991, 2007), peer-reviewed scientific literature (Lebo *et al*. 1999, Burgess *et al*. 2000), and experience among participating labs. Baseline toxicity tests of untreated sediment were tested to compare against the treated sediments and to identify any changes in toxicity that may have occurred during storage. Seven manipulations of the whole sediment were applied to separate portions of the sample (Table B-2). Three general characterization treatments were used: carbon addition, cation exchange resin addition, and dilution/aeration. Four treatments were used to evaluate the influence of organophosphate or pyrethroid pesticides: PBO addition, temperature reduction, carboxyesterase enzyme addition, and protein addition (blank for enzyme treatment). For all treatments, a sample of amphipod home sediment was also manipulated to verify that the procedures themselves were not causing toxicity.

Pore Water

Sediment was centrifuged at 3000 x g for 30 minutes to extract the pore water. The pore water samples were tested using a 10-day *E. estuarius* survival test. Exposures were conducted in glass shell vials with 20 ml of sample at a temperature of 15ºC (or 10ºC for the temperature reduction TIE treatment). Five *E. estuarius* were added to each vial and tests were conducted under constant darkness without aeration. Five replicates were tested for the controls. Three replicates were tested for the baselines and treatments. The baseline toxicity was measured at three concentrations (100%, 50%, and 25%) while the TIE treatments were applied to the 100% sample. Dissolved oxygen, pH, salinity and ammonia samples were taken from surrogate water quality vials at the beginning and end of the exposure. At the end of the test, surviving amphipods were counted to determine percentage survival.

Pore water TIE methods were based on a combination of published manuals (USEPA 1991, 2007), peer-reviewed scientific literature, and internal experience among participating labs. Two

general characterization treatments were applied (Table B-3): solid phase extraction (C18 column) and EDTA addition. Four pesticide-specific treatments were applied, using similar methods as described for the sediment. There was insufficient pore water volume available for the Marina del Rey station to conduct all treatments, therefore the only manipulations that were tested were the C18 column and PBO addition. Laboratory seawater was subjected to all of the TIE treatments to verify that the procedures were not causing toxicity. Samples of 32 ‰ seawater were tested as controls.

Data Analysis

Percentage point differences were calculated and plotted to quantify the effects of the treatments. The percent survival of the baseline was subtracted from the percent survival of each treatment, resulting in either a positive (less toxic) or negative value (more toxic). A 20 percentage point difference criterion was established to indentify whether the treatment had substantially removed or increased toxicity. For example, if survival increased by at least 20 percentage points in comparison to the baseline, then the treatment was considered to have removed a substantial amount of toxicity. Plots of this data show the increase or decrease in toxicity by each TIE treatment. Reference lines indicate whether the change was considered substantial or if complete removal or zero survival had be attained.

RESULTS

Ballona Creek

The Ballona Creek station baseline whole sediment result indicated a similar level of toxicity to the initial sample, with 5% survival (Figure B-3). The addition of coconut carbon was the only treatment that substantially removed toxicity (Figure B-4), which increased survival to 73% (Table B-4). The CEE treatment also removed toxicity, but not a substantial amount (< 20 percentage point difference). The PBO treatment increased toxicity (0% survival)

The 100% baseline pore water sample for Ballona Creek was toxic with 40% survival. The C18 SPE treatment decreased toxicity, increasing amphipod survival to 87% (Table B-5). Each of the pesticide specific treatments affected amphipod survival relative to the baseline. Toxicity was reduced in the CEE treatment and increased in the temperature reduction and PBO treatments (Figure B-5). Reduced survival was also present in the PBO blank, but the effect was less than that observed for the pore water sample.

Marina del Rey

The Marina del Rey baseline whole sediment did not have a substantial amount of toxicity with 73% amphipod survival. This was a substantial decrease in toxicity from what was observed in the initial testing, suggesting that the toxicity of the sample decreased while in storage. Due to the high survival in the baseline, the TIE treatments for Marina del Rey did not provide useful information (Table B-4).

Due to the limited amount of whole sediment available and low extraction efficiency an insufficient amount of pore water was available for from this sample to do all of the TIE treatments. Therefore, only the C18 and PBO treatments were conducted. The 100% baseline pore water sample for Marina del Rey showed no toxicity with 93% survival (Table B-5). However, there was an increase in toxicity with the addition of PBO. This enhancement of toxicity suggests that pyrethroid pesticides were present in the sample.

Mugu Lagoon

The whole sediment baseline sample for Mugu Lagoon was toxic with 30% survival (Table B-4). Both the dilution and carbon treatments reduced a substantial amount of toxicity (Figure B-6). An increase in toxicity resulted from the PBO treatment. The other pesticide-specific treatments had either minor or no effect on the sample toxicity (Figure B-6).

The 100% baseline pore water sample for Mugu Lagoon was highly toxic (0% survival). None of the TIE treatments substantially reduced toxicity (Figure B-7). Due to the high toxicity present in the sample at the end of the 10-day test, survival results after four days were reviewed in an attempt to identify TIE treatment effects. None of the TIE treatments showed effectiveness after four days; there was 0% survival in all treatments.

DISCUSSION

The Bight'08 TIE study had limited success in expanding the spatial scope of our TIE studies, due to the low incidence of toxicity in the focus areas. The TIE treatments were successful in characterizing a possible cause of toxicity for both the Ballona Creek and Mugu Lagoon stations. Similar patterns were seen for whole sediment tests for these two stations, specifically the possibility of a pyrethroid pesticide. However, the toxicity in the Marina del Rey sample had decreased since the initial testing and therefore the TIE treatments did not provide useful information.

Ballona Creek

The toxicity results from both the whole sediment and pore water tests for Ballona Creek, suggest pyrethroid pesticides were the principal cause of toxicity. A decrease in whole sediment toxicity following carbon treatment indicated the presence of a nonpolar organic toxicant. The PBO treatment increased whole sediment toxicity, which is the response expected in the presence of pyrethroid pesticides. The pore water TIE results confirmed the results of the whole sediment test, with changes in toxicity due to C18 column extraction and pyrethroid-specific treatments.

The results for the Ballona Creek station are consistent with other Ballona Creek TIE studies conducted in Bight'03 project and in other SCCWRP research. Sediment TIEs conducted at two Bight'03 stations in Ballona Creek indicated that the likely cause of toxicity was caused by organics, possibly pyrethroid pesticides (Bay *et al*. 2005). In addition, in 2007 and 2008, SCCWRP conducted TIE tests with samples from several Ballona Creek locations in which some sites also appeared to be affected by pyrethroids.

Mugu Lagoon

At the Mugu Lagoon station, there was less agreement between the TIE results from the whole sediment and pore water samples. The increase in survival for the carbon treatment and decreased survival in the PBO treatment for the whole sediment test with the Mugu Lagoon station suggests that the toxicity was due in part to an organic compound, likely a pyrethroid pesticide. However, the sediment dilution treatment also reduced toxicity, suggesting that the effect observed in the carbon treatment may have been due to physical factors such as dilution or aeration of the sediment. There is evidence that other toxicants were present in the Mugu Lagoon sample since none of the TIE treatments completely removed toxicity.

It is likely that high ammonia levels contributed to the toxicity in the Mugu Lagoon sample. Concentrations of un-ionized ammonia in the pore water were elevated, up to 2.47 mg/L, which is higher than the *E. estuarius* LC_{50} for un-ionized ammonia (1.12 mg/L; SCCWRP unpublished). Calculations of the ammonia toxicity units (TU) indicated that these concentrations were likely to cause high amphipod mortality (Table B-6). Mugu Lagoon pore water contained 2.2 TUs of ammonia, much more than was present in the Ballona Creek and Marina del Rey samples. The lack of a substantial effect of the carbon and cation exchange resin treatments, but a reduction in toxicity with dilution is consistent with the pattern expected for ammonia toxicity. Use of additional TIE treatments, such as zeolite addition/extraction, is needed to confirm the influence of ammonia.

Pyrethroids and TIE Development

The results of this study indicate that pyrethroid pesticide contamination is an important factor in Southern California Bight embayment sediment toxicity, consistent with studies in other regions of California (Holmes *et al*. 2008). However, chemistry data from these stations is needed to confirm these results. As the use of organophosphates has declined, the use of pyrethroid pesticides has increased (Amweg *et al*. 2005). In addition to agricultural applications, pyrethroids are widely used in urban areas for landscaping and pest control (Holmes *et al*. 2008). In 2007, the pyrethroid pesticides, permethrin and cypermethrin were two of the top 100 pesticides used statewide in California, in which approximately 341,000 kg combined were reported to have been applied (www.cdpr.ca.gov).

The pesticide-specific treatments included in this TIE study (PBO, CEE, temperature reduction) provided helpful supporting information that aided in the interpretation of the results. These treatments did not always correspond with each other, however, indicating that further development of these treatments is needed to increase their reliability and performance.

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Table B-1. Summary of sample collection, initial testing dates, holding time of sediment and initial test results for TIE stations.

Location	Collection Date	Testing Date	Holding time (days)	Mean (% survival)	StdDev
Ballona Creek	9/11/2008	9/12/2008	1	3	4.47
Marina del Rey	9/29/2008	10/3/2008	4	20	7.91
Mugu Lagoon	8/21/2008	9/5/2008	15	55	18.71

Table B-2. Sediment TIE treatments

Treatment Details	Treatment Details	Purpose	Expected Result
Coconut carbon	15% by weight	Binding of organic contaminants	Decrease toxicity if organics are present
Cation exchange	20% by weight	Binding of cationic metals	Decrease toxicity if metals are present
Piperonyl butoxide (PBO)	400 µg/L	Inhibits amphipod pesticide metabolism pathway	Renders organophosphorus pesticides nontoxic; enhances/increases toxicity of pyrethroid pesticides
Temperature reduction	10°C	Inhibits amphipod pesticide metabolism pathway	Increased toxicity if pyrethroid pesticides present Decrease if organophosphorus pesticides present
Carboxylesterase enzyme (CEE)	1.0 Units/ml - powderized form	Hydrolyzes pyrethroid pesticides	Decrease toxicity if pyrethroid pesticides are present in the sample
Bovine serum albumin (BSA)	Match concentration to CEE enzyme addition	Control for nonspecific binding of toxicants to carboxylesterase	No change in toxicity
Sediment dilution	20% dilution of sample with amphipod home sediment	Control for sample dilution and mixing of the carbon and SIR treatments	No change or small decrease in toxicity

Table B-3. Pore water TIE treatments

Treatment	Treatment Details	Purpose	Expected Result
C18 column extraction	C18 SPE columns	Removal of non-polar organics	Decrease toxicity if organics are present
EDTA	60 mg/L	Chelation of cationic metals (e.g.,Zn, Cu)	Decrease toxicity if metals are present
Carboxylesterase enzyme (CEE)	1.0 Units/ml - powderized form	Hydrolyzes pyrethroid pesticides	Decrease toxicity if pyrethroid pesticides are present in the sample
Bovine serum albumin (BSA)	Match concentration to CEE enzyme addition	Control for nonspecific binding of toxicants to carboxylesterase	No change in toxicity
Piperonyl butoxide (PBO)	200 µg/L	Inhibits amphipod pesticide metabolism pathway	Renders organophosphorus pesticides nontoxic; enhances/increases toxicity of pyrethroid pesticides
Temperature reduction	10°C	Detect presence of pyrethroids by alteration of pyrethroid potency	Increased toxicity if pyrethroid pesticides present Decrease if organophosphorus pesticides present

Table B-4. Whole sediment TIE test results with *Eohaustorius estuarius*.

Treatment	Ballona Creek		Marina del Rey		Mugu Lagoon	
	Mean (% survival)	Std. Dev.	Mean (% survival)	Std. Dev.	Mean (% survival)	Std. Dev.
Control	82	8.4	92	4.5	82	8.4
Baseline	5	10	73	15	30	14.1
Dilution Control	3	5.8	83	15.3	70	20
Temp. Reduction Blank (10°C)	93	5.8	100	0	93	5.8
Temp. Reduction (10°C)	3	5.8	77	15.3	30	26.5
CEE blank	67	5.8	97	5.8	67	5.8
CEE	10	10	67	20.8	43	25.2
BSA Blank	73	23.1	90	0	73	23.1
BSA	3	5.8	83	20.8	43	15.3
PBO Blank	90	0	97	5.8	90	0
PBO	0	0	83	5.8	7	11.5
Cation exchange blank	83	5.8	97	5.8	83	5.8
Cation exchange	3	5.8	87	5.8	47	5.8
Carbon Blank	67	5.8	93	5.8	67	5.8
Carbon	73	5.8	83	20.8	77	15.3

Table B-5. Pore water test results with *Eohaustorius estuarius*.

Treatment	Ballona Creek		Marina del Rey		Mugu Lagoon	
	Mean (% survival)	Std. Dev.	Mean (% survival)	Std. Dev.	Mean (% survival)	Std. Dev.
Control	96	8.9	92	11	96	8.9
Baseline 100%	40	34.6	93	11.5	0	0
Baseline 50%	33	41.6	100	0	33	11.5
Baseline 25%	73	30.6	NA	NA	80	20
EDTA Blank	100	0	NA	NA	100	0
EDTA	27	11.5	NA	NA	0	0
C18 Blank	100	0	73	32.1	53	30.6
C18	87	11.5	93	11.5	7	11.5
C18-PBO	NA	NA	93	11.5	NA	NA
CEE Blank	87	11.5	NA	NA	87	11.5
CEE	60	20	NA	NA	0	0
BSA Blank	87	11.5	NA	NA	87	11.5
BSA	20	20	NA	NA	0	0
PBO Blank	53	46.2	93	11.5	53	46.2
PBO	0	0	67	30.6	0	0
PBO 50%	NA	NA	100	0	NA	NA
Temp. Reduction Blank	73	30.6	NA	NA	73	30.6
Temp. Reduction	7	11.5	NA	NA	0	0

NA = Not enough sample available

Table B-6. Toxicity units (TU) due to un-ionized ammonia levels in sediment pore water based on *Eohaustorius estuarius* toxicity. TUs are defined as: TU = Un-ionized ammonia pore water concentration/ *E. estuarius* LC_{50}.

Sediment Concentration	Ballona Creek		Marina del Rey		Mugu Lagoon	
	UNH_3 (mg/L)	TU	UNH_3 (mg/L)	TU	UNH_3 (mg/L)	TU
100%	0.44	0.39	0.25	0.22	2.47	2.2
50%	0.46	0.41	0.15	0.13	1.15	1.0
25%	0.23	0.21	NA	NA	0.69	0.62

UNH3= Un-ionized ammonia

NA = Insufficient sample

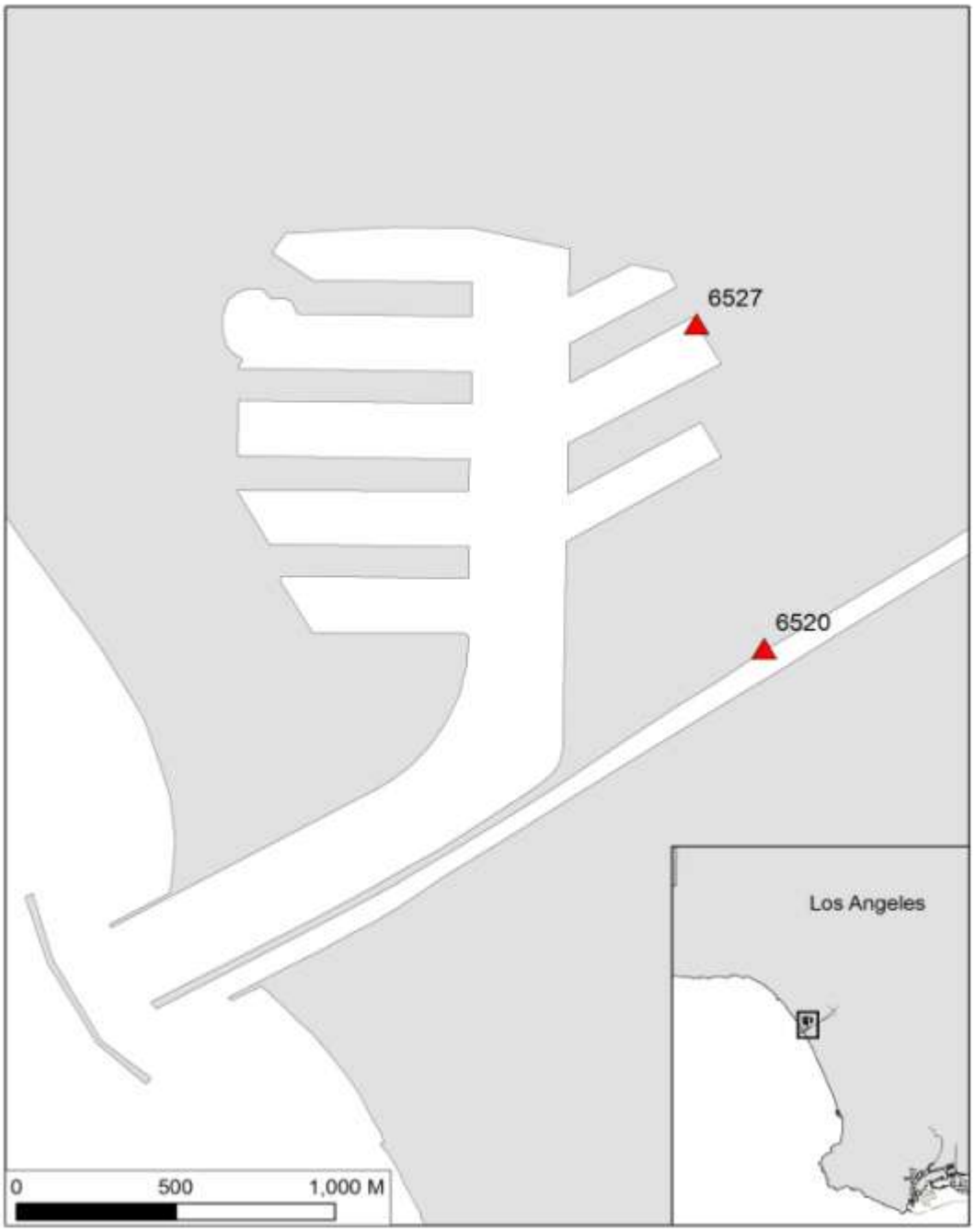

Figure B-1. Stations Ballona Creek (6520) and Marina del Rey (6527) in Los Angeles County.

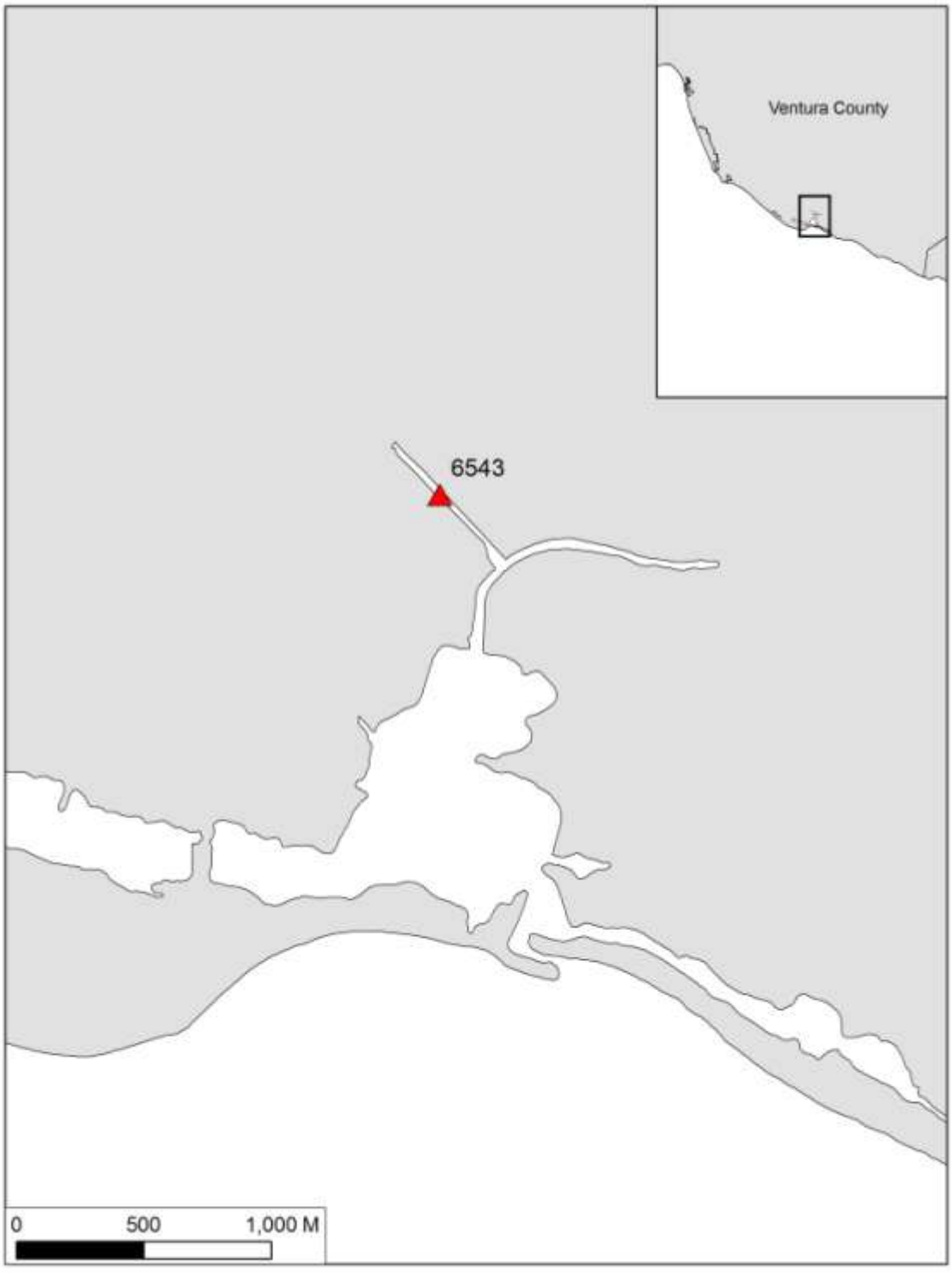

Figure B-2. Station Mugu Lagoon (6543) in Ventura County.

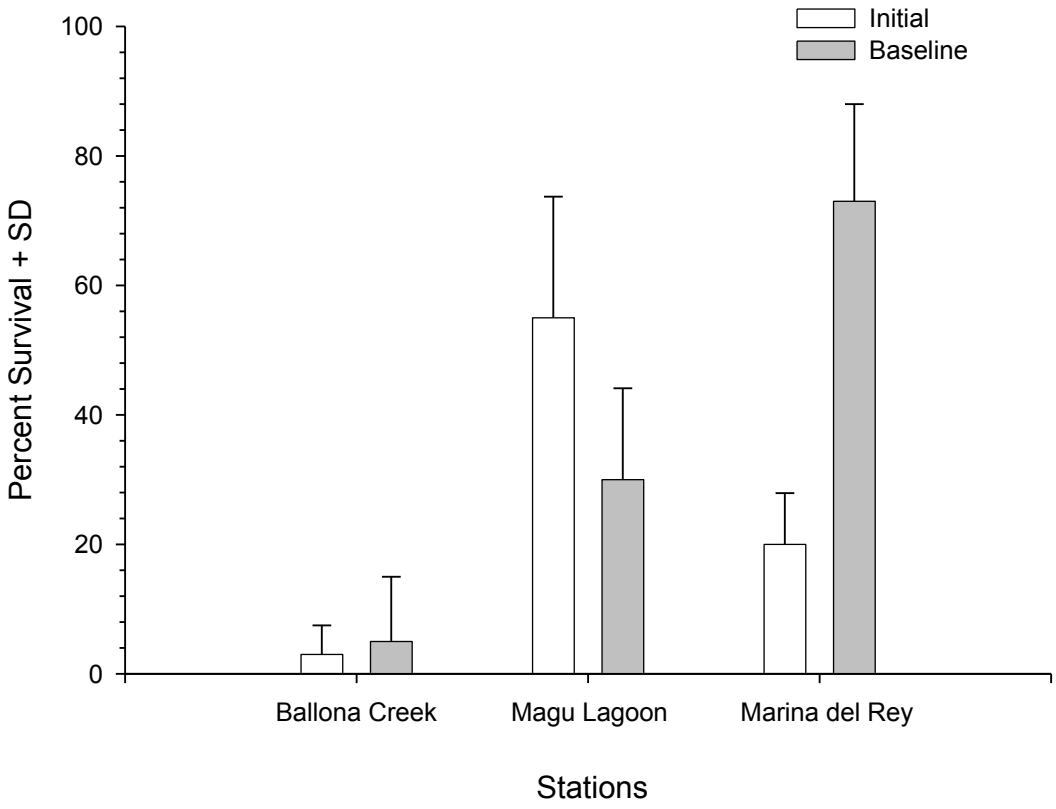

Figure B-3. Results of initial and baseline whole sediment *E. estuarius* toxicity testing on Bight'08 stations.

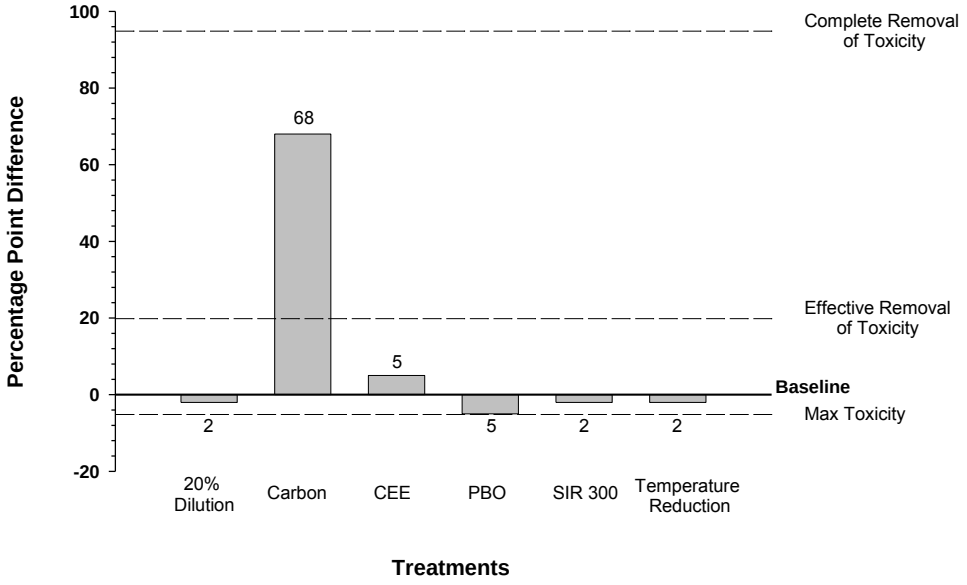

Figure B-4. Ballona Creek whole sediment TIE treatment effectiveness. Values associated with bars indicate the percentage point difference from the baseline.

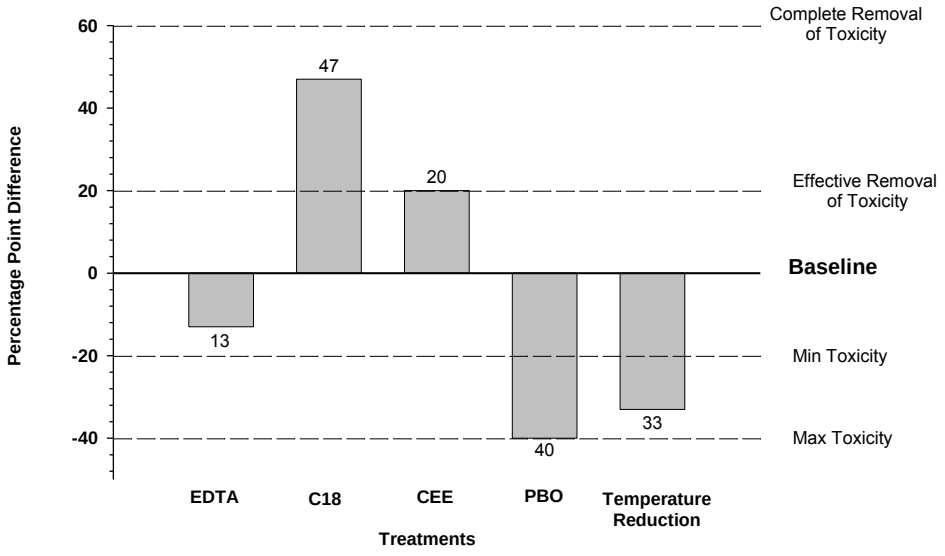

Figure B-5. Ballona Creek pore water TIE treatment effectiveness. Values associated with bars indicate the percentage point difference from the baseline.

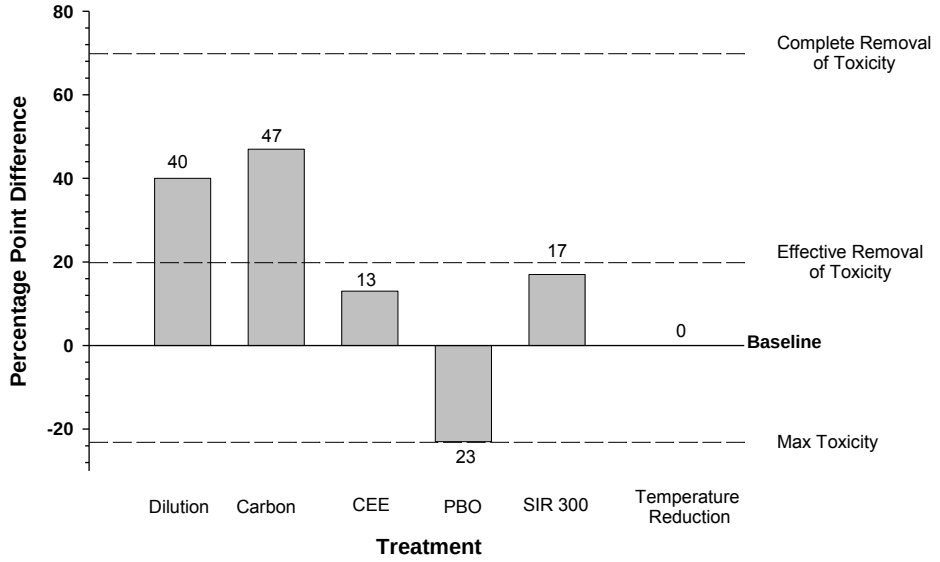

Figure B-6. Mugu Lagoon whole sediment TIE treatment effectiveness. Values associated with bars indicate the percentage point difference from the baseline.

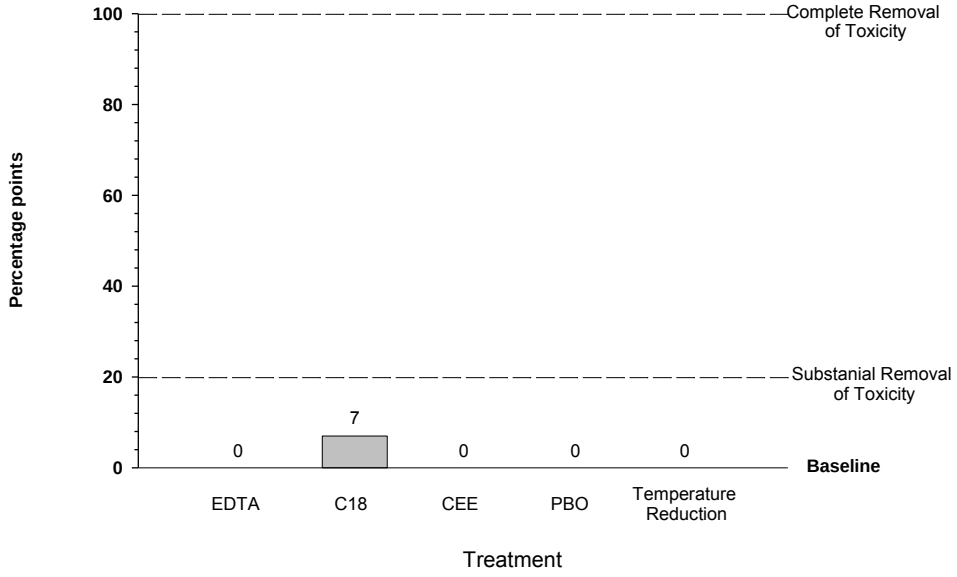

Figure B-7. Mugu Lagoon pore water TIE treatment effectiveness. Values associated with bars indicate the percentage point difference from the baseline.

APPENDIX C. AUDIT FORM

Bight'08 Toxicology Laboratory Audit

Laboratory_____ Date_____ Methods_____

Personnel_____ Inspector_____

Samples stored at 5ºC (See storage area)_____

Sediment sieved 2 mm (Method observed, or screen inspected) _____

Method of press sieving (What pushes sediment through) _____

Test animals are acclimated properly (Records checked)_____

Acceptable test chambers are used_____

Seawater source and filtration (Natural)_____(20 um for Eo)_____
(1 um for SWI)_____

Proper replication and randomization_____

Proper number of animals used(2 males/2 females SWI)_____(20/10 Eo)_____

Proper sediment depth used(2 cm Eo)_____(5 cm SWI)_____

Proper temperature control for exposure (Equipment and records)_____

Proper light cycle used (16:8 Eo sed & SWI)_____(Dark Eo Ref)_____

Proper aeration method used (Rate)_____(Method)_____Placement_____

Pore water collection method (Equipment)_____

Were pore water measurements made at receipt (Sal and NH_3 records)_____

Water quality instruments calibrated (Records, proper equipment)_____

Records (daily checks)_____(Breakdown)_____(Water quality)_____

Breakdown Equipment (Sieve for Eo)_____(Vials for SWI)_____

Other comments_____

_____

APPENDIX D. MAPS OF TOXICITY TESTING RESULTS

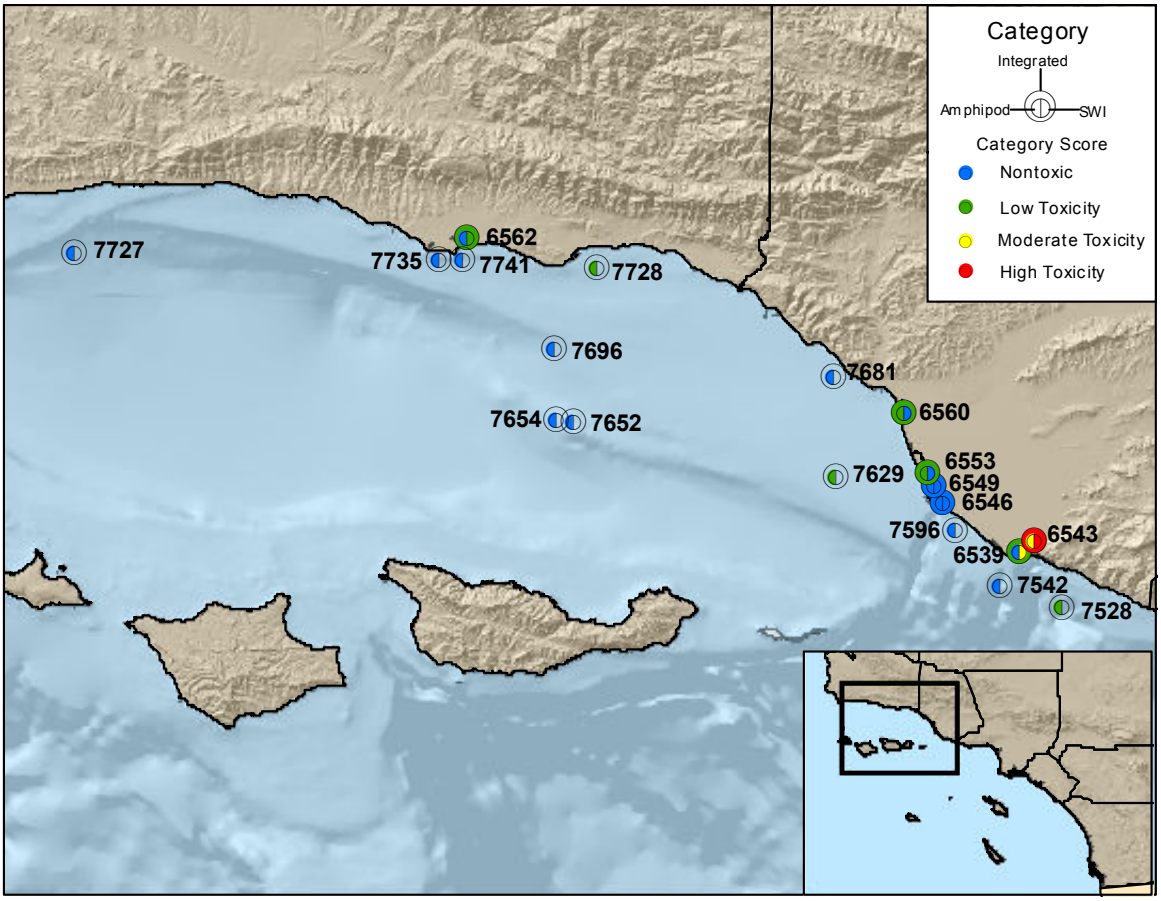

Figure D-1. Map of toxicity testing results Santa Barbara and Ventura Counties

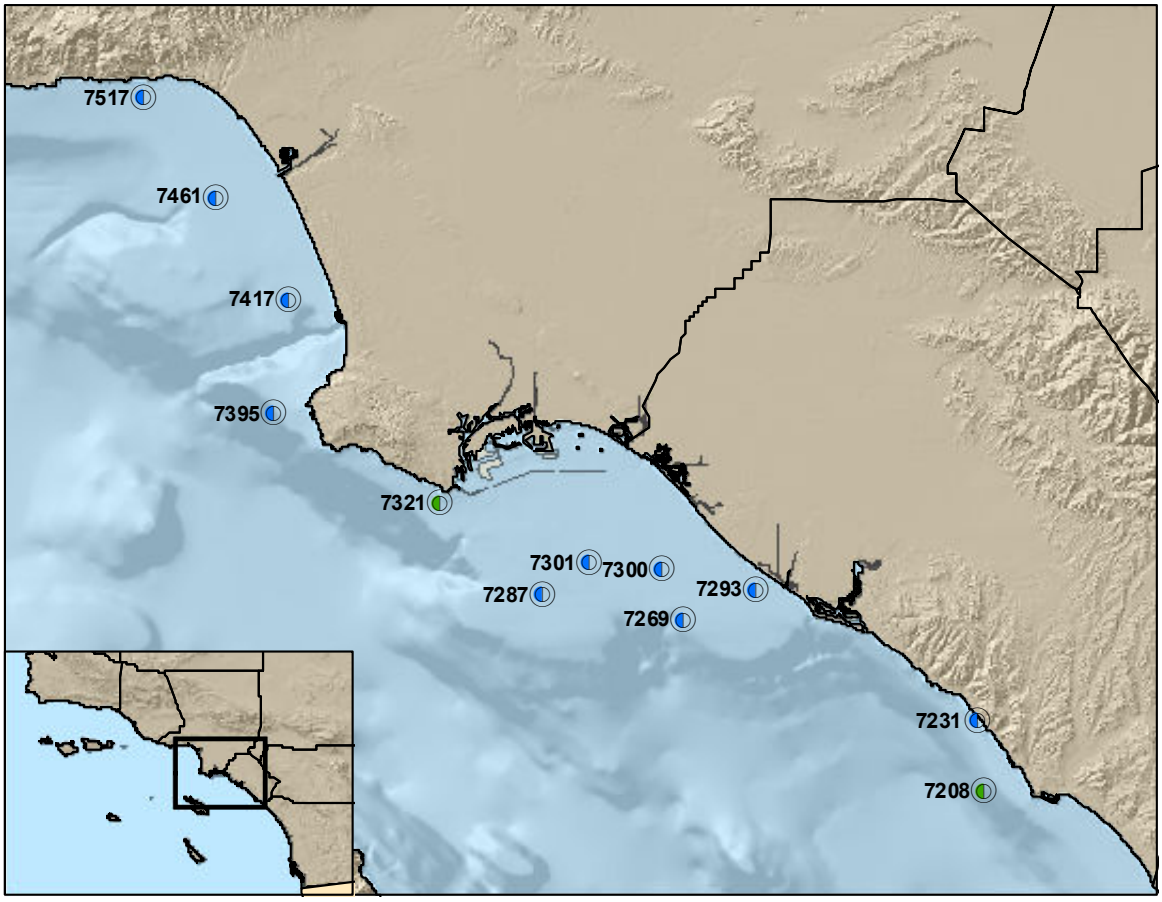

Figure D-2. Map of toxicity testing results for offshore Los Angeles and Orange County stations

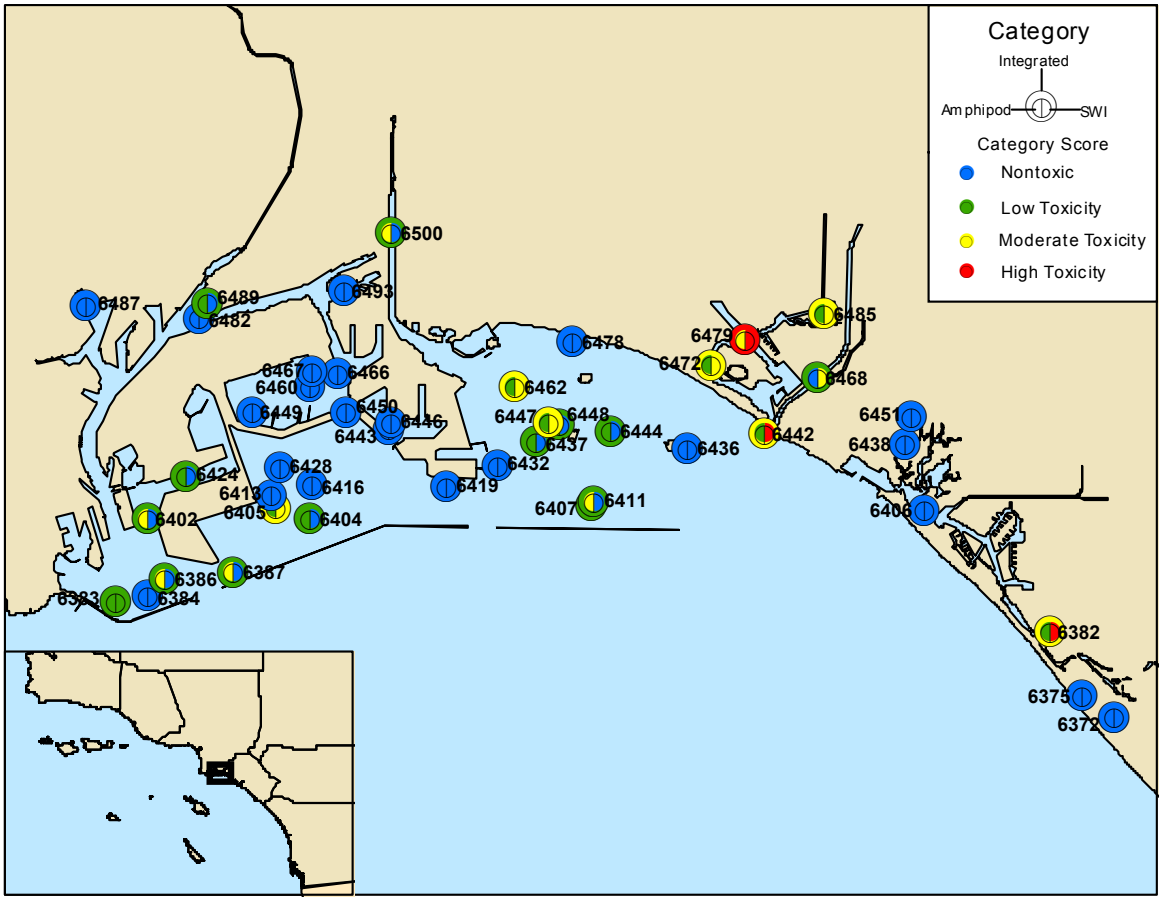

Figure D-3. Map of toxicity testing results for Los Angeles and Long Beach harbors, Alamitos Bay, Anaheim Bay, and Bolsa Chica.

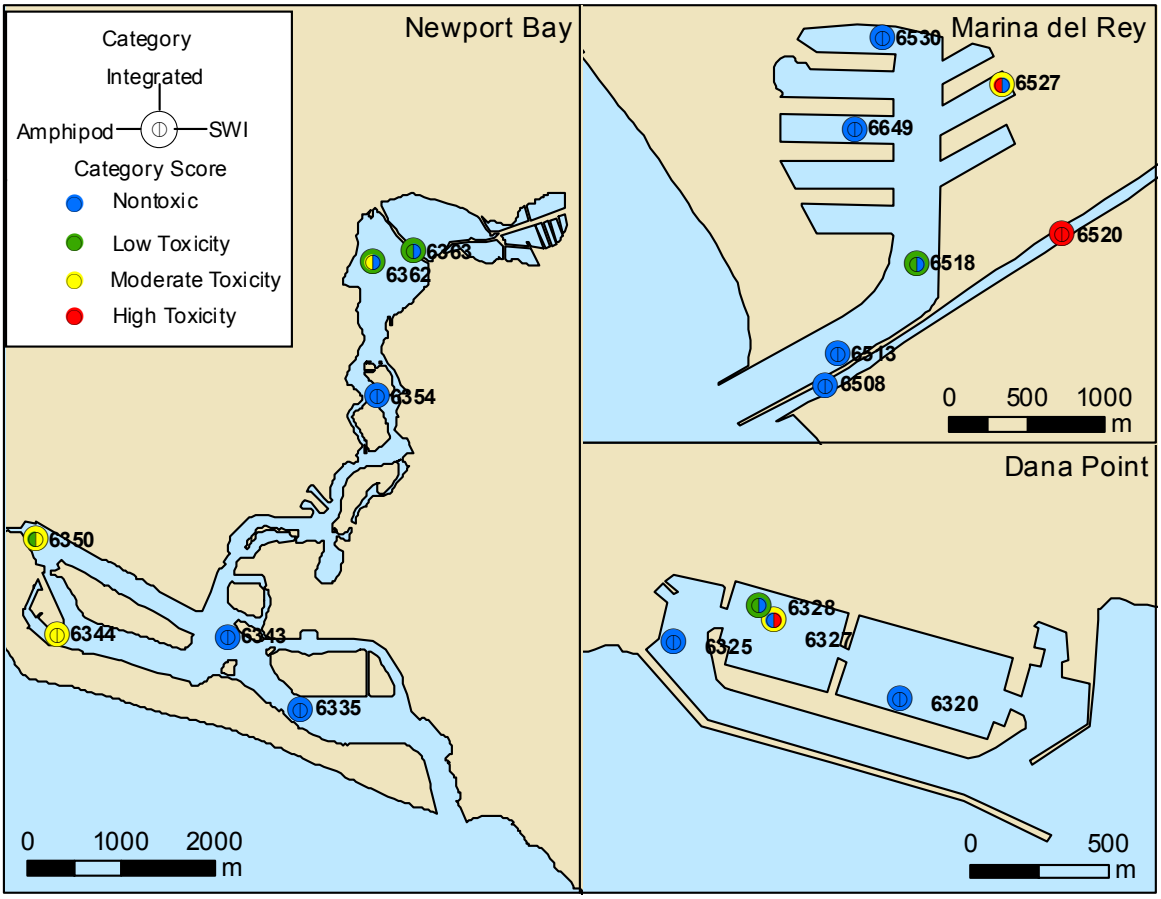

Figure D-4. Maps of toxicity testing results for Newport Bay, Marina del Rey, and Dana Point

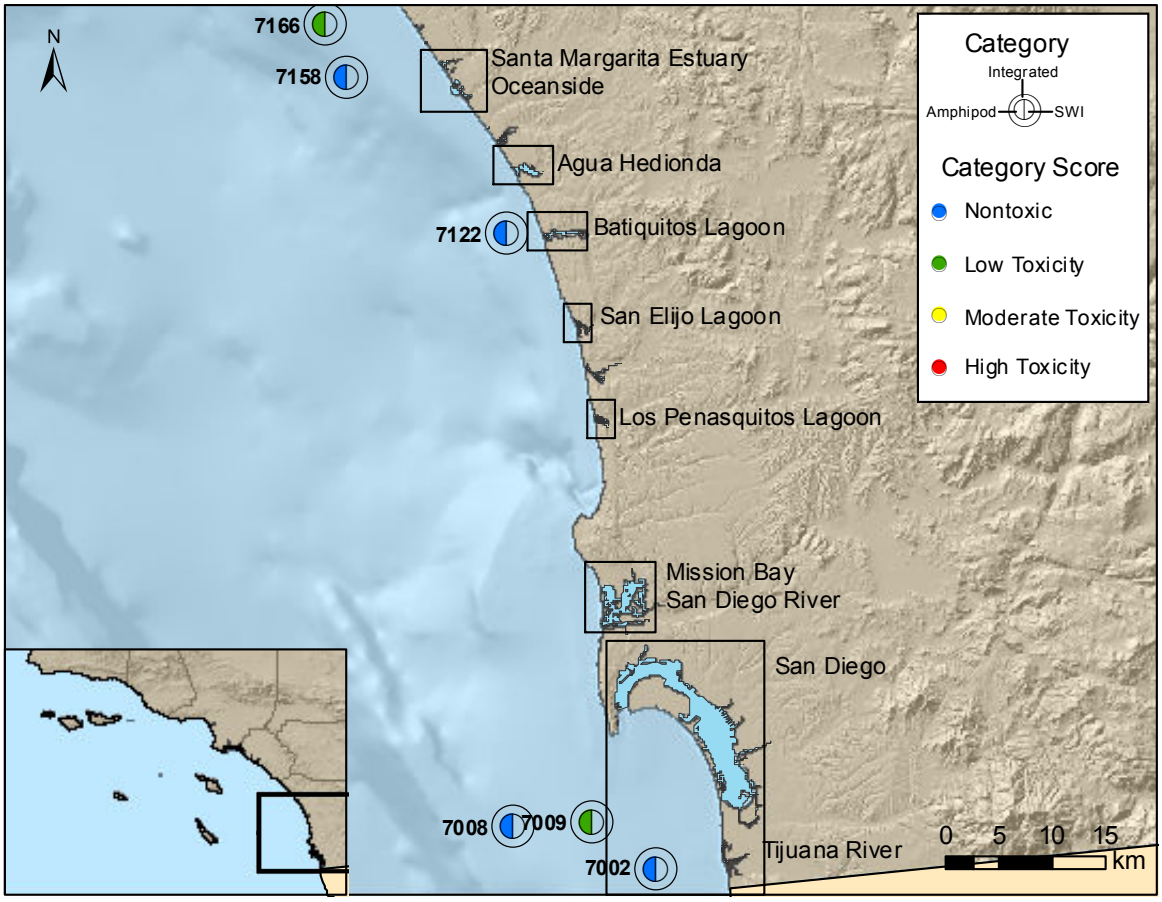

Figure D-5. Map of toxicity testing results for offshore San Diego County

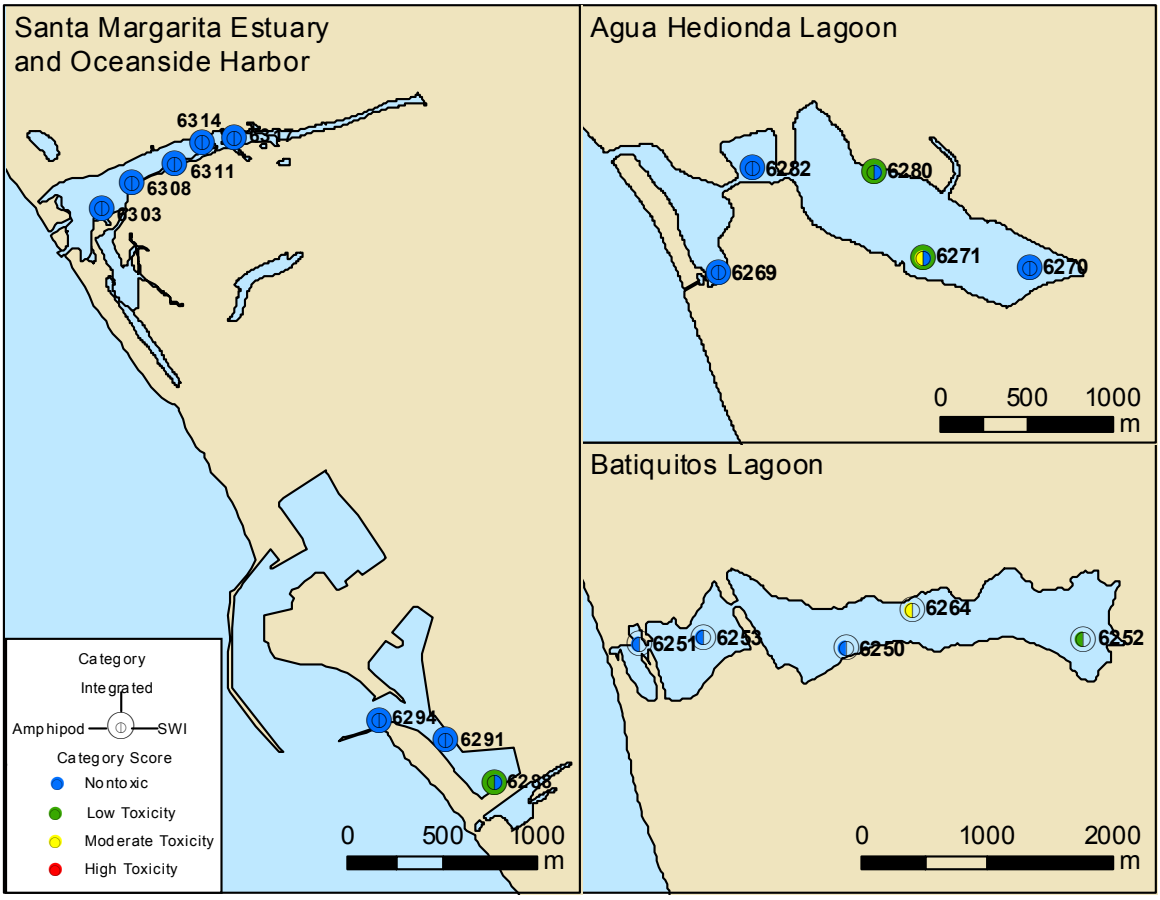

Figure D-6. Maps of toxicity testing results for northern San Diego County embayment stations

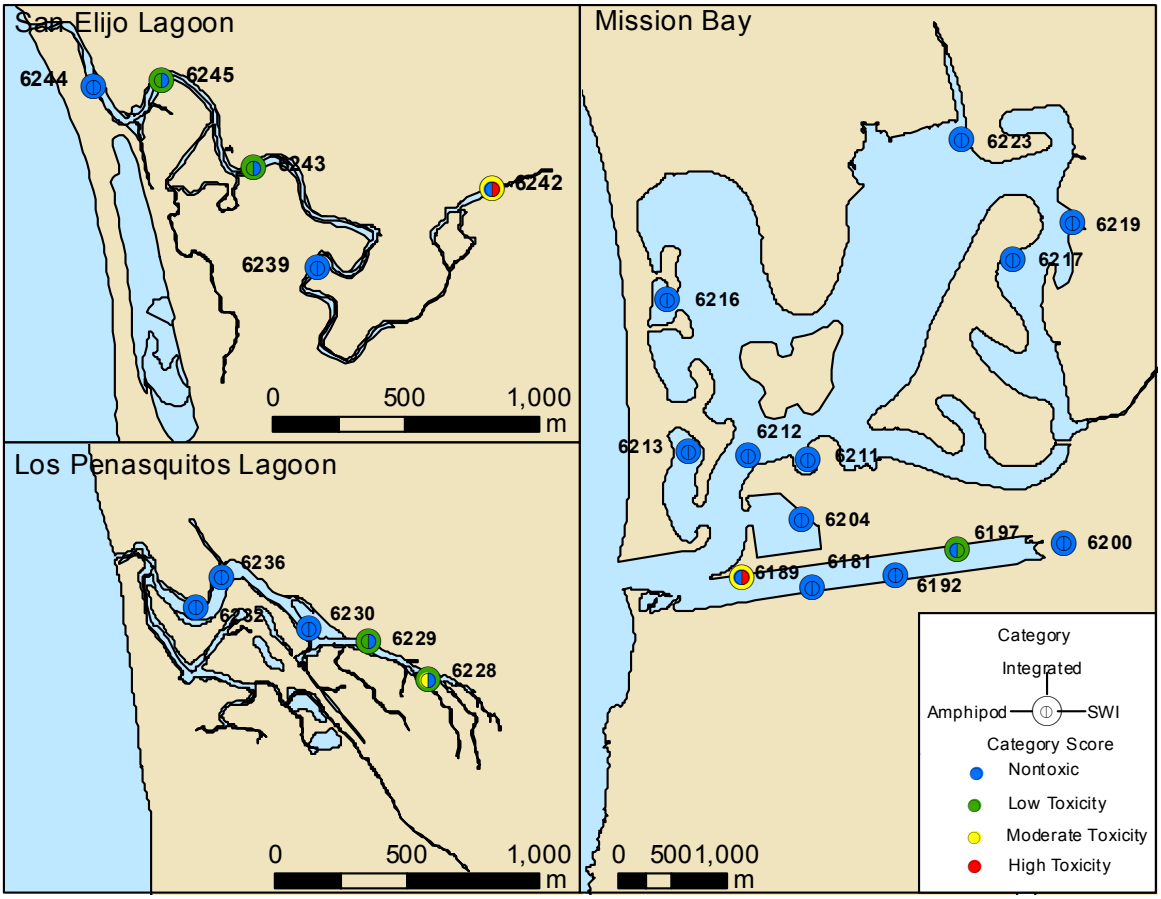

Figure D-7. Map of toxicity testing results for southern San Diego County embayment stations.

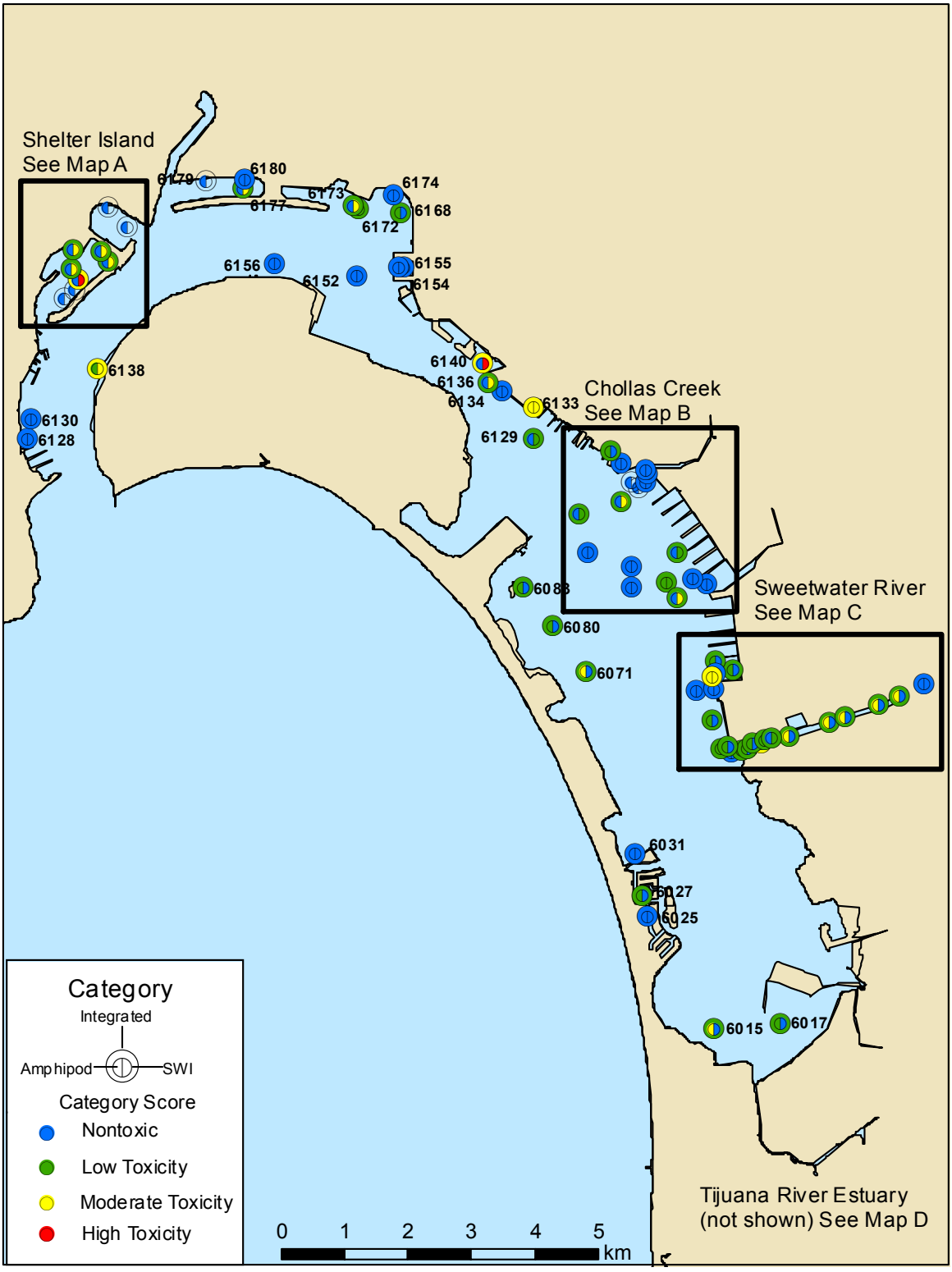

Figure D-8. Map of toxicity testing results for San Diego Bay.

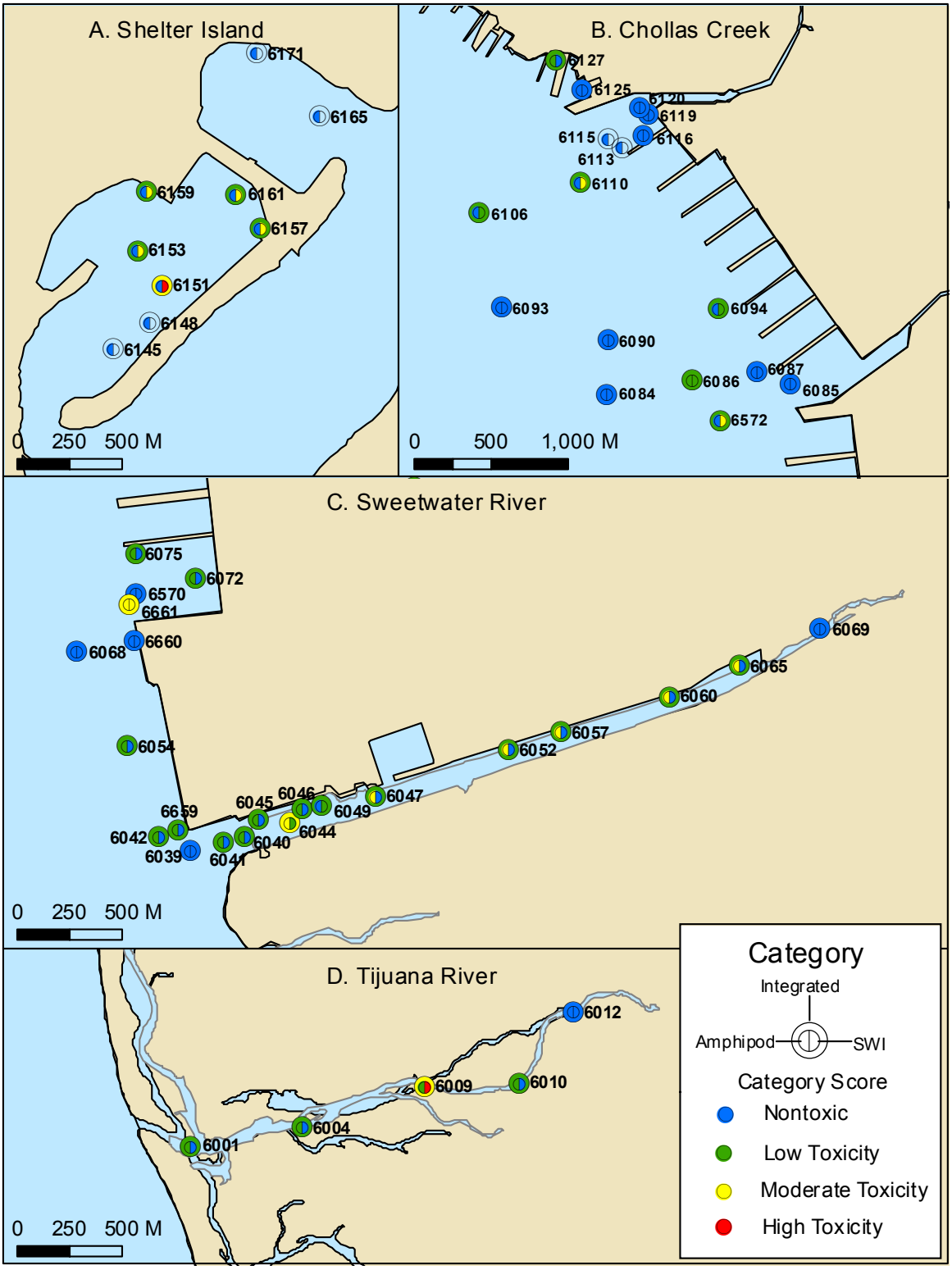

Figure D-9. Inset maps of toxicity testing results for San Diego Bay.

APPENDIX E. TOXICITY RESULTS BY STATION

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (%Control)	Mussel Embryo Develop./Surv. (%Control)	Integrated Toxicity Category[1]	Fines (%)	TOC (%)
6001	32.5566	-116.8718	Estuary	0.9	Tijuana River Estuary	91	90	2	0.0	0.11
6004	32.5574	-117.1224	Estuary	0.8	Tijuana River Estuary	92	100	2	13.7	0.18
6009	32.5592	-117.1160	Estuary	0.6	Tijuana River Estuary	90	0	3	9.5	0.38
6010	32.5594	-117.1112	Estuary	0.6	Tijuana River Estuary	87	95	2	3.0	0.06
6012	32.5624	-117.1084	Estuary	0.8	Tijuana River Estuary	95	95	1	4.0	0.35
6015	32.6076	-117.1224	Bay	1.8	San Diego Bay	81	90	2	76.7	1.82
6017	32.6084	-117.1114	Bay	1.5	San Diego Bay	90	98	2	50.3	0.35
6025	32.6235	-117.1337	Marina	3.7	San Diego Bay	89	90	1	57.4	0.90
6027	32.6265	-117.1347	Marina	3.5	San Diego Bay	91	97	2	63.4	0.99
6031	32.6325	-117.1357	Bay	1.8	San Diego Bay	92	91	1	65.3	1.12
6039	32.6469	-117.1196	Bay	12.6	San Diego Bay	95	90	1	61.4	0.68
6040	32.6472	-117.1178	Bay	11.2	San Diego Bay	91	79	2	57.4	0.79
6041	32.6475	-117.1169	Bay	10.8	San Diego Bay	90	90	2	55.8	0.80
6042	32.6475	-117.1213	Port	12.2	San Diego Bay	83	93	2	65.0	0.54
6044	32.6482	-117.1145	Estuary	12.1	San Diego Bay	81	86	3	67.8	1.09
6045	32.6483	-117.1161	Estuary	11.0	San Diego Bay	90	99	2	67.1	1.09
6046	32.6488	-117.1139	Estuary	11.8	San Diego Bay	88	85	2	56.8	0.72
6047	32.6493	-117.1100	Estuary	4.6	San Diego Bay	80	89	2	75.3	1.05
6049	32.6489	-117.1128	Estuary	10.8	San Diego Bay	95	85	2	67.7	1.05
6052	32.6511	-117.1031	Estuary	2.3	Sweetwater River	61	113	2	71.5	1.29
6054	32.6514	-117.1229	Port	11.8	San Diego Bay	86	94	2	72.2	0.89
6057	32.6521	-117.1005	Estuary	1.7	Sweetwater River	80	105	2	78.4	1.77
6060	32.6537	-117.0949	Estuary	1.0	Sweetwater River	72	116	2	68.8	1.15
6065	32.6550	-117.0913	Estuary	1.2	Sweetwater River	71	111	2	60.7	1.12

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (%Control)	Mussel Embryo Develop./Surv. (%Control)	Integrated Toxicity Category[1]	Fines (%)	TOC (%)
6068	32.6556	-117.1254	Port	9.7	San Diego Bay	91	78	1	75.5	1.12
6069	32.6566	-117.0871	Estuary	1.5	Sweetwater River	92	110	1	2.9	0.24
6071	32.6583	-117.1442	Bay	5.1	San Diego Bay	73	99	2	78.7	0.99
6072	32.6589	-117.1193	Port	5.6	San Diego Bay	89	102	2	61.7	0.71
6075	32.6599	-117.1224	Port	6.6	San Diego Bay	92	84	2	48.6	1.16
6080	32.6649	-117.1498	Bay	4.6	San Diego Bay	88	99	2	53.2	0.55
6083	32.6703	-117.1548	Bay	4.4	San Diego Bay	85	91	2	81.7	0.80
6084	32.6704	-117.1365	Bay	4.8	San Diego Bay	94	99	1	31.5	0.22
6085	32.6710	-117.1238	Port	12.2	San Diego Bay	98	96	1	84.5	1.14
6086	32.6712	-117.1306	Port	8.0	San Diego Bay	86	87	2	83.4	1.14
6087	32.6716	-117.1262	Port	12.6	San Diego Bay	96	93	1	67.9	0.84
6090	32.6735	-117.1364	Bay	4.5	San Diego Bay	100	93	1	44.7	0.51
6093	32.6754	-117.1438	Bay	4.6	San Diego Bay	95	100	1	39.5	0.54
6094	32.6754	-117.1288	Port	11.4	San Diego Bay	96	81	2	81.6	1.19
6106	32.6810	-117.1453	Bay	4.5	San Diego Bay	101	86	2	28.0	0.21
6110	32.6827	-117.1384	Port	12.0	San Diego Bay	96	65	2	60.5	0.61
6113	32.6847	-117.1354	Port	10.5	San Diego Bay	100	D	NA	73.8	1.21
6115	32.6853	-117.1365	Port	10.0	San Diego Bay	95	D	NA	24.4	0.58
6116	32.6856	-117.1340	Port	10.8	San Diego Bay	94	102	1	80.6	2.03
6119	32.6867	-117.1337	Port	10.0	San Diego Bay	99	96	1	57.6	1.35
6120	32.6870	-117.1341	Port	8.2	San Diego Bay	95	94	1	73.0	2.24
6125	32.6881	-117.1382	Port	7.9	San Diego Bay	90	94	1	91.6	1.86
6127	32.6900	-117.1400	Port	12.6	San Diego Bay	91	92	2	91.3	2.33
6128	32.6914	-117.2382	Port	14.4	San Diego Bay	101	106	1	80.4	1.92
6129	32.6916	-117.1529	Port	12.6	San Diego Bay	98	78	2	18.0	0.13
6130	32.6942	-117.2378	Port	12.9	San Diego Bay	94	96	1	65.8	1.24
6133	32.6962	-117.1530	Port	12.5	San Diego Bay	78	60	3	84.0	1.70

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (%Control)	Mussel Embryo Develop./Surv. (%Control)	Integrated Toxicity Category[1]	Fines (%)	TOC (%)
6134	32.6983	-117.1584	Bay	13.7	San Diego Bay	95	84	1	40.4	0.51
6136	32.6996	-117.1609	Bay	14.1	San Diego Bay	102	50	2	40.9	0.56
6138	32.7015	-117.2266	Bay	10.3	San Diego Bay	86	63	3	50.5	1.23
6140	32.7022	-117.1617	Port	9.4	San Diego Bay	93	41	3	80.7	1.33
6145	32.7115	-117.2322	Marina	7.0	San Diego Bay	102	D	NA	88.8	1.82
6148	32.7127	-117.2303	Marina	4.8	San Diego Bay	101	D	NA	28.8	0.32
6151	32.7143	-117.2297	Marina	4.7	San Diego Bay	101	18	3	51.4	0.60
6152	32.7149	-117.1829	Bay	12.3	San Diego Bay	95	80	1	52.3	0.43
6153	32.7158	-117.2309	Marina	5.8	San Diego Bay	101	49	2	83.3	1.11
6154	32.7160	-117.1748	Port	9.9	San Diego Bay	102	89	1	64.6	1.32
6155	32.7159	-117.1759	Port	10.9	San Diego Bay	98	83	1	51.6	0.47
6156	32.7164	-117.1966	Port	11.8	San Diego Bay	93	107	1	44.8	0.70
6157	32.7168	-117.2248	Marina	3.6	San Diego Bay	100	57	2	90.5	1.59
6159	32.7184	-117.2306	Marina	3.3	San Diego Bay	104	57	2	69.2	0.97
6161	32.7183	-117.2259	Marina	5.0	San Diego Bay	101	53	2	90.6	1.42
6165	32.7217	-117.2216	Marina	4.6	San Diego Bay	102	D	NA	33.8	0.38
6168	32.7239	-117.1756	Marina	6.5	San Diego Bay	84	93	2	79.4	1.13
6171	32.7244	-117.2249	Marina	4.7	San Diego Bay	97	D	NA	76.5	1.43
6172	32.7242	-117.1827	Bay	4.7	San Diego Bay	92	52	2	44.7	1.38
6173	32.7249	-117.1835	Marina	4.7	San Diego Bay	100	58	2		0.27
6174	32.7263	-117.1767	Marina	5.7	San Diego Bay	96	100	1	61.3	0.92
6177	32.7271	-117.2022	Marina	3.8	San Diego Bay	96	49	2	74.5	1.23
6179	32.7281	-117.2084	Marina	2.5	San Diego Bay	104	D	NA	51.9	0.49
6180	32.7284	-117.2019	Marina	2.5	San Diego Bay	103	84	1	53.9	0.58
6181	32.7568	-117.2350	Estuary	1.1	San Diego River	89	96	1	67.7	2.49
6189	32.7577	-117.2420	Estuary	0.3	San Diego River	103	6	3	19.0	0.08

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (%Control)	Mussel Embryo Develop./Surv. (%Control)	Integrated Toxicity Category[1]	Fines (%)	TOC (%)
6192	32.7579	-117.2269	Estuary	0.5	San Diego River	100	101	1	60.7	1.30
6197	32.7601	-117.2207	Estuary	0.5	San Diego River	97	88	2	33.9	0.23
6200	32.7605	-117.2101	Estuary	1.7	San Diego River	98	107	1	56.4	0.45
6204	32.7625	-117.2362	Marina	6.5	Mission Bay	102	101	1	66.7	1.27
6211	32.7675	-117.2354	Marina	2.6	Mission Bay	100	105	1	65.9	1.79
6212	32.7678	-117.2413	Bay	6.2	Mission Bay	101	92	1	8.4	0.08
6213	32.7683	-117.2472	Marina	5.3	Mission Bay	102	94	1	52.2	1.00
6216	32.7808	-117.2493	Marina	3.8	Mission Bay	96	101	1	61.8	1.00
6217	32.7844	-117.2153	Bay	3.6	Mission Bay	101	103	1	89.6	2.62
6219	32.7874	-117.2092	Bay	2.9	Mission Bay	99	108	1	79.1	2.24
6223	32.7943	-117.2204	Bay	1.5	Mission Bay	95	105	1	66.9	3.33
6228	32.9304	-117.2486	Estuary	0.5	Los Penasquitos	71	119	2	74.9	0.62
6229	32.9316	-117.2510	Estuary	1.5	Los Penasquitos	93	109	2	81.1	1.18
6230	32.9321	-117.2534	Estuary	0.4	Los Penasquitos	100	122	1	79.1	1.51
6232	32.9328	-117.2579	Estuary	0.6	Los Penasquitos	95	120	1	3.3	0.04
6236	32.9338	-117.2568	Estuary	1.4	Los Penasquitos	97	123	1	46.1	1.13
6239	33.0080	-117.2706	Estuary	0.4	San Elijo Lagoon	94	106	1	18.0	0.26
6242	33.0106	-117.2636	Estuary	2.4	San Elijo Lagoon	99	4	3	80.7	2.86
6243	33.0113	-117.2732	Estuary	0.7	San Elijo Lagoon	82	109	2	24.0	0.58
6244	33.0141	-117.2797	Estuary	1.0	San Elijo Lagoon	95	99	1	1.0	0.14
6245	33.0143	-117.2769	Estuary	2.3	San Elijo Lagoon	85	101	2	7.1	0.14
6250	33.0878	-117.2929	Estuary	0.6	Batiquitos Lagoon	95	D	NA	70.5	0.32
6251	33.0881	-117.3105	Estuary	1.7	Batiquitos Lagoon	100	D	NA	0.1	0.03
6252	33.0885	-117.2726	Estuary	1.5	Batiquitos Lagoon	90	D	NA	95.5	1.69
6253	33.0885	-117.3050	Estuary	1.6	Batiquitos Lagoon	97	D	NA	29.2	0.43
6264	33.0906	-117.2872	Estuary	0.5	Batiquitos Lagoon	63	D	NA	92.8	1.11

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (%Control)	Mussel Embryo Develop./Surv. (%Control)	Integrated Toxicity Category[1]	Fines (%)	TOC (%)
6269	33.1392	-117.3377	Estuary	6.7	Agua Hedionda Lagoon	90	98	1	48.5	0.52
6270	33.1396	-117.3186	Estuary	0.6	Agua Hedionda Lagoon	100	98	1	38.6	0.64
6271	33.1402	-117.3251	Estuary	2.4	Agua Hedionda Lagoon	66	105	2	85.6	1.01
6280	33.1446	-117.3281	Estuary	2.7	Agua Hedionda Lagoon	88	108	2	49.3	0.57
6282	33.1447	-117.3356	Estuary	2.4	Agua Hedionda Lagoon	95	99	1	20.7	0.61
6288	33.2049	-117.3907	Marina	4.5	Oceanside Harbor	88	93	2	78.5	1.45
6291	33.2069	-117.3936	Marina	6.0	Oceanside Harbor	102	91	1	78.0	1.50
6294	33.2078	-117.3973	Marina	7.9	Oceanside Harbor	100	98	1	55.5	0.61
6303	33.2323	-117.4132	Estuary	0.6	Santa Margarita	105	82	1	0.0	0.01
6308	33.2334	-117.4115	Estuary	0.8	Santa Margarita	105	89	1	44.0	1.06
6311	33.2344	-117.4090	Estuary	0.9	Santa Margarita	103	88	1	48.9	1.26
6314	33.2354	-117.4075	Estuary	1.1	Santa Margarita	101	83	1	5.9	0.04
6317	33.2356	-117.4057	Estuary	0.9	Santa Margarita	99	85	1	3.4	0.02
6320	33.4588	-117.6972	Marina	3.0	Dana Point Harbor	95	127	1	97.7	1.95
6325	33.4606	-117.7059	Marina	5.6	Dana Point Harbor	98	107	1	63.5	0.67
6327	33.4613	-117.7021	Marina	3.7	Dana Point Harbor	95	3	3	74.7	1.45
6328	33.4619	-117.7027	Marina	3.1	Dana Point Harbor	92	116	2	77.8	1.41
6335	33.6030	-117.8965	Marina	3.9	Newport Bay	97	90	1	74.0	0.42
6343	33.6099	-117.9051	Marina	3.8	Newport Bay	98	95	1	80.2	1.43
6344	33.6100	-117.9244	Marina	3.1	Newport Bay	69	74	3	99.8	1.94
6350	33.6191	-117.9270	Marina	5.8	Newport Bay	83	70	3	99.2	2.21
6354	33.6329	-117.8881	Estuary	3.0	Upper Newport Bay	88	107	1	59.6	0.58
6355	33.6366	-117.9538	Estuary	1.2	Santa Ana River	99	109	1	74.0	0.96
6362	33.6457	-117.8888	Estuary	3.9	Upper Newport Bay	72	102	2	96.2	1.47
6363	33.6468	-117.8841	Estuary	3.0	Upper Newport Bay	83	112	2	82.1	1.10
6372	33.6872	-118.0339	Estuary	1.1	Bolsa Chica	95	92	1	9.9	0.22

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (%Control)	Mussel Embryo Develop./Surv. (%Control)	Integrated Toxicity Category[1]	Fines (%)	TOC (%)
6375	33.6913	-118.0415	Estuary	0.8	Bolsa Chica	87	95	1	68.8	1.50
6382	33.7037	-118.0496	Estuary	1.2	Bolsa Chica	91	8	3	81.3	5.27
6383	33.7073	-118.2690	Bay	4.0	Los Angeles/Long	84	74	2	35.2	0.09
6384	33.7090	-118.2618	Bay	5.0	Los Angeles/Long	94	103	1	70.1	0.44
6386	33.7122	-118.2583	Bay	16.0	Los Angeles/Long	76	97	2	82.4	1.29
6387	33.7135	-118.2418	Bay	23.0	Los Angeles/Long	78	92	2	82.0	1.30
6402	33.7241	-118.2623	Port	26.0	Los Angeles/Long	59	95	2	90.4	1.34
6404	33.7245	-118.2240	Bay	17.0	Los Angeles/Long	86	91	2	85.0	0.96
6405	33.7266	-118.2326	Port	13.0	Los Angeles/Long	86	74	3	76.0	1.13
6406	33.7276	-118.0792	Marina	4.0	Huntington Harbor	99	96	1	18.0	0.33
6407	33.7275	-118.1579	Bay	15.0	Los Angeles/Long	87	83	2	91.2	1.69
6411	33.7284	-118.1572	Bay	17.0	Los Angeles/Long	77	83	2	86.3	1.56
6413	33.7291	-118.2341	Port	12.0	Los Angeles/Long	95	96	1	72.3	0.89
6416	33.7313	-118.2237	Bay	12.0	Los Angeles/Long	96	97	1	56.3	0.26
6419	33.7310	-118.1918	Port	15.0	Los Angeles/Long	96	94	1	57.7	0.78
6424	33.7327	-118.2532	Port	17.0	Los Angeles/Long	85	102	2	68.9	0.92
6428	33.7345	-118.2316	Port	11.0	Los Angeles/Long	104	110	1	53.1	0.53
6432	33.7358	-118.1800	Bay	17.0	Los Angeles/Long	92	81	1	61.8	0.45
6436	33.7394	-118.1346	Bay	8.0	Los Angeles/Long	94	90	1	71.0	0.64
6437	33.7397	-118.1718	Bay	14.0	Los Angeles/Long	88	83	2	90.5	1.52
6438	33.7402	-118.0843	Estuary	1.8	Seal Beach	95	97	1	59.8	0.87
6442	33.7420	-118.1174	Estuary	4.0	San Gabriel River	87	6	3	24.0	1.00
6443	33.7417	-118.2054	Port	20.0	Los Angeles/Long	94	105	1	69.6	0.33
6444	33.7421	-118.1530	Bay	11.0	Los Angeles/Long	85	88	2	91.7	1.34
6446	33.7431	-118.2048	Port	18.0	Los Angeles/Long	95	107	1	84.8	1.08
6447	33.7433	-118.1650	Bay	12.0	Los Angeles/Long	91	89	2	95.0	1.76

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (%Control)	Mussel Embryo Develop./Surv. (%Control)	Integrated Toxicity Category[1]	Fines (%)	TOC (%)
6448	33.7445	-118.1691	Bay	13.0	Los Angeles/Long	83	71	3	89.3	1.18
6449	33.7450	-118.2384	Port	10.0	Los Angeles/Long	95	108	1	74.4	0.72
6450	33.7457	-118.2156	Port	18.0	Los Angeles/Long	94	98	1	58.2	0.36
6451	33.7458	-118.0826	Estuary	0.4	Seal Beach	93	101	1	87.8	2.56
6460	33.7501	-118.2249	Port	16.0	Los Angeles/Long	96	99	1	34.0	0.32
6462	33.7510	-118.1761	Bay	11.0	Los Angeles/Long	88	76	3	72.4	1.75
6466	33.7526	-118.2179	Port	23.0	Los Angeles/Long	92	111	1	81.1	1.09
6467	33.7531	-118.2237	Port	15.0	Los Angeles/Long	91	92	1	82.7	1.28
6468	33.7530	-118.1051	Estuary	3.4	San Gabriel River	97	72	2	42.8	0.65
6472	33.7554	-118.1299	Marina	5.0	Alamitos Bay	87	54	3	93.4	2.02
6478	33.7596	-118.1624	Bay	6.0	Los Angeles/Long	96	91	1	57.7	0.34
6479	33.7605	-118.1217	Marina	4.0	Alamitos Bay	81	27	4	88.5	2.06
6482	33.7633	-118.2510	Marina	14.0	Los Angeles/Long	92	100	1	86.7	1.42
6485	33.7658	-118.1036	Estuary	3.5	Los Alamitos Estuary	83	44	3	59.6	2.04
6487	33.7660	-118.2775	Port	17.0	Los Angeles/Long	95	99	1	82.0	1.09
6489	33.7667	-118.2485	Marina	4.0	Los Angeles/Long	82	103	2	88.8	3.71
6493	33.7692	-118.2171	Port	15.0	Los Angeles/Long	92	99	1	79.0	1.12
6500	33.7806	-118.2058	Estuary	2.8	Los Angeles River	72	86	2	41.8	1.38
6508	33.9628	-118.4542	Estuary	3.3	Ballona Creek	96	93	1	48.3	5.07
6513	33.9647	-118.4533	Marina	7.0	Marina del Rey	97	99	1	67.6	3.07
6518	33.9702	-118.4480	Marina	6.8	Marina del Rey	89	99	2	82.8	2.16
6520	33.9713	-118.4396	Estuary	2.3	Ballona Creek	3	0	4	58.3	0.09
6527	33.9805	-118.4422	Marina	4.4	Marina del Rey	21	95	3	99.5	1.55
6530	33.9831	-118.4507	Marina	3.8	Marina del Rey	96	101	1	99.2	1.42
6539	34.1040	-119.1124	Estuary	0.5	Mugu Lagoon-south	96	64	2	91.1	1.46
6543	34.1142	-119.0937	Estuary	1.2	Mugu Lagoon-south	59	0	4	64.2	5.23

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (%Control)	Mussel Embryo Develop./Surv. (%Control)	Integrated Toxicity Category[1]	Fines (%)	TOC (%)
6546	34.1529	-119.2098	Port	8.4	Port Hueneme	90	90	1	75.4	1.35
6549	34.1712	-119.2235	Marina	4.2	Oxnard/Channel I.	98	88	1	90.7	2.47
6553	34.1846	-119.2309	Estuary	3.8	Channel Islands Harbor	94	82	2	92.4	2.16
6560	34.2487	-119.2641	Marina	4.2	Ventura Harbor	91	89	2	98.7	1.00
6562	34.4218	-119.8294	Estuary	1.8	Goleta Slough	99	79	2	61.9	2.08
6570	32.6581	-117.1224	Port	11.6	San Diego Bay	93	94	1	82.9	1.39
6572	32.6689	-117.1287	Port	8.0	San Diego Bay	93	69	2	88.1	1.43
6649	33.9777	-118.4527	Marina	3.0	Marina del Rey	94	116	1	85.9	1.21
6659	32.6478	-117.1203	Port	6.5	San Diego Bay	88	89	2	46.2	0.79
6660	32.6561	-117.1225	Port	13.3	San Diego Bay	93	93	1	64.5	0.74
6661	32.6576	-117.1228	Port	12.0	San Diego Bay	69	69	3	72.2	0.89
7002	32.5510	-117.1991	Shelf	35.0	South San Diego Shelf	90	NS	NA	29.4	0.28
7008	32.5863	-117.3412	Shelf	181.0	South San Diego Shelf	95	NS	NA	64.4	2.21
7009	32.5891	-117.2634	Shelf	57.0	South San Diego Shelf	90	NS	NA	44.4	0.71
7122	33.0881	-117.3509	Shelf	73.2	North San Diego Shelf	93	NS	NA	63.2	0.65
7158	33.2207	-117.5121	Shelf	192.9	North San Diego Shelf	95	NS	NA	70.5	1.49
7166	33.2650	-117.5336	Shelf	63.0	North San Diego Shelf	94	NS	NA	68.1	0.88
7208	33.4642	-117.7622	Shelf	161.0	Orange Shelf	89	NS	NA	67.2	0.95
7231	33.5213	-117.7696	Shelf	14.6	Orange Shelf	98	NS	NA	7.9	0.28
7269	33.6024	-118.0566	Shelf	38.0	San Pedro Shelf	96	NS	NA	24.6	0.21
7287	33.6210	-118.1951	Shelf	42.0	San Pedro Shelf	98	NS	NA	17.3	0.41
7293	33.6271	-117.9871	Shelf	13.0	San Pedro Shelf	102	NS	NA	23.9	0.34
7300	33.6431	-118.0781	Shelf	27.0	San Pedro Shelf	99	NS	NA	27.3	0.24
7301	33.6480	-118.1493	Shelf	31.0	San Pedro Shelf	100	NS	NA	20.5	0.50
7321	33.6954	-118.2960	Shelf	28.0	Palos Verdes Shelf	91	NS	NA	31.0	4.25

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (%Control)	Mussel Embryo Develop./Surv. (%Control)	Integrated Toxicity Category[1]	Fines (%)	TOC (%)
7395	33.7667	-118.4604	Shelf	133.0	Santa Monica Bay	93	NS	NA	59.1	3.03
7417	33.8610	-118.4475	Shelf	59.0	Santa Monica Bay	103	NS	NA	32.1	0.76
7461	33.9436	-118.5195	Shelf	49.0	Santa Monica Bay	98	NS	NA	58.7	0.65
7517	34.0237	-118.5931	Shelf	24.0	Santa Monica Bay	97	NS	NA	69.6	0.82
7528	34.0443	-119.0552	Shelf	203.0	Hueneme to Dume	89	NS	NA	79.6	1.11
7542	34.0664	-119.1341	Shelf	174.0	Hueneme to Dume	99	NS	NA	64.9	1.16
7596	34.1251	-119.1925	Shelf	15.0	Hueneme to Dume	99	NS	NA	2.6	0.09
7629	34.1784	-119.3471	Shelf	25.9	E. Santa Barbara	94	NS	NA	68.8	0.64
7652	34.2301	-119.6874	Shelf	139.0	E. Santa Barbara	100	NS	NA	41.4	2.71
7654	34.2326	-119.7068	Shelf	158.2	E. Santa Barbara	101	NS	NA	40.2	0.88
7681	34.2841	-119.3546	Shelf	20.7	E. Santa Barbara	97	NS	NA	35.2	0.36
7696	34.3078	-119.7128	Shelf	139.9	E. Santa Barbara	100	NS	NA	79.3	1.22
7727	34.3946	-120.3315	Shelf	185.9	W. Santa Barbara	98	NS	NA	54.1	0.32
7728	34.3956	-119.6622	Shelf	25.9	E. Santa Barbara	93	NS	NA	40.8	0.53
7735	34.3983	-119.8643	Shelf	28.3	E. Santa Barbara	96	NS	NA	25.9	0.75
7741	34.4010	-119.8328	Shelf	29.9	E. Santa Barbara	101	NS	NA	35.0	1.13

[1] Toxicity categories: 1= Nontoxic; 2= Low Toxicity; 3= Moderate Toxicity; 4= High Toxicity

D= Data removed because of testing quality assurance issues.

NA= Calculation of integrated toxicity category is not applicable if only one toxicity test was performed

NS= No sample taken for the mussel embryo sediment-water interface test; offshore stations only evaluated using amphipod test.