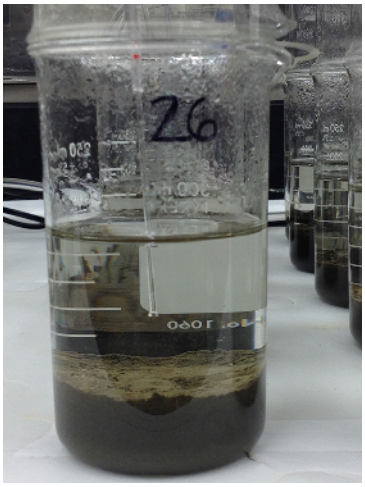
Sediment Toxicity

BIGHT '13



Southern California Bight
2013 Regional Monitoring
Program
Volume I

SCCWRP Technical Report 899

Southern California Bight 2013 Regional Monitoring Program: Volume I. Sediment Toxicity

Steven M. Bay[1], Lan Wiborg[2], Darrin J. Greenstein[1], Nick Haring[2], Christina Pottios[3], Chris Stransky[4] and Kenneth Schiff[1]

[1] *Southern California Coastal Water Research Project*

[2] *City of San Diego Public Utilities Department*

[3] *Sanitation Districts of Los Angeles County*

[4] *AMEC Foster Wheeler*

December 2015
SCCWRP Technical Report 899

SEDIMENT TOXICITY TECHNICAL COMMITTEE

Chris Stransky	AMEC Foster Wheeler
Joe Freas	Aquatic Bioassay and Consulting Laboratories
Michael Machuzak	Aquatic Bioassay and Consulting Laboratories
Matthew Jacobe	City of LA, EMD
Lan Wiborg	City of San Diego
Nick Haring	City of San Diego
Christina Pottios	Sanitation Districts of Los Angeles County
Josh Westfall	Sanitation Districts of Los Angeles County
Adrienne Cibor	Nautilus Environmental
Katie Flocken-Payne	Nautilus Environmental
Peter Arth	Nautilus Environmental
Rob Gamber	Orange County Sanitation District
Jian Peng	Orange County Public Works
Steven Bay	SCCWRP
Darrin Greenstein	SCCWRP
Alvina Mehinto	SCCWRP

FOREWORD

The 2013 Southern California Bight Regional Monitoring Survey (Bight'13) is an integrated, collaborative effort to provide large-scale assessments of the Southern California Bight (SCB). The Bight'13 survey is an extension of previous regional assessments conducted every five years dating back to 1994. The collaboration represents the combined efforts of nearly 100 organizations. Bight'13 is organized into five elements: 1) Contaminant Impact Assessment (formerly Coastal Ecology), 2) Shoreline Microbiology, 3) Nutrients, 4) Marine Protected Areas, and 5) Trash and Debris. This assessment report presents the results of the sediment toxicity portion of the survey, which is one component of the Contaminant Impact Assessment element. Copies of this and other Bight'13 reports, as well as work plans and quality assurance plans, are available for download at www.sccwrp.org.

ACKNOWLEDGEMENTS

This report is a result of the dedication and hard work of many individuals who share a common goal of improving our understanding of the environmental quality of the Southern California Bight. The authors wish to thank the members of the Bight'13 Toxicology Committee for their assistance with study design, sample analysis, data analysis, and report review. We also thank the Bight'13 Executive Advisory Committee and Contaminant Impact Assessment Committee for their guidance and support of sediment toxicity testing in regional monitoring. This study would not have been possible without the remarkable expertise of the field sampling personnel from the following organizations: City of San Diego, Weston Solutions, Orange County Sanitation District, Sanitation Districts of Los Angeles County, City of Los Angeles, Southern California Coastal Water Research Project, Aquatic Bioassay and Consulting Laboratories, and AMEC Foster Wheeler. We also wish to express our gratitude to Abel Santana (SCCWRP) for assistance with map preparation.

Toxicity testing was provided by six laboratories: Aquatic Bioassay and Consulting Laboratories, City of Los Angeles, City of San Diego, Nautilus Environmental, Orange County Sanitation District, and Sanitation Districts of Los Angeles County.

EXECUTIVE SUMMARY

While a substantial expenditure of effort is made to monitor the health of the benthic environment in the Southern California Bight each year, relatively little is spent on toxicity testing. The Southern California Bight Regional Monitoring Surveys represent the most comprehensive effort to determine the toxicity of the region's sediments. The goal of the Bight'13 sediment toxicity studies was to answer three key 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 compare to previous regional surveys? In addition, several special studies were conducted using subsets of the samples to investigate the toxicity results in greater detail and to evaluate alternative toxicity methods.

Sediment was collected from 232 stations for toxicity testing. These stations were sampled between July 1 and September 30, 2013 and located between Point Conception, California, and the United States-Mexico border. The majority of the sites were selected using a stratified random design to ensure representativeness and minimize bias. There were a total of six strata tested throughout the Bight. Two of the strata were offshore; the shelf stratum represented the mainland shelf, while the canyon stratum encompassed deep stations within submarine canyons which had not been sampled in previous monitoring programs. There were four strata within embayments: marina, port, bay, and estuary. Van Veen grab samples were taken at each station and the surficial sediments were tested for toxicity (upper 2 cm for offshore and upper 5 cm for embayments).

Two toxicity tests were used to assess the sediments. A 10-day survival test using the amphipod *Eohaustorius estuarius* was conducted on sediment from all stations. The second test was a sediment-water interface test using embryos of the mussel *Mytilus galloprovincialis*, which was conducted only on the embayment stations. The amphipod test has been used in the previous three Bight programs, while the mussel test was first used in Bight'08. The combination of these two tests provides results that are compatible with the requirements of California's sediment quality objectives (SQO) policy for bays and estuaries.

Bight'13 included a comprehensive quality control and assurance program consisting of an interlaboratory comparison, standardized test methods, laboratory audits, and analysis of split samples to ensure the data were comparable and of high quality. All of the 170 samples tested with mussel embryos met test acceptability criteria. For the amphipod test, 95% of the 232 samples met acceptability criteria. The remaining twelve samples that did not meet acceptability criteria were excluded from the data analyses, but are included in the survey dataset (with the addition of qualifiers and other features to prevent their unintentional use).

Methodology from the SQO program was used to classify the results into one of four categories of toxicity. The Nontoxic category represented results falling within the acceptable range for controls. The Low category corresponded to a small, but significant test organism response. For the purposes of Bight'13 data summarization, these two categories were defined as representing a condition termed "not toxic". The Moderate and High categories indicated a toxicity response of greater magnitude that was considered to be a reliable and substantial level of toxicity. Stations falling in either the Moderate or High categories were termed "toxic".

The prevalence of toxicity in the SCB was quite low. The amphipod test results indicated that 98% of the SCB was not toxic (falling in the Nontoxic or Low SQO categories). An intriguing result was that 17% of the area in the canyon stratum was identified as toxic (Moderate or High SQO categories); a much greater magnitude and spatial extent of toxicity than the surrounding shelf. The cause of the sediment toxicity in the submarine canyons has not been identified, although a toxicity identification evaluation (TIE) is in

progress for one sample from the La Jolla Canyon. Preliminary TIE results indicate that organic contaminants are likely responsible.

Embayments also had a low spatial extent of toxicity. Bivalve embryos were tested only in the embayment strata and 99% of the area was not toxic using this species. The integrated toxicity results using both species classified 96% of the embayment area as not toxic. Within the embayments, the greatest prevalence of toxicity was in the estuary stratum with 7% of the area identified as toxic, followed by the bay stratum at 6%, and the marina stratum at 4%. None of the area within the port stratum was identified as toxic. The amphipod test found more stations to be toxic than the mussel test and it was rare that they both agreed a station was toxic.

Temporal analysis of the results indicated that the trend of decreasing toxicity for the amphipod test observed in Bight'08 continued in Bight'13. With the exception of the Shelf, all strata experienced a marked decrease in the percentage of area identified as toxic. The Shelf stratum indicated a slight increase in toxicity extent which was attributable to one station classified as toxic. For the first time, results of integrated results for the two toxicity tests could be compared temporally. The trend toward decreasing toxicity was again evident, but not as pronounced as for the individual tests. A group of 83 stations has now been tested with *E. estuarius* during three different Bight surveys, enabling more detailed analysis of temporal changes.

Overall, the Bight'13 sediment toxicity survey was quite successful. A high level of test completion was attained and comparability of test results was high among the multiple testing laboratories. This survey represented the first time that testing in the SCB has been repeated on a regional basis using the SQO analysis methods. The results obtained for the canyon stratum provide a valuable baseline for the support of additional investigations in this little studied habitat.

Toxicity is just one of multiple lines of evidence necessary to accurately assess sediment quality. Caution should be applied in using the toxicity results reported here as the only basis for depicting sediment quality in the SCB. All of the stations analyzed for sediment toxicity were also sampled for assessment of sediment chemistry and benthic macrofauna community composition. The results for these additional lines of evidence will be described in other Bight'13 reports. The results from all three lines of evidence will be used to make an integrated assessment of sediment conditions in the SCB.

The encouraging temporal trend of decreasing toxicity in embayments is an example of the value of periodic regional monitoring that uses comparable methods. Continued assessment of sediment toxicity using the methods and study design employed in Bight'13 is recommended. Several recommendations to improve the efficiency and utility of future Bight surveys are provided in this document. Expanded studies in submarine canyons and inclusion of monitoring data from other programs are needed to investigate the extent and cause of the sediment toxicity observed in this study. In addition, investigation of methods for onboard sample homogenization is recommended to increase the comparability of the toxicity and chemistry results. Refinement of methods for interpreting toxicity data from offshore areas and integrating them with other lines of evidence is also needed, as our current approach was developed specifically for use in enclosed bays and estuaries.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. INTRODUCTION

Each year, a substantial effort is put into monitoring the health of the benthic environment in the Southern California Bight (SCB). Most of this effort is focused on assessing the impacts near treated wastewater discharges that the publicly owned treatment works (POTWs) release to the ocean environment (Schiff et al. 2002). The majority of this work is related to chemical evaluations of the sediment and assessment of the biological assemblages in the soft bottom communities. Very little of the effort is expended on sediment toxicity testing. The Southern California Bight Regional Monitoring Surveys have provided the opportunity to expand the assessments beyond the areas affected by the POTWs and into bays and estuaries. They also provide the occasion to do extensive sediment toxicity testing on a regional basis and in multiple habitats.

Sediment toxicity testing is a key component to the overall assessment of sediment quality. While chemical measurements are also important, only a limited number of contaminants are analyzed in monitoring programs. This analysis cannot account for the interactive effects of multiple contaminants, and does not account for bioavailability. Toxicity testing integrates the effects of all chemicals present and accounts for interactive effects and contaminant bioavailability. The other important component is benthic biology, but it cannot account for changes to community structure caused by non-contaminant factors. Toxicity testing also has drawbacks including a limited toolbox of testing species and an uncertain connection between results obtained in the laboratory versus conditions in the environment.

Beginning with the 2008 survey, the Bight surveys have used a study design employing methodologies for chemistry, toxicity, and benthic community analysis compatible with the California Sediment Quality Objectives Program (SQO). This allows for a Bight-wide assessment that is much more quantitative than what had been used in the past. The Bight'13 survey has the goal of assessing sediment quality from Point Conception to the U.S.-Mexico border. Using the standardized SQO assessment methods will allow for comparison of the Bight'13 results to other regions of the state and on a temporal basis, especially with the Bight'08 survey.

The sediment toxicity portion of Bight'13 was designed so that three questions could be answered: 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 compare to previous regional surveys?

Extent and magnitude of sediment toxicity are described by the number of square kilometers or percentage of habitat area with toxic responses in laboratory testing relative to control responses. There were six habitats or strata evaluated for sediment toxicity in Bight'13: bay, marina, port, estuary, shelf, and canyon. The canyon stratum was new for the Bight'13 survey. To be consistent with the SQO program, two toxicity test methods were conducted on each sample from embayment strata, an acute amphipod survival test and a chronic test with mussel embryos exposed at the sediment-water interface. Since the requirements of the SQO program do not apply to offshore waters, only the amphipod test was conducted for the shelf and canyon strata. The SQO program allows for the use of an alternate chronic test: a survival and growth test with a polychaete worm. As part of a special study associated with the Bight survey the polychaete test was conducted on a subset of stations to test its comparability and feasibility in relation to the other test methods. In an additional special study, an attempt was made to determine the cause of observed toxicity at selected stations through the use of toxicity identification evaluations (TIE).

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. Appendices contain reports on the results of the alternate toxicity test special study, electronic maps of results, and a station-by-station summary of the toxicity results.

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 2016.

II. METHODS

A. Sampling Design

There were 232 sites on the continental shelf between Point Conception, California, and the United States-Mexico international border (Figure II-1) that were sampled for toxicity testing between July 1 and September 30, 2013. The study used a Generalized Random Tessellated Stratified sampling design for site selection, which creates a spatially balanced random sampling of resources (Stevens 1997). Toxicity samples were distributed among six strata: shelf, marinas, ports, bays, estuaries, and canyons. Enhancement of the sampling design was achieved through intensified sampling in targeted areas and by resampling of stations from previous surveys. Intensified sampling was applied within portions of San Diego Bay to encompass additional substrata (freshwater influenced, shallow harbor, and deep harbor). These additional stations were not randomly selected and their toxicity results are therefore only included in the descriptive results portion of this report (not used for calculation of spatial extent of toxicity). In order to assess temporal trends, approximately 50% of the Bight'13 samples were new sites while 50% of the sample sites were previously sampled in Bight'08. Of the resampled stations 50% had also been previously sampled in Bight'98 and 50 % from Bight'03. This was the first Bight survey in which samples were collected and tested from the canyon stratum.

Two toxicity tests were used for the regional survey. Whole sediment toxicity was measured for all stations using the amphipod (*Eohaustorius estuarius*) 10-day survival test. In addition, a sediment-water interface test was conducted using mussel (*Mytilus galloprovincialis*) embryos on samples from embayment strata (ports, bays, estuaries, and marinas).

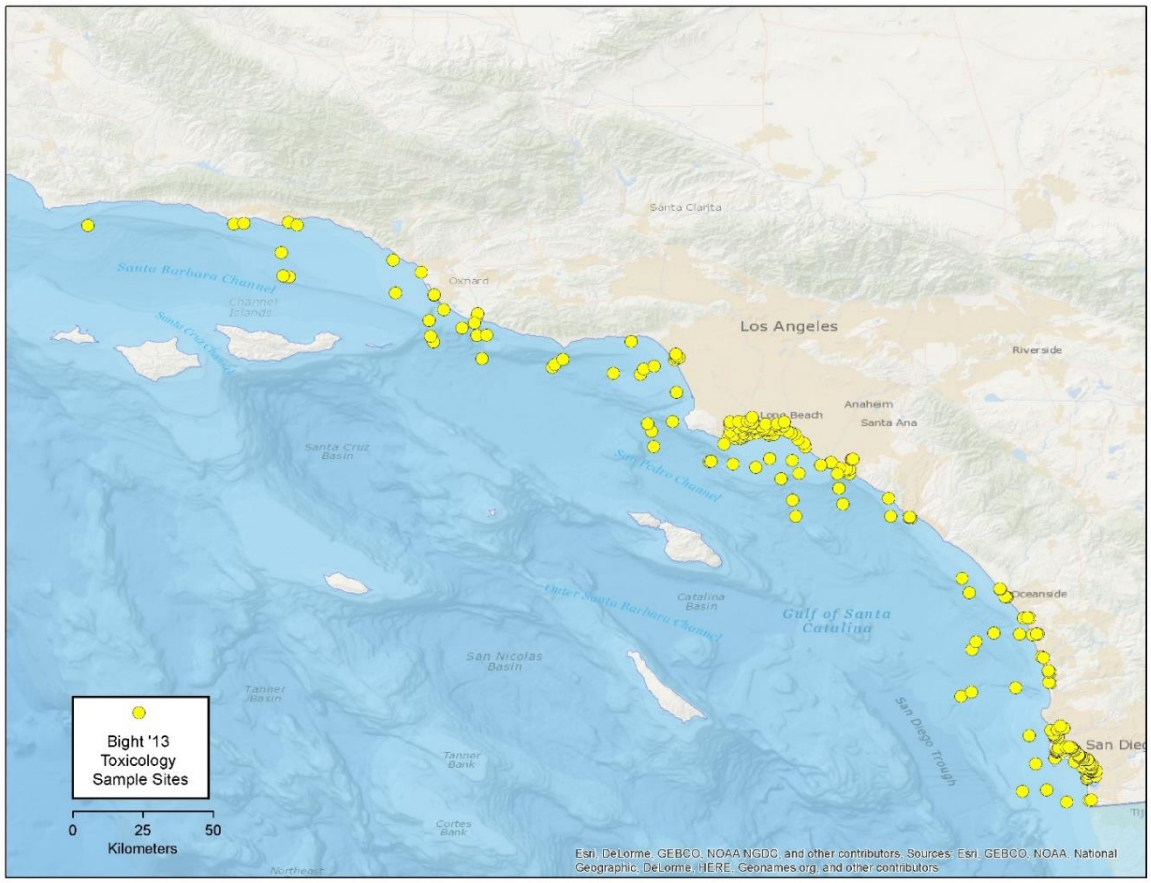


Figure II-1. Locations of all stations targeted for toxicity testing as part of the Bight'13 project.

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. A plastic (high-density polyethylene [HDPE], polycarbonate, or Teflon) scoop was used to collect sediment from the top 2 cm (offshore stations) or top 5 cm (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, in an attempt to make the contents of each comparable. Once collected, the samples were stored in the dark at 4°C in the laboratory for no longer than four weeks prior to testing. Additional subsamples of sediment were taken for chemical and particle size analysis.

C. Laboratory Methods

Whole Sediment Toxicity

The toxicity of whole sediment to amphipods was determined using a 10-d survival test (USEPA 1994, ASTM 2010) with *E. estuarius* (EE) under static conditions. Amphipods and negative control sediment were collected from a non-contaminated estuarine site (Yaquina Bay, OR) by Northwestern Aquatic Sciences (Newport, OR). The amphipods were acclimated to laboratory conditions for at least 2 d, but not longer than 10 d, prior to the initial test date. The amphipods were fed once (0.25 g of Tetramarin® slurry in 100 ml seawater per 1000 amphipods) at receipt. Testing was conducted in 1 L glass containers. Sediment samples were press sieved through a 1 mm mesh screen and homogenized in the laboratory before addition to test chambers. Sediment was 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, measurements of overlying water (temperature, dissolved oxygen, pH, total ammonia, and salinity) and pore water (pH, total ammonia, and salinity). A negative control (amphipod collection site sediment) was included with each batch of samples tested

Overlying water quality measurements of temperature, pH, dissolved oxygen, and salinity were made at time zero and at least every other day for the duration of the exposure. Ammonia measurements in the overlying water were made on day 0 and day 10. Pore water measurements of ammonia and salinity were made at sample receipt and day 0. The measurement at sample receipt was used to determine if adjustments to testing procedures 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, 20 randomly selected amphipods were added to each container. 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. 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. In order for the data from any given test batch to be considered acceptable, the mean control survival had to be at least 90% and the coefficient of variation for the control had to be no more than 11.9%.

A concurrent reference toxicant test was performed with each test batch. The reference toxicant exposure consisted of four replicates of five concentrations (15.6, 31.2, 62.5, 125, and 250 mg/L total ammonia) 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. 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}) for un-ionized ammonia 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 test data for each laboratory. A test result within two standard deviations of the mean control chart LC_{50} for each individual laboratory was considered acceptable. A test falling outside two standard deviations was not considered invalid, but a thorough review of all data and test procedures was triggered to assure that the data were of high quality.

Previous studies have suggested that finer grained sediments may affect the survival of *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 fine grained sediment collected from a relatively clean site prior to the start of the survey. Sediment was collected from a station in Mission Bay, California by AMEC. The sediment was homogenized, placed into 1L HDPE wide mouth containers, put into coolers with ice, and then shipped overnight to the testing laboratories where it was held in the dark at 4 °C until use.

Sediment-Water Interface Toxicity

For the sediment-water interface test, embryos of the mussel, *M. galloprovincialis* (MG), were exposed following the methodology of USEPA (1995) and Anderson et al. (1996). The animals were obtained from either Carlsbad Aquafarms (Carlsbad, CA) or Taylor Shellfish (Shelton, WA). Sediment was added to a glass chamber having dimensions of approximately 7.5 x 15 cm (600 ml tall form beakers). Sediment was passed through a 1 mm sieve and homogenized prior to addition to the test chambers 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°C with a 16 hour 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 nylon or 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 adverse effects to test organisms. In addition, a second control consisting of 10 ml laboratory seawater in an approximately 20 ml glass shell vials was tested to verify organism health. The controls from the concurrent reference toxicant test were often used for this purpose.

On the day of test initiation, spawning was induced, gametes were collected, and fertilization was monitored in a controlled environment. 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 which were immediately fixed for determination of the initial number of embryos. Water quality parameters (temperature, dissolved oxygen, salinity, and pH) were measured daily in the overlying water. Ammonia was analyzed in the overlying water at test initiation and termination. After 48 hours, the embryos were washed from the screen tube into another vessel for preservation and storage. The embryos were then counted and examined for normal development under a microscope. The number of normal embryos divided by the average initial number of embryos inoculated determined the endpoint, termed percent normal-alive (PNA). For the data from any given test batch to be considered acceptable, the mean control PNA had to be ≥70%.

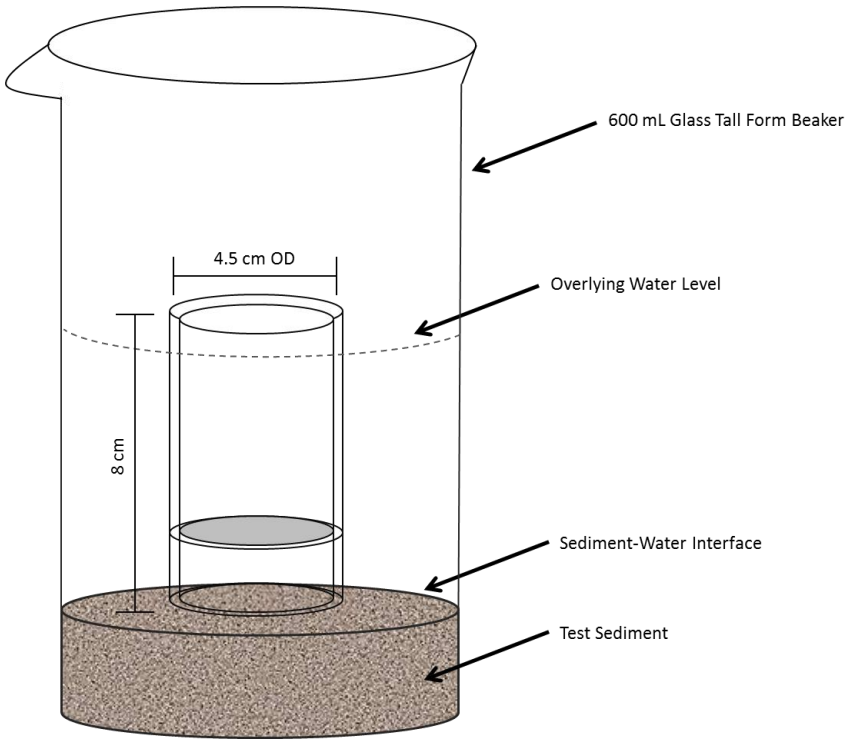


Figure II-2. Schematic diagram of sediment-water interface exposure system.

A concurrent reference toxicant test was conducted with each test batch. The reference toxicant exposure consisted of five replicates of six ammonia concentrations (2.0, 4.0, 6.0, 8.0, 10, and 20 mg/L total ammonia) dissolved in seawater, plus a control. Embryos were added to approximately 20 ml glass shell vials and exposed for 48 hours. At the end of the exposure period, embryos were preserved and stored for microscopic analysis. Water quality for the reference toxicant tests was measured using similar methods as for the sediment test. Samples were examined microscopically as described above to determine the PNA. The median effective concentration for PNA (EC_{50}) for un-ionized ammonia 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 laboratory was considered acceptable. A test falling outside two standard deviations was not considered invalid, but a thorough review of all data and test procedures was triggered to assure that the data were of high quality.

D. Data analysis

Data were analyzed using three methods: 1) calculation of the mean control-normalized response; 2) determination of the toxicity category using SQO thresholds; and 3) assessment of the percent area within each stratum that was classified into each of the SQO toxicity categories.

The control-normalized response for a given sample is calculated as the sample response mean 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 category of toxicity associated with each station was calculated using thresholds established for the SQO program (Bay et al. 2014). 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. The toxicity categories reflect both severity of toxicity and the confidence that the effects are reproducible.

- **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.

The toxicity thresholds described in Table II-1 were developed specifically for application in embayments using a process that included analysis of toxicity data exclusively from bays and estuaries according to a peer-reviewed conceptual approach (Greenstein and Bay 2012). These thresholds were also used for interpretation of the amphipod test results for offshore samples, although their use for offshore sediments has not been specifically validated. Use of these thresholds for offshore samples was considered appropriate in this study because the thresholds separating the nontoxic, low, and moderate categories (which include nearly all of the expected results) are either identical or very similar to those used throughout the United States for regional sediment quality assessment (USEPA 2014).

For stations where both test methods were used, a final toxicity category was established by integrating results from the two methods. This 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 Percent Normal-alive	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 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 having 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 area sampled. 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, which were due to inability to access sites 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. A complete description of the statistical tools used in this analysis as well as a download of scripts for probability-based estimation is available at http://archive.epa.gov/nheerl/arm/web/html/monit_intro.html.

III. QUALITY ASSURANCE EVALUATION

A. Introduction

In order to ensure good data quality and comparability between laboratories, the Toxicology Committee instituted a quality assurance (QA) plan for the Bight'13 survey. This QA plan was developed by the Committee and included in the Toxicology Laboratory Manual which guided all testing. The QA plan describes five elements that were used to ensure data quality. First, was to establish an acceptable level of sampling and testing success. The targeted number of stations within each stratum were successfully sampled. Additionally, samples were required to be tested before the pre-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 response and test procedures were comparable among different testing periods within a laboratory. Third, criteria for test performance and parameters for water quality were established. Deviations from the QA plan were examined by the Toxicology Committee. Those deviations deemed as minor were flagged in the database, while major deviations were excluded from the database. Evaluations of the effects of ammonia and grain size were also examined. Fourth, a laboratory audit was conducted during the survey in order to identify and correct deviations from the Toxicology Laboratory Manual in a timely fashion. Fifth, an interlaboratory study was conducted prior to the survey. Additionally, split samples were tested during the survey which provided information regarding the comparability of data among the participating laboratories.

B. Sample Storage

The optimal sediment storage time for toxicity testing was 14 days or less. The maximum allowable storage time was 28 days. For the EE testing, 88% of the samples were tested within 14 days of sample collection (Table III-1). All samples were tested with EE within 28 days. All samples tested with mussel embryos were initiated within 14 days of sample collection.

Table III-1. Toxicity sample holding time (from sample collection to animal addition).

	Eohaustorius estuarius (EE)		*Mytilus galloprovincialis* (MG)	
Time Interval (days)	**# Samples**	**Percent of Total**	**# Samples**	**Percent of Total**
0-14	204	88	170	100
15-28	27	12	0	0
>28	0	0	0	0

C. Organism Holding

All organisms were held in accordance with the protocols set forth in the Toxicology Laboratory Manual. One amphipod batch was subject to unusual conditions during transport (high temperature and longer travel time). Analysis of this batch indicated no unusual results for controls or the reference toxicant. No unusual occurrences were noted for mussel holding or transport.

D. Reference Toxicant Testing

Each toxicity test batch for both methods was accompanied by a concurrent reference toxicant test. The reference toxicant test served to verify organism health and relative sensitivity throughout the survey period. There were 23 EE and 19 MG reference toxicant batches. The EC_{50}/LC_{50} data were computed for the time zero measured un-ionized ammonia using the maximum likelihood probit method in the CETIS™ statistical software package.

For two EE test batches, spurious un-ionized ammonia values were reported. In both cases, erroneous data was caused by pH measurements which were too high to be deemed likely. In each case, the final time point un-ionized ammonia data was used for LC_{50} calculations instead of the initial.

The data for each test method was compared to the standard deviation of a large set of historical reference toxicant data submitted by the participating laboratories. All of the EE test results for batches having reported LC_{50} values fell within two historical standard deviations of the historical grand mean (Figure III-1). However, two additional EE test batches were not evaluated because an LC_{50} could not be calculated due to a lack of sufficient toxicity at the highest concentration tested. Most of the mussel reference toxicant test batches were within the historical two standard deviations, except for three tests by Lab 5 (Figure III-2). However, all of data for Lab 5 fell within two standard deviations of their own mean which was the assessment criterion for the survey.

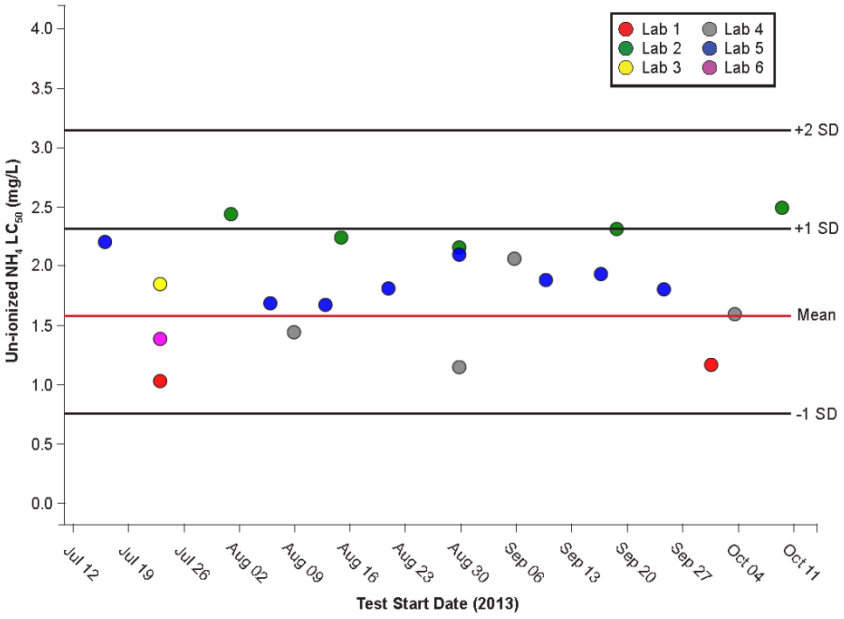


Figure III-1. Results of amphipod 96 hr reference toxicant tests with ammonia.

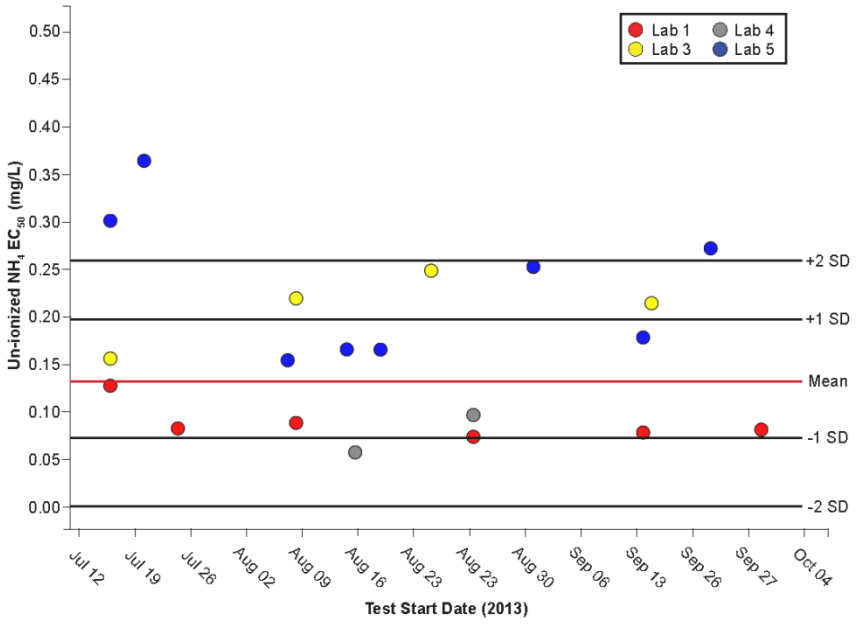


Figure III-2. Results of mussel embryo 48 hr reference toxicant tests with ammonia.

E. Water Quality

There were a relatively small number of water quality measurements that were outside of the limits set in the QA Plan. Analysis of this data versus either amphipod survival or mussel embryo percentage normal-alive found that these excursions from the limits were not associated with samples exhibiting toxicity. Therefore, all the exceedances were deemed minor and the data were considered acceptable for analysis.

F. Test Performance

Two EE test batches did not meet test acceptability criteria. One test batch did not meet the criterion for control survival of ≥90% (mean=89%). A second test batch did not meet the criterion for control variability (coefficient of variation ≤ 11.9%), having a value of 12.3%. Data from both of these test batches were excluded from the descriptive, regional assessment, and temporal analyses. The exclusion of these data led to the loss of at least one station from each stratum, except for the port stratum (Table III-2). It should be noted that mean survival of amphipods exceeded 90% in all samples associated with the batch having low control survival. Most of these samples were associated with the canyon stratum. Likewise, most of the samples associated with the one batch of tests that had a high control CV also had mean survival of greater than 90% (ranging from 88 to 98%, n=7). Control normalized data for these results are provided in Appendix A and the final database (with qualifiers and protections for unintentional use) for comparison purposes. The data quality objective of 90% completeness for the amphipod test was met for the survey as a whole, as well as for each stratum, with the exception of the canyon stratum. All test batches for the mussel embryo method met the control acceptability criterion of a percentage normal-alive ≥ 70%.

Table III-2. Toxicity sample testing success.

	Eohaustorius estuarius			*Mytilus galloprovincialis*		
	Targeted	**Tested**	**Testing Success Percentage**	**Targeted**	**Tested**	**Testing Success Percentage**
Bay	38	36	95	38	38	100
Marina	43	41	95	43	43	100
Port	45	45	100	45	45	100
Estuary	44	41	93	44	44	100
Shelf	32	31	97	0	0	-
Canyon	30	26	87	0	0	-
Total	232	220	95	170	170	100

G. Interlaboratory Study and Split Samples

Interlaboratory Study

Prior to the Bight'13 survey period, an interlaboratory study was conducted under the auspices of the Southern California Regional Chapter of the Society of Environmental Toxicology and Chemistry. This study was performed to ensure comparability of data produced by the multiple laboratories likely to participate in the survey. The study used a combination of split field samples, duplicate samples, and reference toxicants to assess interlaboratory comparability of both the amphipod and mussel embryo tests. As a result of the interlaboratory study, two laboratories were found to be not comparable and were excluded from carrying out amphipod survival tests on Bight'13 samples. The lack of comparability was based on greater than expected variation in survival results compared to the other laboratories and higher than expected variability between duplicate samples. While excluding these two laboratories made the logistics of performing the survey more difficult, it ultimately increased confidence in the data quality. All laboratories participating in the mussel embryo test interlaboratory study were found to be comparable.

Split Samples

Split samples from stations B13-8325 and B13-9197 were tested by all laboratories. The laboratories which had been excluded from testing as a result of the interlaboratory study also participated in the split sample testing as an opportunity to demonstrate improved comparability. While the results of these split samples were used to monitor interlaboratory variability, the outcomes 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'13 samples and were tested by all laboratories within two weeks of collection. The comparison criteria used to evaluate laboratory performance were similar to those used for the pre-survey interlaboratory comparison; however, no duplicate samples were included. Details of the assessment methods can be found in Appendix E. The maximum point score for overall comparability was 12. The ranges used for assessment were: 11.0-12.0 points, very high comparability; 10.5-9.5 points, high comparability; 9.0-8.0 points, moderate comparability; and <8.0 points, low comparability.

For the amphipod testing, all of the laboratories were in good agreement on station B13-8325, with five classifying the sample as Nontoxic and one in the Low category (Figure III-3). For B13-9197, five of the laboratories agreed the sample was Nontoxic, but Laboratory 2 placed it in the High category. No explanation has been determined for this difference, but poor homogenization of the sample prior to distribution to the laboratories has been suggested. It should be noted that the test batch for Laboratory 4 which included B13-9197 did not pass test acceptability for control survival, but is included for comparison.

The testing of the split samples with the mussel test showed good agreement between the laboratories for both stations (Figure III-3). All three participating laboratories found both samples to be Nontoxic. Note that due to scheduling difficulties, one laboratory was unable to test split samples for the mussel test. The remaining two laboratories which tested Bight'13 samples did not conduct the mussel test for the survey.

Five of the six laboratories were found to have either high or very high comparability for the amphipod tests of the split samples (Table III-3). Laboratory 2 was found to have low comparability all of which was a consequence of the differences discussed earlier for B13-9197. All three laboratories had very high comparability for the mussel test (Table III-4).

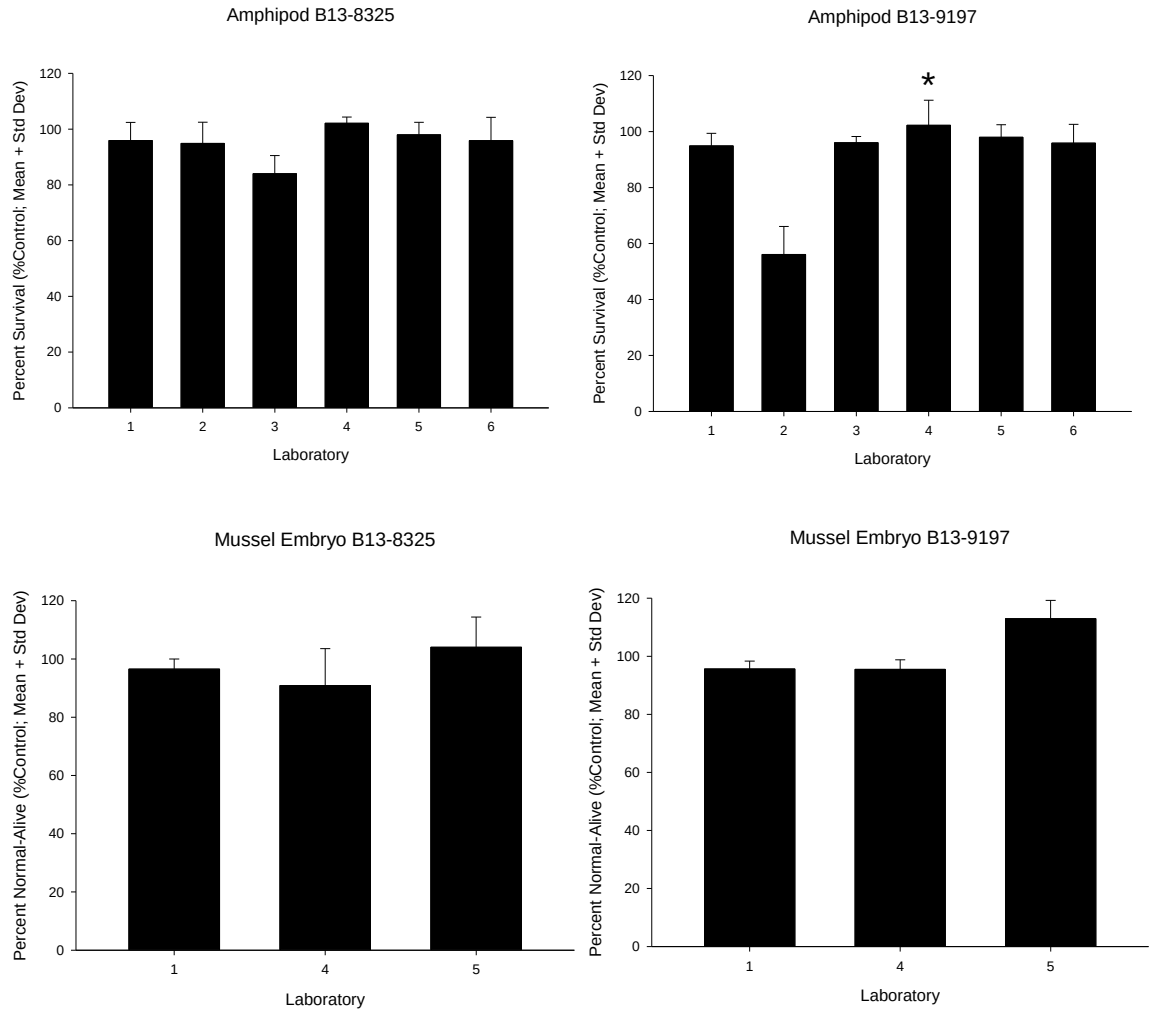


Figure III-3. Results of split sample testing of two Bight stations for the amphipod and mussel embryo tests.

***= test did not meet control acceptability criterion.**

Table III-3. Split sample assessment of each laboratory's comparability using the *Eohaustorius estuarius* sediment toxicity test.

Laboratory	8325 Difference[1]	8325 Category[2]	9197 Difference[1]	9197 Category[2]	Reference Toxicant[3]	Total	Comparability Category
1	3	1.5	3	1.5	3	12.0	Very High
2	3	1.5	0	0.0	3	7.5	Low
3	2	1.0	3	1.5	3	10.5	High
4	3	1.5	3	1.5	3	12.0	Very High
5	3	1.5	3	1.5	3	12.0	Very High
6	3	1.5	3	1.5	3	12.0	Very High

[1]Assessment based on the difference between the laboratories' percentage survival and the grand mean for all participating laboratories.

[2]Assessment based on the difference between the laboratories' identification of SQO category versus the category calculated from the grand mean of all participating laboratories.

[3]Assessment based on the difference between the laboratories' reference toxicant LC_{50} and the standard deviation of a historical group of data.

Table III-4. Split sample assessment of each laboratory's comparability using the *Mytilus galloprovincialis* sediment toxicity test.

Laboratory	8325 Difference[1]	8325 Category[2]	9197 Difference[1]	9197 Category[2]	Reference Toxicant[3]	Total	Comparability Category
1	3	1.5	3	1.5	2	11	Very High
4	3	1.5	3	1.5	2	11	Very High
5	3	1.5	2	1.5	3	11	Very High

[1]Assessment based on the difference between the laboratories' percentage survival and the grand mean for all participating laboratories.

[2]Assessment based on the difference between the laboratories' identification of SQO category versus the category calculated from the grand mean of all participating laboratories.

[3]Assessment based on the difference between the laboratories' reference toxicant LC50 and the standard deviation of a historical group of data.

H. Laboratory Audit

Onsite audits of each laboratory were conducted. An effort was made to conduct the audit during the first test batch of the survey for each species, so that corrections or clarifications to the protocol could be made to all laboratories. Very few deviations from the test protocols were observed. One laboratory used a 2 mm sieve instead of a 1 mm for its first test batch. Another laboratory was not using a device to avoid turbulence of the sediment during addition of the overlying water. Neither of these deviations likely affected test outcomes and both were corrected in subsequent test batches.

IV. DESCRIPTIVE RESULTS

A. Frequency of Toxicity

The toxicity results were evaluated using the SQO framework and the results were classified into one of four categories: Nontoxic, Low Toxicity, Moderate Toxicity, or High Toxicity. For the purposes of Bight'13, sediment toxicity is defined as stations determined to be in the Moderate or High SQO Toxicity categories. Therefore, samples in the Nontoxic or Low Toxicity categories are considered to be "not toxic". This reflects the level of uncertainty associated with the Low Toxicity category.

Of the 220 stations successfully tested with the amphipod survival test, 203 (92%) were in the Nontoxic or Low categories and thus deemed to be not toxic (Table IV-1, Figure IV-1). A total of 37 stations (17%) fell into the Low category. Toxicity (Moderate or High categories) was observed in at least one station in each stratum for the amphipod test. The greatest prevalence of toxic stations was within the embayment strata (bay, marina, port and estuary).

Of the 170 stations tested with mussel embryos, 167 (98%) were classified as being not toxic (Table IV-2, Figure IV-2). Toxicity was only observed in the estuary stratum for stations tested with the mussel embryo sediment-water interface test. There were 9 (5%) stations in the Low category throughout the embayments for the mussel test.

The canyon stratum was a new addition for the Bight'13 survey. As with other offshore strata, only the amphipod test was utilized for canyon stations. The majority of the stations were classified as Nontoxic (17 stations, 65%). However, three (12%) stations were toxic (Table IV-1, Figure IV-1) and another six (23%) were in the Low category. These findings are greater than that for the shelf strata where one (3%) station was found to be toxic and three (10%) were in the Low category. It should be noted that four stations were excluded from the canyon strata because they were in a test batch that did not meet the acceptability criterion for control survival. Each of these stations had greater than 90% survival and would likely have been in the Nontoxic category. Had these data been used in the analysis, it would have lowered the percentage of stations found to be toxic to 10% and those in Low category to 20%; still a higher percentage than what was observed in the shelf strata.

Within the embayment strata (bay, marina, port, and estuary), the greatest prevalence of toxic stations was observed in the estuary stratum (Tables IV-1 and IV-2, Figures IV-1 and IV-2). This trend was detected for both test species, with the amphipod test finding six (15%) and the mussel embryos three (7%) stations to be toxic. An additional nine (22%) stations for the amphipods and two (5%) for the mussel embryo test were in the Low category.

When the results of the two toxicity test methods were integrated, most stations were not toxic (158/93%), of which 44 (27%) were in the Low category (Table IV-3, Figure IV-3). Five (3%) embayment stations were in the Moderate or High categories. The port stratum had the lowest prevalence of toxicity with the amphipod test identifying one (2%) station as toxic and the mussel embryo test none. There were an additional eight (18%) amphipod and five (11%) mussel embryo stations from the port stratum in the Low category.

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

Stratum	Nontoxic	Low Toxicity	Moderate Toxicity	High Toxicity	Total
Bay	30	3	1	2	36
Marina	30	8	2	1	41
Port	36	8	1	0	45
Estuary	26	9	4	2	41
Shelf	27	3	1	0	31
Canyon	17	6	1	2	26
Total Embayment	122	28	8	5	163
Total Bight	166	37	10	7	220

Table IV-2. Sediment-water interface toxicity to mussel (*Mytilus galloprovincialis*) embryos, expressed as number of stations.

Stratum	Nontoxic	Low Toxicity	Moderate Toxicity	High Toxicity	Total
Bay	37	1	0	0	38
Marina	42	1	0	0	43
Port	40	5	0	0	45
Estuary	39	2	1	2	44
Total Embayment	158	9	1	2	170

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

Stratum	Nontoxic	Low Toxicity	Moderate Toxicity	High Toxicity	Total
Bay	30	4	2	0	36
Marina	29	11	1	0	41
Port	32	13	0	0	45
Estuary	23	16	0	2	41
Total Embayment	114	44	3	2	163

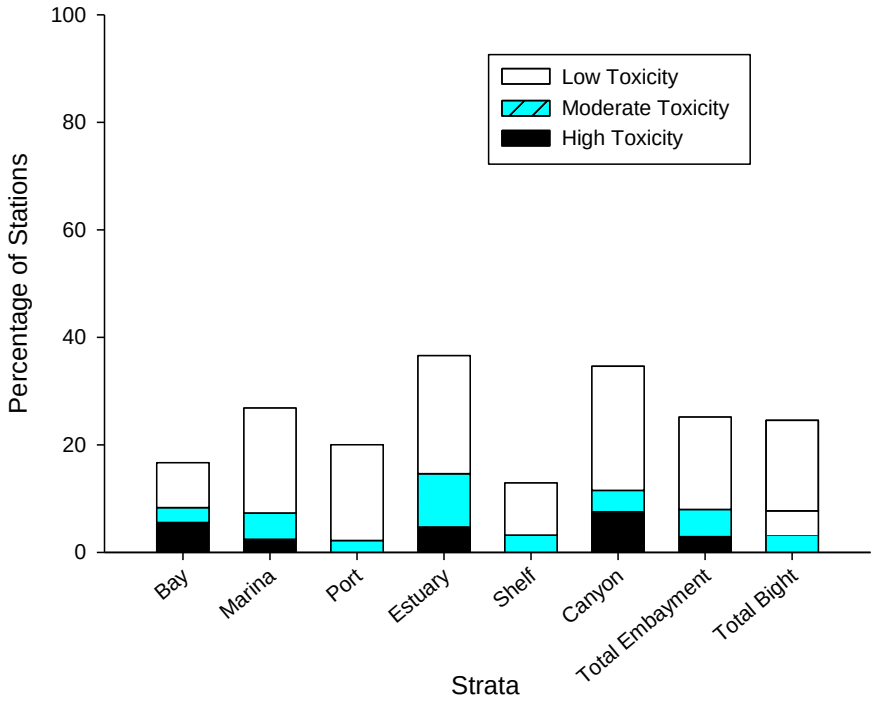


Figure IV-1. The percentage of stations in each sediment quality objective category by stratum for *Eohaustorius estuarius* survival.

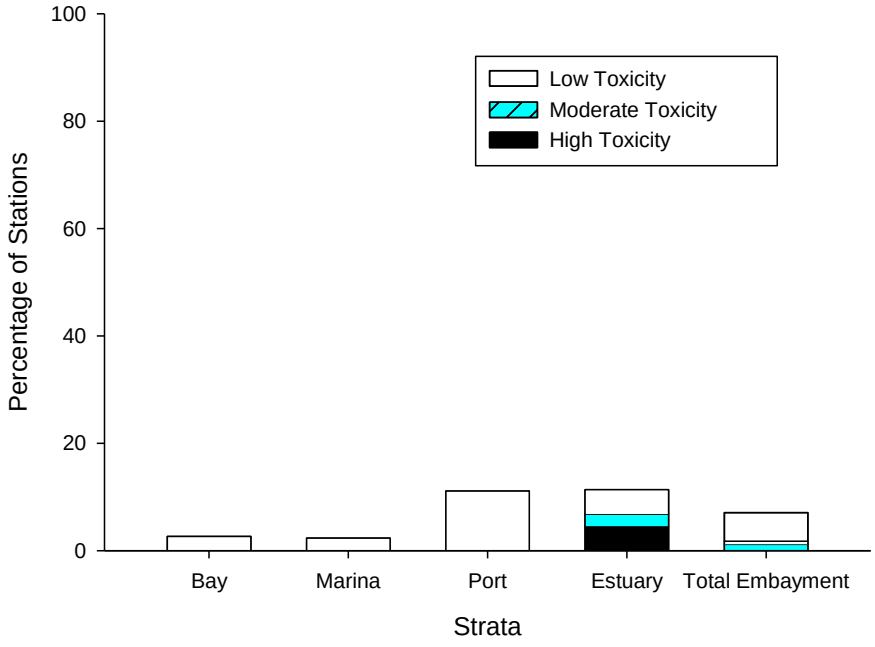


Figure IV-2. The percentage of stations in each sediment quality objective category by stratum for *Mytilus galloprovincialis* embryo sediment-water interface test.

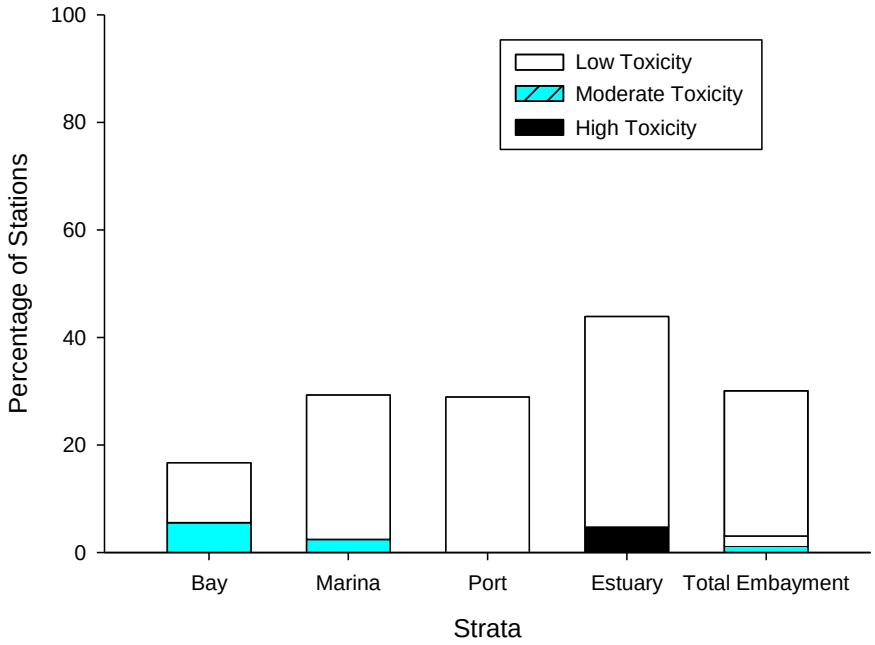


Figure IV-3. The percentage of stations in each sediment quality objective category by stratum after integration of the results from the amphipod survival and mussel sediment-water interface tests.

B. Magnitude of Toxicity

The magnitude of toxicity for each stratum is described by the control-adjusted response data for each test method. For the amphipod test, each stratum had at least one station that fell into the Moderate category, with the estuary stratum containing the greatest number (4) of stations in this category (Table IV-4). The port and shelf strata had no stations in the High category. No amphipod station had survival less than 44% of the control.

Only the estuary stratum had stations in the Moderate or High Toxicity categories for the mussel embryo test. The two stations in the High Category were both in the Los Angeles River estuary and had percentage normal-alive of 29% and 2%.

There was not much agreement between the two test methods as to the magnitude of toxicity. There were only three stations where both methods suggested toxicity as indicated by the lower left quadrant of Figure IV-4. The mussel test showed a greater magnitude of toxicity for two of these stations. There was one additional station where the mussel test showed toxicity but the amphipod test did not (lower right quadrant). There were many stations that the amphipod test found a greater degree of toxicity than did the mussel test (upper left quadrant). These results indicate the amphipod test was more sensitive than the mussels for this survey. This is a similar outcome to what was observed in the Bight'08 survey.

Table IV-4. Mean control-adjusted survival of amphipods in each stratum and for stations in the Moderate or High categories.

	Moderate Toxicity			High Toxicity		
Stratum	**Mean**	**Range**	**n**	**Mean**	**Range**	**n**
Bay	77	na	1	53	44-48	2
Marina	73	73-73	2	53	na	1
Port	71	na	1	na	na	0
Estuary	76	69-81	4	48	45-52	2
Shelf	64	na	1	na	na	0
Canyon	80	na	1	55	52-58	2
All Strata	74	64-81	10	50	44-58	2

na=not applicable. Either zero or one station in the category.

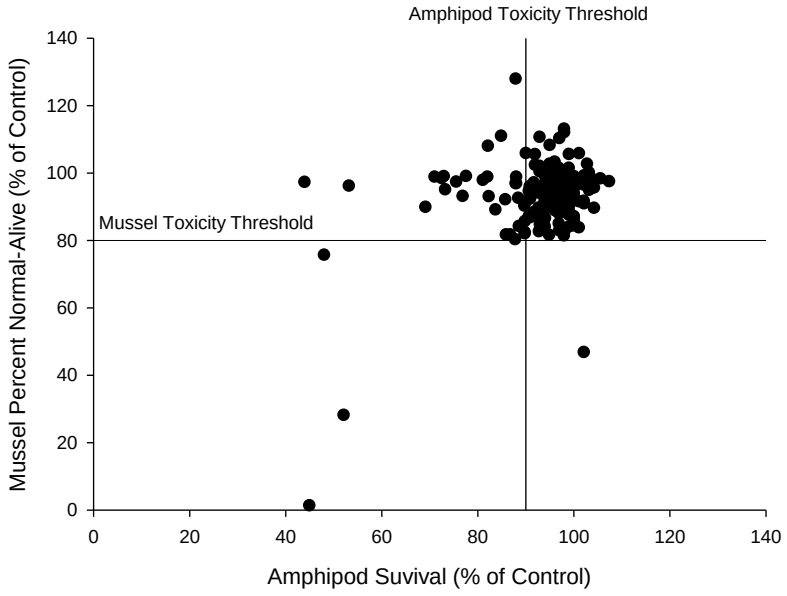


Figure IV-4. Comparison of response results between the amphipod and mussel embryo toxicity test methods. Note that samples falling below the thresholds may not be identified as toxic, since a statistical difference from the control is also necessary to establish toxicity.

C. Toxicity Characterization

Additional testing was conducted in an effort to determine the cause of amphipod toxicity. After review of the survival data, three stations were selected for follow up toxicity identification evaluations (TIE). They were B13-8403 in the Los Angeles River estuary, B13-9076 in La Jolla Canyon, and B13-8275 in Upper Newport Bay. In addition, stations B13-8290, B13-8291, and B13-8292 in Upper Newport Bay were targeted because of observed toxicity at these locations in previous Bight surveys. For logistical reasons, resampling for the TIEs did not occur until about a year after the initial sampling. Toxicity screening tests were conducted on all of the samples prior to conducting TIEs. Toxicity was not detected in the samples from the Los Angeles River and Upper Newport Bay; all stations had greater than 90% survival. A small response (17% mortality) was obtained for the La Jolla Canyon station and a TIE was initiated. The results of the TIE indicated that organic chemicals were the likely cause of toxicity.

D. Grain Size Controls

A grain size control (Mission Bay, California sediment) was included with most of the amphipod test batches throughout the survey. It was tested at least once by five different laboratories and after as much as five months of storage. The grain size control was found to be in the Nontoxic category in all of the test batches, with greater than 90% survival in all cases (Figure IV-5). Previous analysis of this sediment indicated it was composed of 77% silt and 15% clay (92% fines). The fines content of the grain size control was greater than that measured in 90% of samples from previous Bight surveys. Assuming that the Bight'13 samples had similar size characteristics as previous surveys, it is unlikely that their fines content substantially influenced the toxicity results.

The grain size control also served as an additional QA element since it was tested by all laboratories that assessed Bight samples using the amphipod test. It is a good indication of laboratory testing comparability that this sample was tested more than 30 times by five different laboratories and an equivalent result was achieved in each case.

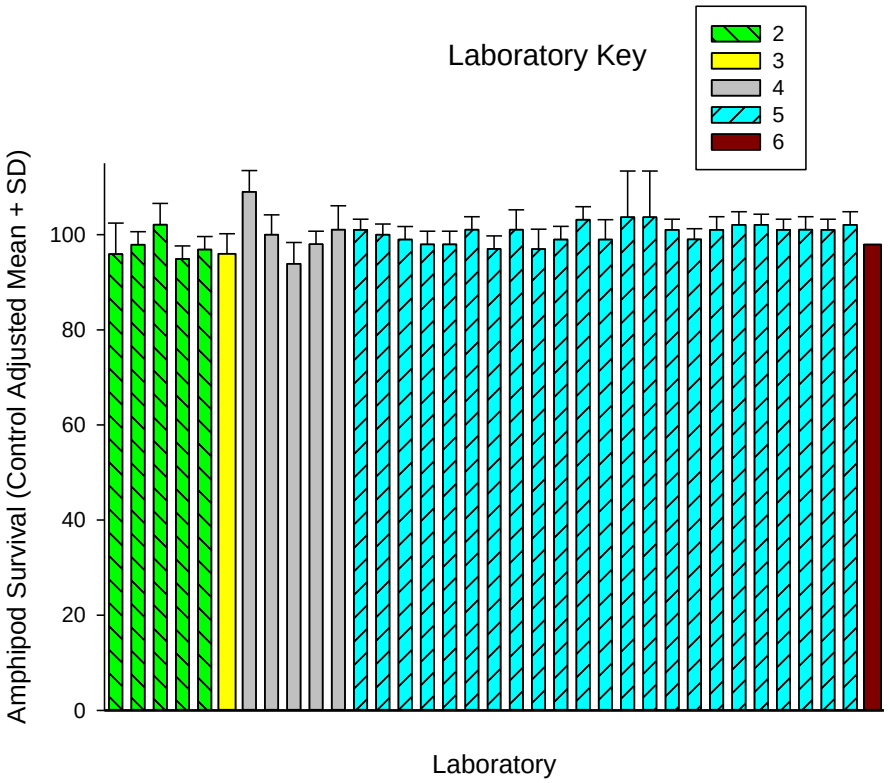


Figure IV-5. Results of grain size control testing using the amphipod, *Eohaustorius estuarius*. All samples were found to be nontoxic. A grain size control was not tested by Laboratory 1.

V. REGIONAL ASSESSMENT OF TOXICITY

A. Extent

There are a few important notes about the area data presented in this section. The exclusion of stations for the amphipod test due to QA issues leaves a total of 67.3 km^2 for which assessments cannot be made. This represents less than 2% of the SCB. Most of this area is accounted for by one shelf station representing 60.1 km^2. The remaining area is 6.7 km^2 in the canyons and 0.5 km^2 in marinas. The percentage data presented represents the area successfully surveyed. Additionally there were 34 stations in San Diego Bay which were not randomly assigned and therefore were not area weighted; the amphipod data for five of these stations was excluded due to QA issues. The mussel data for all 34 stations and the amphipod data for 29 stations were included in the descriptive results, but these data were not included in the regional assessment due to the lack of area weights. Finally, it should be noted that for all of the area estimates within any toxicity category that are represented by only a few stations, the uncertainty of the area estimate is quite high. This situation exists for any statistical calculation with a small number of samples. In many cases the lower bounds of the 95% confidence limit includes zero.

The amphipod survival test identified most of the SCB as being in the Nontoxic category. The total area successfully surveyed represented 3985 km^2 of which 3513 km^2 or 88% was in the Nontoxic category using an area-weighted average approach (Table V-1 and Figure V-1). Another 380 km^2 or 9.5% of the total fell into the Low category. Therefore, the total area considered to be not toxic (Nontoxic and Low categories) was 3893 km^2 or 97.7% of the Bight. The area classified as toxic (i.e., in the Moderate or High categories) was 92.4 km^2 or 2.3% of the Bight. For the embayment strata (bay, marina, port, and estuary), the total area was 122 km^2 of which 96.8 km^2 or 79% was in the Nontoxic category with another 15.7 km^2 or 13% being in the Low category for a total area identified as not toxic of 112 km^2 or 92%. The amphipod test identified 9.5 km^2 or 7.8% of the embayments as toxic.

For the amphipod test results, the canyon stratum had both the greatest area and percentage area in the High category (Table V-1 and Figure V-1). If the excluded stations for the canyon had been deemed to be in the Nontoxic category and included in the calculations, the percentage area in the Nontoxic category would have increased by less than 2% to 69.2% and the percent area in the High category would have decreased to 14.7%. The shelf stratum had the largest area identified as toxic with 60 km^2, but this represented only one station and 1.6% of the stratum's area. Within the embayments, the estuary stratum had the largest percentage of area (19.1%) identified as toxic. No calculated area within the port strata was identified as toxic by the amphipods.

The mussel embryo SWI test was only conducted on embayment strata samples. The embayment area found to be in the Nontoxic category by mussel embryo test was 114 km^2 or 93% of the total (Table V-2 and Figure V-2). Another 8.1 or 6.6% of the embayments were in the Low category for a total area of 121.6 km^2 or 99% of the area being not toxic. The area identified as toxic was 1.0 km^2 or 0.9%.

The only stratum where the mussel embryo test identified sediments as toxic (Moderate and High categories) was the estuary (Table V-2 and Figure V-2). However, the toxic area was small at 1.0 km^2 or 9.7% of the stratum. The port stratum had the largest area in the uncertain Low category with 4.8 km^2 or 19% of the stratum.

The results from the two toxicity tests were integrated only for embayments where both tests were performed. The area found to be in the Nontoxic category was reduced to 75% of the area (Table V-3 and Figure V-3); a little lower than found for the amphipod test and considerably lower than that based on the mussel test results alone. This reduction was caused by the effect of averaging the category results for each station and then rounding up if the mean was between two categories. The extent of the Low category at 25.3 km^2 or 20.7% of the embayment area was substantially higher than for either of test methods by itself. The combination of Nontoxic and Low resulted in 96% of the area being found to be

not toxic. The integrated area classified as toxic was 5.3 km^2 or 4.3% of the area. This was higher than found by the mussel embryo test but lower than for the amphipods.

When the data for the two tests were integrated, the largest area of toxic sediment was located in the bay stratum with 4.0 km^2 representing 5.6% of the stratum (Table V-3 and Figure V-3). However, the estuary stratum while having a smaller area identified as toxic at 0.7 km^2, had a slightly higher percent area considered to be toxic at 6.9%. This is due to the small total area of the estuary stratum at just over 10 km^2. Again the port stratum had both the largest area and percentage area in the uncertain Low category at 8.5 km^2 and 33% respectively.

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	59.1	9.7	6.0	4.4	2.0	3.5	4.0	4.8
Marina	11.2	2.2	2.3	1.5	0.9	1.1	0.6	1.1
Port	21.0	3.3	4.7	3.3	0	-	0	-
Estuary	5.5	1.6	2.7	1.3	1.3	1.0	0.7	0.7
Shelf	3325	615	344	323	60.1	105	0	-
Canyon	91.3	34.5	20.7	13.0	2.0	3.3	20.8	24.1
Total	3513	1057	380	322	66.3	93.0	26.1	23.8

Table V-2. Estimated area of SCB sediment classified by toxicity category using the sediment-water interface test with mussels. 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	69.1	7.7	2.0	3.4	0	-	0	-
Marina	14.9	1.1	0.6	1.1	0	-	0	-
Port	20.9	3.6	4.8	3.4	0	-	0	-
Estuary	8.6	1.5	0.7	0.7	0.3	0.3	0.7	0.7
Total	113.5	13.7	8.1	5.0	0.3	0.5	0.7	0.7

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	59.1	9.7	8.0	5.6	4.0	4.8	0	-
Marina	10.6	2.3	3.8	2.0	0.6	1.1	0	-
Port	17.2	4.3	8.5	4.3	0	-	0	-
Estuary	4.5	1.7	5.0	1.5	0	-	0.7	0.7
Total	91.4	14.5	25.3	7.5	4.6	5.0	0.7	0.7

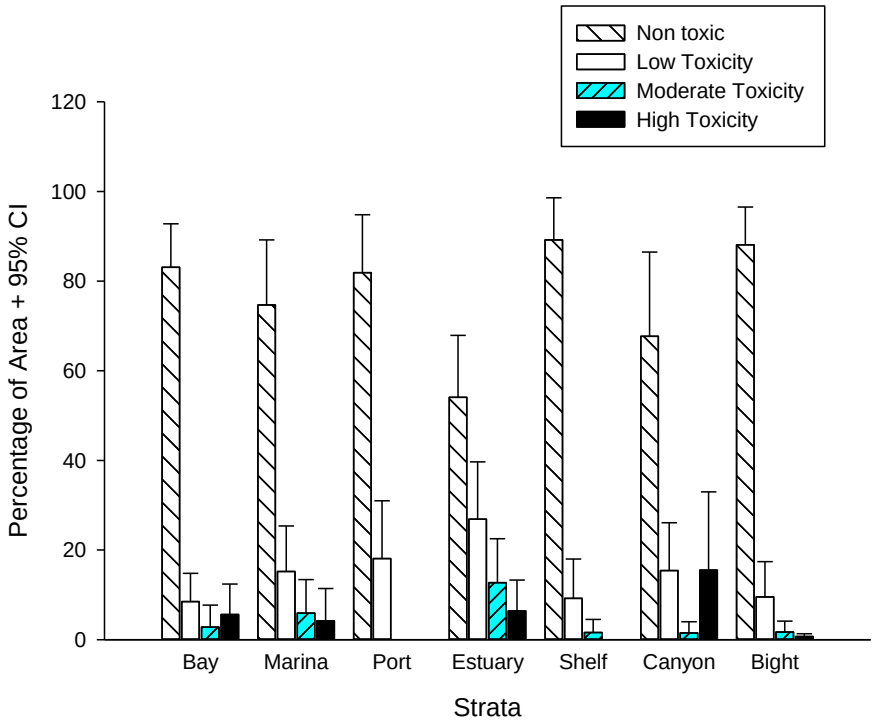


Figure V-1. Percentage of area falling into each of the sediment quality objective categories by strata using the amphipod survival test.

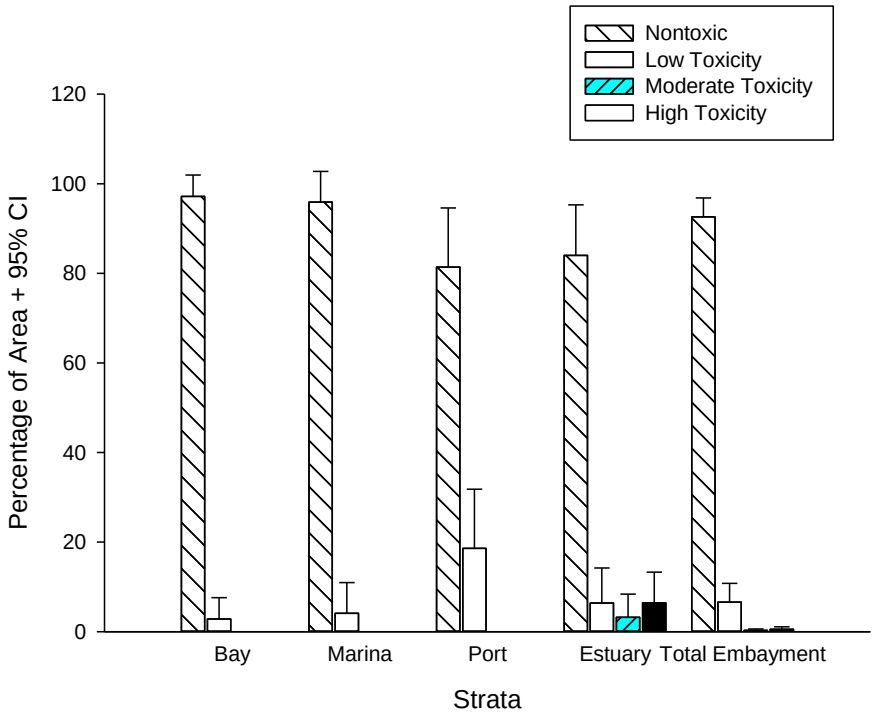


Figure V-2. Percentage of area falling into each of the sediment quality objective categories by strata using the mussel embryo sediment-water interface test.

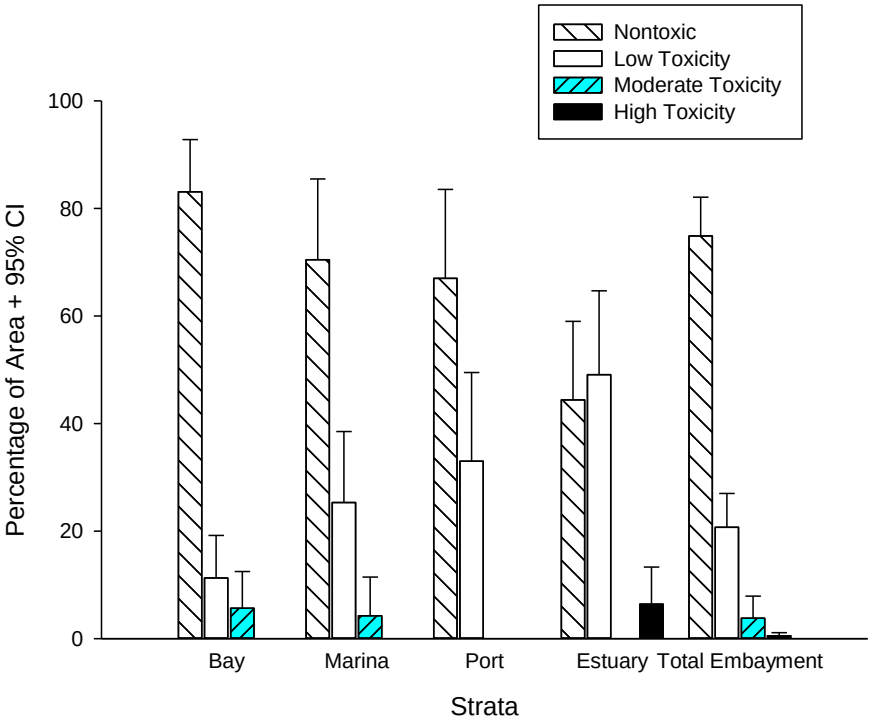


Figure V-3. Percentage of area falling into each of the sediment quality objective categories by strata when the results of the amphipod and mussel embryo tests are integrated.

B. Temporal Variation

Three previous toxicity surveys of the SCB have been conducted using a similar probabilistic sampling design and the amphipod *Eohaustorius estuarius* toxicity test method: the 1998, 2003, and 2008 Southern California Bight regional surveys (Bay et al. 2000, Bay et al. 2005, Bay et al. 2011). In addition, the last two surveys have included the mussel embryo sediment-water interface test in the embayments. This historical data allows for temporal comparisons to be made for each test method and of integrated toxicity data. 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 1998 and 2003 surveys were reevaluated using the SQO thresholds employed in 2008 and 2013. Another complication in making temporal comparisons among surveys is that the areas within each of the strata have not been consistent. The temporal comparisons were therefore made on a percent of area basis in order to minimize the influence of the difference in areas. The port and bay strata were not separated for the earliest survey, so they have been combined for all (Figure V-4). The offshore designation represents a combination of shelf and slope stations over the various surveys. The canyon stations were not included in the temporal comparison since that stratum has not been sampled previously.

The trend towards decreasing toxicity over time identified in Bight'08 continued with the Bight'13 survey. Most strata exhibited a marked decrease in the percentage area identified as toxic (Moderate and High categories; Figure V-4). The only stratum exhibiting an increase in the toxic area was the offshore, but this was quite small and represented only one station. The only stratum not showing appreciable change from Bight'08 was the estuary.

With the results of the Bight'13 survey, temporal comparisons can be made for the mussel embryo test for the first time. A trend toward a decrease in toxicity within each stratum as indicated by a lower percentage of area in the Moderate and High Toxicity categories in 2013 (Figure V-5). Only the estuary stratum was found to have area identified as toxic for the Bight'13 survey, whereas all embayment strata had some toxic samples in Bight'08.

Temporal comparisons can be made for the first time for the integrated SQO Toxicity Line of Evidence. While the trend again was toward decreased toxicity, the results were less pronounced than for the individual test methods (Figure V-6). The marina and port strata showed a substantial decrease in the area identified as toxic (Moderate and High categories), whereas the bay stratum did not. However, the bay stratum showed a substantial decrease in the area falling into the Low category. While the estuary strata showed a marked decline in the area identified as toxic, the area with the greatest magnitude of toxicity was similar between surveys.

Looking at temporal comparisons for the amphipod test on an individual station basis gives some insight into the level of consistency of the results. Appendices B and C contains links to scalable maps of the SCB showing results for all Bight'13 stations and the results from previous surveys for samples from the same locations. There are 83 stations which have now been sampled and tested with the *E. estuarius* survival test during three Bight surveys (no station has been sampled for all four). All of these stations were tested in both Bight'13 and Bight'08. Each of these stations was also sampled for the first time in either Bight'03 or Bight'98. None of the 83 stations were found to be toxic (Moderate or High Toxicity categories) for all three surveys (Figure V-7). The majority (67%) of stations were found to be not toxic (Nontoxic or Low categories) for all three samplings. Another 16% of the stations were found to be not toxic in Bight'13, but had been toxic in either the first sample period alone, or in both the first sampling and in Bight'08 (termed trending not toxic in the figure). Stations which were toxic in Bight'13, but not toxic either in the first sampling or both the first sampling and Bight'08 accounted for 5% of the total (trending toxic in the figure). The final 8% of the stations had a different outcome in Bight'08 than in the first sampling and Bight'13 (e.g. toxic in both the first sampling and Bight'13, but not toxic in Bight'08).

There were 91 stations sampled in both Bight'13 and Bight'08 on which both the amphipod and mussel embryo SWI test were performed. The integrated results from the two tests found that none of them were toxic (Moderate and High categories) for both surveys. A majority of the stations (86%) were not toxic in either survey. An additional 12% of the stations were toxic in Bight'08, but not in Bight'13. The remaining 2% of the stations were toxic in Bight'13, but had not been in Bight'08.

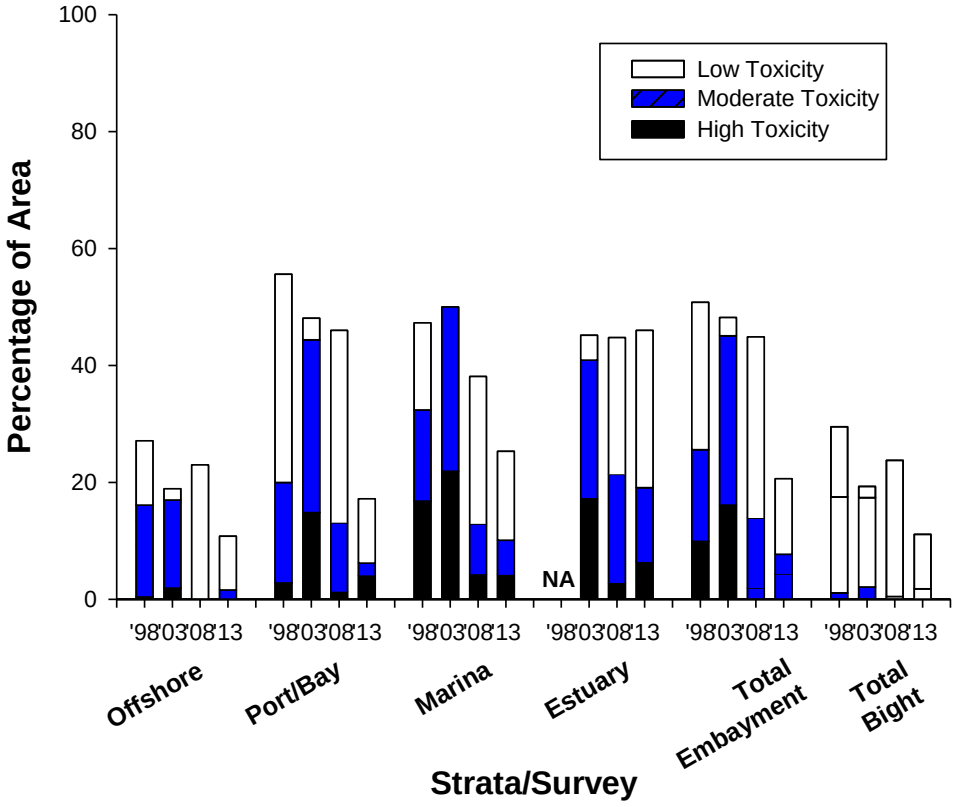


Figure V-4. Comparison of percentage areas found to be toxic with amphipod survival testing, shown by stratum over multiple years.

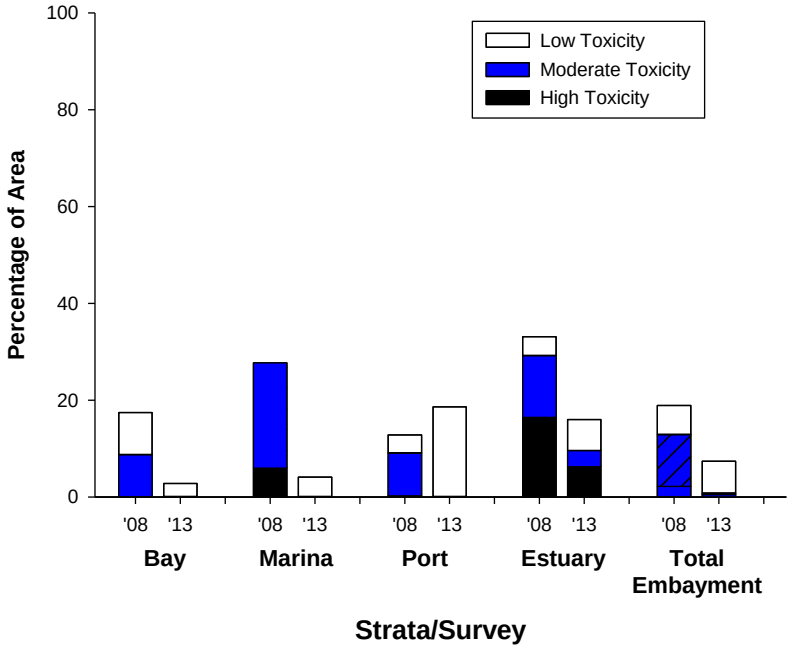


Figure V-5. Comparison of percentage areas found to be toxic with mussel embryo development testing, shown by stratum over in Bight'08 and Bight'13.

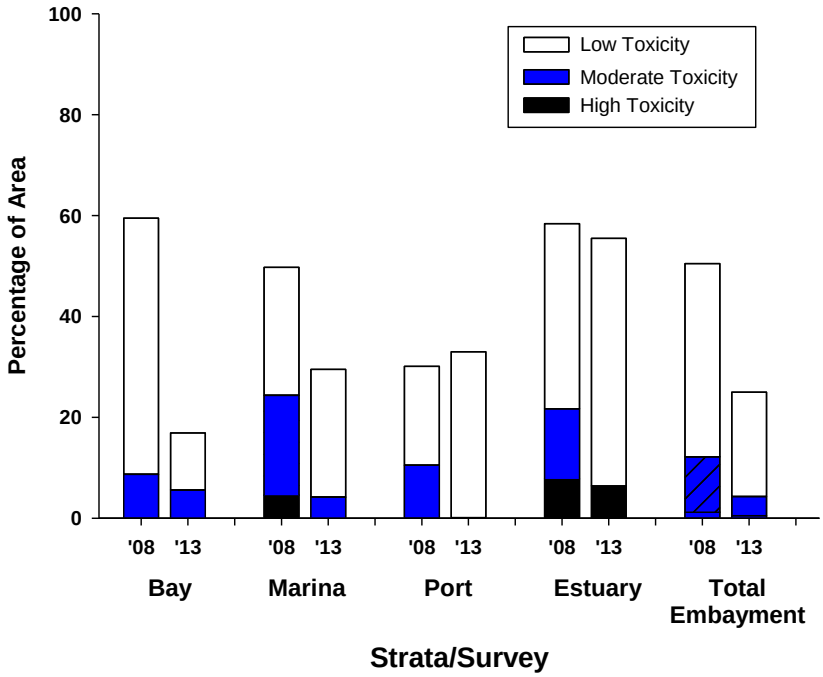


Figure V-6. Comparison of percentage areas found to be toxic when the results of the amphipod survival and mussel embryo development tests are integrated, shown by stratum over in Bight'08 and Bight'13.

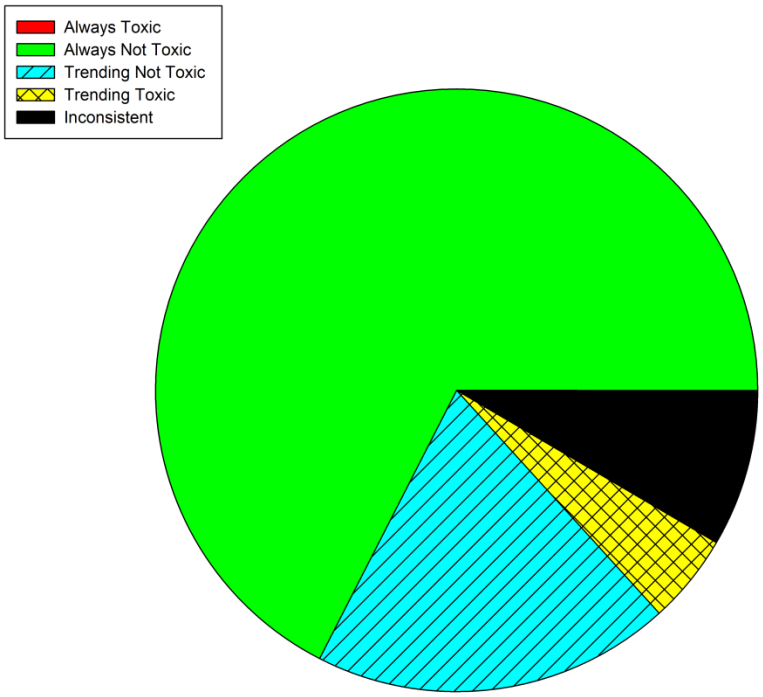


Figure V-7. Temporal trends for the amphipod survival test for individual stations over three sampling periods, Bight'13, Bight'08, and either Bight '03 or Bight'98. No stations were found to be always toxic.

VI. DISCUSSION

The Bight'13 sediment toxicity survey confirmed the trend toward improved sediment quality throughout the SCB observed in the past two surveys. Toxicity continues to be most prevalent in the embayments, but this group of strata is also showing improvement. There was a slight uptick in toxicity in the shelf stratum, but this change is within the range of statistical variation characteristic of the study design. The offshore strata are represented by relatively few stations spread over a large area.

The canyon stratum provided some of the most intriguing results. The extent and magnitude of the toxicity in the canyons was much greater than for the surrounding shelf stratum. No previous survey has found greater than 1% of the area in the High Toxicity category for any offshore stratum. The canyon stratum had 16% of the area in the High category. However, it should be noted this high magnitude of toxicity was represented by only two stations in large structurally complex canyons, both of which were in the southern part of the Bight (Appendix B). A TIE was performed on a station in La Jolla Canyon (B08-9076). Preliminary results indicate that organic chemicals were the cause of toxicity. This station is in fairly close proximity to a dredged materials disposal site. Since so little is known about the canyons and in light of the toxicity results, continued study of this stratum in future Bight surveys is warranted.

Within the embayments, the estuary stratum continues to have the largest extent and magnitude of toxicity (Figure V-6). The magnitude declined from 2008 to 2013 for the integrated toxicity line of evidence. This appears to be driven by the mussel test results which showed considerable reduction in toxicity (Figure V-5), while the amphipod test had very little change (Figure V-4). This difference between the tests may be indicative of the source of the toxicity since the test organisms have differential sensitivity to contaminants. In general, the amphipods are more sensitive to organic contaminants and the mussel embryos are more sensitive to metals.

Toxicity is far less prevalent in Southern California embayments than in San Francisco Bay. The San Francisco Bay Regional Monitoring Program (RMP) uses the same set of toxicity tests as are used for the Bight survey. San Francisco Bay had 70 to 80% of monitored stations identified as being toxic between 2008 and 2012 based on yearly monitoring (SFEI 2013). This is in contrast with the Bight'13 results where only 3% of the embayment stations were considered toxic based on the integrated toxicity results (Figure IV-3). In the 2012 RMP toxicity survey, more stations were toxic to amphipods than mussels, which was the opposite of some of their earlier assessments (SFEI 2014), but consistent with findings in the last two Bight surveys.

The Bight'13 survey is the second to use two toxicity tests in the embayments as prescribed by the California Sediment Quality Objectives Program (SWRCB 2009). A comparison between the results of the two surveys indicates that the integrated results show a trend of improvement which falls between that of the individual test results; an outcome that might be expected but would not occur if there were not general agreement between the two methods. This illustrates the increased confidence gained by using multiple toxicity test methods.

Use of the SQO toxicity thresholds to interpret the offshore sample results represents an application that is not currently intended by the SQO program. There is some uncertainty as to whether the thresholds distinguishing among the Low, Moderate, and High toxicity categories are the most appropriate ones for use with offshore samples since the statistical analyses used to derive them did not include offshore sediment test data. Considering the presence of Moderate and High toxicity in some Bight'13 canyon samples, it would be prudent to validate and/or refine the toxicity thresholds for offshore application prior to the next Bight survey.

While results for the two toxicity test methods agreed at the vast majority of the stations, this agreement was most prevalent for stations that were not toxic to either species (Figure IV-4). There was not very

good agreement when toxicity was encountered; only three stations were toxic for both species. As was seen in Bight'08, the occurrence of toxicity to the amphipods was more common, but the magnitude of toxicity was greater for the mussel embryos in two of the three stations. The mussel embryo test measures a sub-lethal endpoint (development) and consequently is often thought of as being more sensitive. Two surveys have now confirmed this to not be the case. The difference in results between the species may well be caused by the dissimilarities in sensitivity to various classes of contaminants, or to the different exposure routes inherent to the methods; direct contact for the amphipod test verses diffusion into the overlying water for the sediment-water interface test. Additionally, as part of a special study, selected stations were tested with a third toxicity test, a 28-day survival and growth test with a polychaete worm (Appendix F). Results of the polychaete test found that it was often the most sensitive of the three methods. However, there were a few stations where the worm test was the least sensitive, further indicating that no single test can provide a complete assessment of toxicity.

The majority of stations that were resamples of previous Bight stations had either the same assessment for toxicity at each sampling (i.e., always not toxic) or showed a trending direction. However, 8% of the stations showed a pattern of flip-flopping between toxic and not. This pattern may be due to small-scale spatial or temporal variability within the sampling site, or inherent variability within the test method. One of the sampling crews documented visibly different sediment characteristics between adjacent Van Veen grab samples (Figure VI-1). Additionally, the Bight program resamples stations over the relatively long time scale of five years. For some environments that have high rates of sediment transport or deposition, changes can occur rapidly. Indeed for the Bight'13 TIE studies, five of the six stations that were identified as toxic were found to no longer be so when resampled approximately one year later.

The results for sediment toxicity provide only one 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 a more accurate assessment of sediment quality (SWRCB 2009). Toxicity tests are valuable because they integrate biological response to the sediment characteristics, both natural and anthropogenic. 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'13 measured biological responses under controlled laboratory conditions, which may not be fully reflective of 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 toxicity, chemistry, and benthic community structure to assess sediment quality, known as the sediment quality triad, maximizes 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 integrated assessment of sediment quality in the SCB for Bight'13.

Figure VI-1. Double Van Veen grab sample from Bight'13 sampling showing different sediment types on each side (photo courtesy of AMEC Foster Wheeler).

VII. CONCLUSIONS

The Bight'13 survey presented a comprehensive regional assessment of sediment toxicity in the SCB using two common marine test species. Analysis of the results by the Toxicology Technical Committee produced the following conclusions:

- **Most of the SCB sediments were not toxic.**

  More than 88% of the SCB was found to be in the Nontoxic SQO toxicity category. Less than 3% of the SCB was found to be in the Moderate or High Toxicity categories. The remaining area fell into the less certain Low Toxicity category

- **Embayment sediments had the greatest extent and magnitude of toxicity.**

  The estuary stratum exhibited the greatest magnitude of toxicity in the embayments with 7% of the area being in the High Toxicity category. The bay stratum had a similar percentage of area found to be toxic (6%), but all of that area was in Moderate Toxicity category.

- **The canyon stratum, which was new for this survey, had a greater extent and magnitude of toxicity than previously encountered in the offshore environment.**

  It was estimated that 16% of the canyon stratum was in the High Toxicity category. No offshore strata have previously had more than 1% of its sampled area in the High Toxicity category.

- **The trend from recent Bight surveys towards decreasing sediment toxicity continued.**

  Except for the shelf stratum which had a small uptick in toxicity, all strata were less toxic in 2013 than 2008, regardless of testing species. The amphipod test indicates that toxicity was highest in 2003 and has subsequently declined.

- **The toxicity at 8% of the revisited stations was transitory in nature.**

  Of the stations that have now been tested during three surveys, some show intermittent toxicity with no apparent pattern. This lack of consistency may be due to multiple factors, including sedimentation, changing contaminant inputs, spatial variability, sampling techniques, degradation of contaminants to less toxic forms, or inherent variability within the test method.

VIII. RECOMMENDATIONS

- **Increase emphasis in particular habitats**

  The general design of the Bight'13 survey is sound, but some enhancements are needed to address knowledge gaps. It is important to continue and possibly expand sampling in the canyons to verify the results for this poorly understood stratum. Intensified studies in high depositional areas, such as river and creek mouths, would help to understand small scale variability, possibly through the use of field duplicates.

- **Strengthen basis for toxicity data interpretation**

  Use of sediment toxicity testing in NPDES monitoring programs is increasing due to implementation of the SQO program and changes to the Ocean Plan. Future Bight surveys should make use of these data for interpreting the results, as they are likely to provide important context for evaluating spatial and temporal trends. Selection of the thresholds used for offshore toxicity data interpretation should also be reconsidered as part of the planning for the next Bight survey. The increased availability of offshore toxicity data from prior Bight surveys and other monitoring should make it feasible to verify or refine these thresholds, resulting in greater confidence in the data interpretation.

- **Investigate use of onboard homogenization of chemistry and toxicity samples**

  For all Bight surveys to present, subsamples for chemistry and toxicity analyses have been created by qualitatively adding proportional amounts of sediment from multiple grabs to various containers in order to achieve approximate similarity between subsamples. Small scale variability between or even within grabs makes it uncertain whether each subsample is in fact representative of the others. From a quality assurance perspective, it would be preferable to use onboard compositing and homogenization of sediment to prepare subsamples. The Committee recognizes that this may be an extra burden to the sampling crews that could increase the costs of sampling, but feels that the increased connection between the chemistry and toxicity samples will make the added effort worthwhile. An evaluation of the feasibility and effectiveness of onboard homogenization should be conducted prior to the next Bight survey so that improved methods can be incorporated into the program.

- **Consider use of the *Neanthes* growth test in future surveys**

  The Bight'13 special study on alternative test methods demonstrated that the *Neanthes* 28-day growth test was feasible for routine use and produced different results for a limited number of samples, relative to the amphipod and mussel tests. Use of this test on a wider scale in future Bight regional surveys should be considered, as it may help to provide a more complete assessment of the extent, magnitude, or cause of sediment toxicity in the region.

- **Improve data entry and upload quality**

  Multiple errors in data submission were discovered during the process of data analysis that should have been either prevented or discovered earlier in the process. It is suggested that data entry templates be developed that will trap certain common errors, such as by setting range limits on appropriate data types. Double entry procedures should also be required. The data upload portal should also be modified to prevent many types of common errors from getting into the database (e.g. duplicate data and out of range data).

- **Revise split sample testing plan.**

  The differences in results between laboratories that were observed for the split samples were difficult to explain. Due to the way the samples were collected, it was impossible to separate sampling artifacts from interlaboratory variability. For future surveys it is recommended that the split samples be homogenized before distribution to the laboratories to eliminate differences which may occur during collection. Increased representativeness of the split samples will improve the ability to use the results to identify and control factors affecting the comparability of survey data. To provide greater utility for describing lab comparability, the split sample study should include both toxic and nontoxic samples. This can be accomplished through the use of spiked sediment or field sediment with a known level of toxicity.

- **Improve training and analysis methods for water quality parameters.**

  There were several cases where suspect water quality data were reported at the end of the survey. These fallacious results led to the incorrect analysis of results for the reference toxicants. These errors should have been caught at the time of sampling and the analysis immediately repeated. There is a need for additional training of technicians to recognize water quality readings that are spurious and not allow them to be reported without reanalysis. Secondly, QA review by supervisory staff should happen on a timely basis so that reanalysis is feasible. Finally, water quality analysis should be part of the intercalibration exercises that occur before the Bight surveys commence.

IX. REFERENCES

Anderson, B.S., J.W. Hunt, M. Hester, and B.M. Phillips. 1996. Assessment of sediment toxicity at the sediment-water interface. pp. 609-624 *in*: G.K. Ostrander (ed.), Techniques in aquatic toxicology. CRC Press Inc. Boca Raton, FL.

American Society for Testing and Materials ASTM. 2010. Standard test method for measuring the toxicity of sediment-associated contaminants with estuarine and marine invertebrates. pp. 400-461, 2010 Annual Book of ASTM Standards, Vol. 11.05. American Society for Testing and Materials. West Conshohocken, PA.

Bay, S.M., D. Lapota, J. Anderson, J. Armstrong, T. Mikel, A. Jirik, and S. Asato. 2000. Southern California Bight 1998 Regional Monitoring Program: IV. Sediment toxicity. Southern California Coastal Water Research Project. Westminster, CA.

Bay, S.M., T. Mikel, K. Schiff, S. Mathison, B. Hester, D. Young, and D. Greenstein. 2005. Southern California Bight 2003 regional monitoring program: I. Sediment toxicity. Southern California Coastal Water Research Project. Westminster, CA.

Bay, S.M., D.J. Greenstein, M. Jacobe, C. Barton, K. Sakamoto, D. Young, K.J. Ritter, and K.C. Schiff. 2011. Southern California Bight 2008 regional monitoring program: I. Sediment toxicity. . Southern California Coastal Water Research Project. Costa Mesa, CA.

Bay, S.M., D.J. Greenstein, J.A. Ranasinghe, D.W. Diehl, and A.E. Fetscher. 2014. Sediment quality assessment technical support manual. Technical Report Number 777. Southern California Coastal Water Research Project. Costa Mesa, CA.

Chapman, P.M., B. Anderson, S. Carr, B. Engle, R. Green, J. Hameedi, M. Harmon, P. Haverland, J. Hyland, C. Ingersoll, E. Long, J. Rodgers, M. Salazar, P.K. Sibley, P.J. Smith, R.C. Swartz, B. Thompson, and H. Windom. 1997. General guidelines for using the Sediment Quality Triad. *Marine Pollution Bulletin* 34:368-372.

DeWitt, T.H., R.C. Swartz, and J.O. Lamberson. 1989. Measuring the acute toxicity of estuarine sediments. *Environmental Toxicology and Chemistry* 8:1035-1048.

Greenstein, D.J. and S.M. Bay. 2012. Selection of methods for assessing sediment toxicity in California bays and estuaries. *Integrated Environmental Assessment and Management* 8:625-637.

Schiff, K., S.B. Weisberg, and V. Raco-Rands. 2002. Inventory of ocean monitoring in the Southern California Bight. *Environmental Management* 29:871-876.

San Francisco Esturay Institute SFEI. 2013. The pulse of the Bay: Contaminants of emerging conern. SFEI Contribution 701. San Francisco Estuary Institute. Richmond, CA.

San Francisco Estuary Institute SFEI. 2014. 2012 annual monitoring results. The Regional Monitoring Program for Water Quality in the San Francisco Bay (RMP). Contribution #710. San Francisco Estuary Institute. Richmond, CA.

Stevens, D.L., Jr. 1997. Variable density grid-based sampling designs for continuous spatial populations. *Envirometrics* 8:167-195.

Stevens, D.L., Jr. and A.R. Olsen. 2003. Variance estimation for spatially balanced samples of environmental resources. *Envirometrics* 14:593-610.

State Water Resources Control Board SWRCB. 2009. Water quality control plan for enclosed bays and estuaries; Part I: Sediment quality. State Water Resources Control Board. Sacramento, CA.

Tay, K.L., K. Doe, P. Jackman, and A. McDonald. 1998. Assessment and evaluation of the effects of particle size, ammonia, and sulfide on the acute lethality test. Environment Canada Atlantic Division.

U.S. Environmental Protection Agency USEPA. 1994. Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA/600/R-94/025. Office of Research and Development, U.S. Environmental Protection Agency. Narragansett, RI.

U.S. Environmental Protection Agency USEPA. 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. EPA/600/R-95/136. Office of Research and Development. Cincinnati, OH.

U.S. Environmental Protection Agency USEPA. 2014. National Coastal Condition Assessment 2010: Draft Technical Appendix. U.S. Environmental Protection Agency, Office of Water. Washington, DC.

APPENDIX A. TOXICITY RESULTS BY STATION

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B13-8002	32.55662	-117.12821	Estuary	1.0	Tijuana River Estuary	97	89	Nontoxic
B13-8008	32.558283	-117.12053	Estuary	1.0	Tijuana River Estuary	97	89	Nontoxic
B13-8013	32.623601	-117.13346	Marina	3.0	San Diego Bay	(98)*	87	NA
B13-8014	32.626539	-117.13468	Marina	3.0	San Diego Bay	(108)*	100	NA
B13-8017	32.631569	-117.13084	Bay	4.0	San Diego Bay	96	95	Nontoxic
B13-8018	32.63417	-117.10733	Port	1.0	San Diego Bay	98	88	Nontoxic
B13-8020	32.641792	-117.13141	Bay	5.0	San Diego Bay	94	94	Nontoxic
B13-8028#	32.646603	-117.11935	Bay	12.0	San Diego Bay	(107)*	99	NA
B13-8029	32.646936	-117.11824	Bay	10.0	San Diego Bay	94	92	Nontoxic
B13-8030#	32.647272	-117.11667	Bay	13.0	San Diego Bay	(108)*	92	NA
B13-8031#	32.647579	-117.12148	Port	13.0	San Diego Bay	71	99	Low
B13-8033#	32.647521	-117.11945	Port	8.0	San Diego Bay	94	90	Nontoxic
B13-8036#	32.647856	-117.11614	Estuary	12.0	San Diego Bay	(108)*	100	NA
B13-8038#	32.648344	-117.11401	Estuary	12.0	Sweetwater Channel	(96)*	101	NA
B13-8040#	32.649219	-117.11006	Estuary	4.0	Sweetwater Channel	(103)*	97	NA
B13-8043	32.65037	-117.10509	Estuary	1.0	Sweetwater Channel	96	95	Nontoxic
B13-8045	32.65155	-117.12246	Port	12.0	San Diego Bay	82	99	Low
B13-8049#	32.656156	-117.12262	Port	12.0	San Diego Bay	95	94	Nontoxic
B13-8050#	32.657727	-117.12311	Port	12.0	San Diego Bay	103	95	Nontoxic
B13-8052	32.658339	-117.14422	Bay	5.0	San Diego Bay	100	98	Nontoxic
B13-8053#	32.658371	-117.11977	Port	5.0	San Diego Bay	100	94	Nontoxic
B13-8056	32.660613	-117.12339	Port	10.0	San Diego Bay	96	103	Nontoxic
B13-8058	32.661471	-117.14410	Bay	5.0	San Diego Bay	96	104	Nontoxic
B13-8060	32.665184	-117.14980	Bay	4.0	San Diego Bay	107	98	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B13-8064#	32.670959	-117.12396	Port	11.0	San Diego Bay	86	82	Low
B13-8065	32.671353	-117.11913	Port	7.0	San Diego Bay	91	92	Nontoxic
B13-8066#	32.671711	-117.12532	Port	12.0	San Diego Bay	97	96	Nontoxic
B13-8068	32.675472	-117.14384	Bay	5.0	San Diego Bay	90	106	Nontoxic
B13-8069#	32.676137	-117.12796	Port	12.0	San Diego Bay	93	89	Nontoxic
B13-8073	32.680331	-117.17476	Marina	5.0	San Diego Bay	98	99	Nontoxic
B13-8074#	32.685488	-117.13652	Port	10.0	San Diego Bay	97	102	Nontoxic
B13-8075#	32.68561	-117.13393	Port	9.0	San Diego Bay	99	102	Nontoxic
B13-8076#	32.686389	-117.13332	Port	10.0	San Diego Bay	97	100	Nontoxic
B13-8077#	32.686514	-117.13409	Port	10.0	San Diego Bay	104	96	Nontoxic
B13-8078	32.686723	-117.14859	Bay	13.0	San Diego Bay	94	94	Nontoxic
B13-8085	32.691687	-117.23824	Port	15.0	San Diego Bay	94	84	Nontoxic
B13-8087	32.691721	-117.15322	Port	13.0	San Diego Bay	96	99	Nontoxic
B13-8090#	32.692885	-117.14758	Port	8.0	San Diego Bay	88	99	Low
B13-8093	32.695601	-117.16256	Bay	14.0	San Diego Bay	99	84	Nontoxic
B13-8095#	32.696061	-117.15345	Port	13.0	San Diego Bay	91	95	Low
B13-8096#	32.698521	-117.15879	Bay	14.0	San Diego Bay	93	102	Nontoxic
B13-8098#	32.699765	-117.16098	Bay	15.0	San Diego Bay	97	92	Nontoxic
B13-8099#	32.702034	-117.16082	Bay	9.0	San Diego Bay	95	100	Nontoxic
B13-8100	32.7024	-117.16178	Port	9.0	San Diego Bay	94	97	Nontoxic
B13-8102	32.711543	-117.23255	Marina	7.0	San Diego Bay	90	83	Low
B13-8105	32.712275	-117.21397	Bay	4.0	San Diego Bay	98	89	Nontoxic
B13-8106	32.712329	-117.23213	Marina	6.0	San Diego Bay	90	83	Low
B13-8108#	32.714498	-117.23011	Marina	5.0	San Diego Bay	100	84	Nontoxic
B13-8109	32.714963	-117.18291	Bay	12.0	San Diego Bay	104	98	Nontoxic
B13-8111	32.716092	-117.17395	Port	12.0	San Diego Bay	92	95	Nontoxic
B13-8112	32.71619	-117.17624	Port	13.0	San Diego Bay	99	96	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B13-8113	32.716887	-117.22521	Marina	5.0	San Diego Bay	86	92	Low
B13-8116	32.718402	-117.23040	Marina	4.0	San Diego Bay	96	93	Nontoxic
B13-8117	32.718569	-117.22611	Marina	6.0	San Diego Bay	84	89	Low
B13-8118	32.719885	-117.17874	Bay	11.0	San Diego Bay	106	99	Nontoxic
B13-8121#	32.724358	-117.22482	Marina	3.0	San Diego Bay	96	91	Nontoxic
B13-8122	32.724148	-117.18298	Bay	5.0	San Diego Bay	103	103	Nontoxic
B13-8123	32.725018	-117.18368	Marina	6.0	San Diego Bay	102	92	Nontoxic
B13-8124	32.726301	-117.18664	Marina	4.0	San Diego Bay	97	96	Nontoxic
B13-8127	32.726737	-117.20252	Marina	4.0	San Diego Bay	73	95	Low
B13-8128	32.727123	-117.19192	Marina	5.0	San Diego Bay	97	95	Nontoxic
B13-8129	32.756983	-117.23530	Estuary	1.0	San Diego River	96	89	Nontoxic
B13-8134	32.757373	-117.23792	Estuary	1.0	San Diego River	99	96	Nontoxic
B13-8136	32.757755	-117.22732	Estuary	1.0	San Diego River	96	90	Nontoxic
B13-8145	32.761632	-117.23822	Marina	8.0	Mission Bay	97	92	Nontoxic
B13-8146	32.762461	-117.23621	Marina	8.0	Mission Bay	95	101	Nontoxic
B13-8151	32.767196	-117.23565	Marina	4.0	Mission Bay	92	106	Nontoxic
B13-8152	32.767905	-117.24148	Bay	7.0	Mission Bay	97	110	Nontoxic
B13-8156	32.780705	-117.24928	Marina	3.0	Mission Bay	92	102	Nontoxic
B13-8157	32.782939	-117.23000	Bay	3.0	Mission Bay	94	101	Nontoxic
B13-8159	32.784475	-117.21536	Bay	4.0	Mission Bay	95	102	Nontoxic
B13-8160#	32.787378	-117.20944	Bay	3.0	Mission Bay	96	95	Nontoxic
B13-8163#	32.794357	-117.22016	Bay	2.0	Mission Bay	95	108	Nontoxic
B13-8169	32.931677	-117.25208	Estuary	2.0	Los Penasquitos Lagoon	94	87	Nontoxic
B13-8176	32.933665	-117.25676	Estuary	1.0	Los Penasquitos Lagoon	98	95	Nontoxic
B13-8179	32.966143	-117.25237	Estuary	1.0	San Dieguito Lagoon	88	97	Low
B13-8180	32.966314	-117.25775	Estuary	1.0	San Dieguito Lagoon	98	96	Nontoxic
B13-8187	32.970816	-117.25821	Estuary	1.0	San Dieguito Lagoon	99	90	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B13-8188	33.016151	-117.28073	Estuary	1.0	San Elijo Lagoon	101	84	Nontoxic
B13-8189	33.012169	-117.27461	Estuary	1.0	San Elijo Lagoon	97	85	Nontoxic
B13-8200	33.08509	-117.30970	Estuary	2.0	Batiquitos Lagoon	90	90	Low
B13-8202	33.088069	-117.29129	Estuary	1.0	Batiquitos Lagoon	69	90	Low
B13-8205	33.088812	-117.29595	Estuary	2.0	Batiquitos Lagoon	95	82	Nontoxic
B13-8218	33.139112	-117.33757	Estuary	6.0	Agua Hedionda Lagoon	98	82	Nontoxic
B13-8219	33.139452	-117.31874	Estuary	1.0	Agua Hedionda Lagoon	93	87	Nontoxic
B13-8222	33.140126	-117.32438	Estuary	3.0	Agua Hedionda Lagoon	89	84	Low
B13-8233#	33.204705	-117.39083	Marina	4.0	Oceanside Harbor	93	90	Low
B13-8236#	33.207124	-117.39361	Marina	6.0	Oceanside Harbor	100	95	Nontoxic
B13-8239#	33.207931	-117.39744	Marina	7.0	Oceanside Harbor	100	86	Nontoxic
B13-8248	33.23197	-117.41291	Estuary	1.0	Santa Margarita Estuary	98	97	Nontoxic
B13-8250	33.232007	-117.41242	Estuary	1.0	Santa Margarita Estuary	100	87	Nontoxic
B13-8253	33.233248	-117.41343	Estuary	1.0	Santa Margarita Estuary	100	87	Nontoxic
B13-8259#	33.459462	-117.69730	Marina	4.0	Dana Point Harbor	93	94	Low
B13-8263#	33.460296	-117.70574	Marina	5.0	Dana Point Harbor	104	90	Nontoxic
B13-8265#	33.460884	-117.70213	Marina	4.0	Dana Point Harbor	97	88	Nontoxic
B13-8267#	33.462071	-117.70212	Marina	3.0	Dana Point Harbor	101	92	Nontoxic
B13-8269	33.600899	-117.89466	Marina	3.0	Newport Bay	98	96	Nontoxic
B13-8273	33.609098	-117.90464	Marina	6.0	Newport Bay	96	94	Nontoxic
B13-8274	33.613867	-117.91481	Marina	3.0	Newport Bay	97	98	Nontoxic
B13-8275	33.61549	-117.89395	Marina	1.8	Upper Newport Bay	53	96	Moderate
B13-8279	33.61652	-117.90530	Estuary	4.9	Upper Newport Bay	103	100	Nontoxic
B13-8280	33.61925	-117.92692	Marina	6.0	Newport Bay	98	99	Nontoxic
B13-8286	33.635776	-117.95621	Estuary	1.8	Santa Ana River	97	89	Low
B13-8287	33.636618	-117.95375	Estuary	0.9	Santa Ana River	88	92	Low
B13-8290	33.64579	-117.88890	Estuary	5.5	Upper Newport Bay	78	99	Low

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B13-8291	33.64676	-117.88682	Estuary	6.1	Upper Newport Bay	76	97	Low
B13-8292	33.64705	-117.88421	Estuary	5.8	Upper Newport Bay	72	99	Nontoxic
B13-8295	33.68658	-118.03602	Estuary	2.7	Bolsa Chica Lagoon	102	47	Low
B13-8298	33.69742	-118.04052	Estuary	2.4	Bolsa Chica Lagoon	99	88	Nontoxic
B13-8301	33.70983	-118.05969	Estuary	1.5	Bolsa Bay	98	100	Nontoxic
B13-8302	33.71242	-118.25790	Bay	18.0	Los Angeles/Long Beach	85	111	Low
B13-8304	33.71345	-118.24131	Bay	24.0	Los Angeles/Long Beach	93	85	Nontoxic
B13-8306	33.71475	-118.28269	Bay	3.0	Los Angeles/Long Beach	90	91	Low
B13-8308	33.7174	-118.24385	Port	23.0	Los Angeles/Long Beach	94	84	Low
B13-8310	33.71791	-118.23298	Port	14.0	Los Angeles/Long Beach	93	90	Nontoxic
B13-8315	33.72405	-118.15247	Bay	15.0	Los Angeles/Long Beach	44	98	Moderate
B13-8316	33.72387	-118.26270	Port	27.0	Los Angeles/Long Beach	95	102	Nontoxic
B13-8318	33.72421	-118.22437	Bay	18.0	Los Angeles/Long Beach	94	100	Nontoxic
B13-8319	33.7257	-118.13760	Bay	12.0	Los Angeles/Long Beach	97	101	Nontoxic
B13-8321	33.72707	-118.07011	Marina	4.9	Huntington Harbor	91	87	Low
B13-8322	33.72762	-118.21274	Bay	21.0	Los Angeles/Long Beach	90	92	Low
B13-8325	33.728683	-118.15700	Bay	14.0	Los Angeles/Long Beach	102	97	Nontoxic
B13-8326	33.72924	-118.23361	Port	11.0	Los Angeles/Long Beach	97	95	Nontoxic
B13-8328	33.73076	-118.08191	Estuary	4.6	Seal Beach	97	83	Low
B13-8333	33.7311	-118.19240	Port	15.0	Los Angeles/Long Beach	99	98	Nontoxic
B13-8338	33.73515	-118.09149	Port	14.0	Anaheim Bay	95	98	Nontoxic
B13-8340	33.73549	-118.27676	Port	18.0	Los Angeles/Long Beach	88	97	Low
B13-8346	33.739	-118.14465	Bay	10.0	Los Angeles/Long Beach	100	99	Nontoxic
B13-8347	33.73891	-118.21039	Port	27.0	Los Angeles/Long Beach	87	82	Low
B13-8349	33.73906	-118.23651	Port	14.0	Los Angeles/Long Beach	92	89	Low
B13-8350	33.7398	-118.17132	Bay	12.0	Los Angeles/Long Beach	48	76	Moderate
B13-8351	33.740133	-118.15877	Bay	11.0	Los Angeles/Long Beach	96	94	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B13-8353	33.74148	-118.11662	Estuary	2.0	San Gabriel River Estuary	93	95	Low
B13-8355	33.742717	-118.15320	Bay	10.0	Los Angeles/Long Beach	97	99	Nontoxic
B13-8356	33.74337	-118.20448	Port	19.0	Los Angeles/Long Beach	97	95	Nontoxic
B13-8358	33.744217	-118.16873	Bay	10.0	Los Angeles/Long Beach	77	93	Low
B13-8360	33.74553	-118.21570	Port	20.0	Los Angeles/Long Beach	100	96	Nontoxic
B13-8363	33.74719	-118.22137	Port	15.0	Los Angeles/Long Beach	96	103	Nontoxic
B13-8365	33.74767	-118.19819	Port	16.0	Los Angeles/Long Beach	88	128	Low
B13-8367	33.74853	-118.24890	Port	3.0	Los Angeles/Long Beach	98	112	Nontoxic
B13-8371	33.75109	-118.23063	Port	17.0	Los Angeles/Long Beach	98	82	Low
B13-8372	33.751271	-118.11864	Marina	3.7	Alamitos Bay	102	91	Nontoxic
B13-8374	33.75269	-118.21776	Port	18.0	Los Angeles/Long Beach	90	86	Low
B13-8375	33.7529	-118.17743	Bay	9.0	Los Angeles/Long Beach	94	94	Nontoxic
B13-8378	33.75302	-118.10528	Estuary	4.0	San Gabriel River	91	87	Low
B13-8382	33.75512	-118.23012	Port	18.0	Los Angeles/Long Beach	99	89	Nontoxic
B13-8383	33.755483	-118.12989	Marina	6.1	Alamitos Bay	101	106	Nontoxic
B13-8384	33.75686	-118.27742	Port	18.0	Los Angeles/Long Beach	98	113	Nontoxic
B13-8388	33.7594	-118.16267	Bay	5.0	Los Angeles/Long Beach	96	94	Nontoxic
B13-8389	33.760071	-118.12616	Marina	5.8	Alamitos Bay	91	95	Nontoxic
B13-8390	33.76074	-118.20169	Estuary	2.7	Los Angeles River	91	96	Low
B13-8391	33.76273	-118.20478	Estuary	6.1	Los Angeles River	52	29	High
B13-8394	33.766034	-118.10371	Estuary	2.4	Los Alamitos Estuary	82	108	Low
B13-8396	33.7662	-118.27747	Port	15.0	Los Angeles/Long Beach	93	100	Nontoxic
B13-8397	33.767	-118.24938	Marina	4.0	Los Angeles/Long Beach	93	110	Nontoxic
B13-8399	33.76871	-118.22204	Port	19.0	Los Angeles/Long Beach	99	105	Nontoxic
B13-8401	33.77158	-118.21180	Port	14.0	Los Angeles/Long Beach	88	80	Low
B13-8403	33.78083	-118.20569	Estuary	2.7	Los Angeles River	45	2	High
B13-8407	33.9647	-118.45352	Marina	5.0	Marina del Rey	99	92	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B13-8409	33.970367	-118.44768	Marina	5.0	Marina del Rey	97	98	Nontoxic
B13-8411	33.97108	-118.43923	Estuary	2.1	Ballona Creek	92	97	Low
B13-8413	33.975617	-118.44697	Marina	4.0	Marina del Rey	93	83	Low
B13-8417	33.983083	-118.45075	Marina	2.0	Marina del Rey	82	93	Low
B13-8419	34.10065	-119.08708	Estuary	0.9	Mugu Lagoon-south	102	99	Nontoxic
B13-8421	34.11264	-119.08406	Estuary	1.8	Mugu Lagoon-south	81	98	Low
B13-8425	34.1712	-119.22348	Marina	3.0	Channel Islands Harbor	73	99	Low
B13-8426	34.1731	-119.22353	Marina	4.0	Channel Islands Harbor	96	94	Nontoxic
B13-8430	34.24424	-119.26496	Marina	2.0	Ventura Harbor	91	95	Nontoxic
B13-8431	34.40453	-119.68904	Marina	2.0	Santa Barbara	97	96	Nontoxic
B13-8500#	32.727047	-117.17733	Port	6.0	San Diego Bay	96	91	Nontoxic
B13-9007	32.55148	-117.19950	Shelf	35.0	South San Diego Shelf	91	NA	Nontoxic
B13-9011	32.58574	-117.34070	Shelf	183.0	South San Diego Shelf	64	NA	Moderate
B13-9012	32.58969	-117.26429	Shelf	58.0	South San Diego Shelf	101	NA	Nontoxic
B13-9025	32.67264	-117.29913	Shelf	77.0	South San Diego Shelf	101	NA	Nontoxic
B13-9037	32.76406	-117.31932	Shelf	68.0	South San Diego Shelf	101	NA	Nontoxic
B13-9069	32.88862	-117.53744	Canyon	839.0	La Jolla Canyon	95	NA	Nontoxic
B13-9071	32.90256	-117.50521	Canyon	805.0	La Jolla Canyon	97	NA	Nontoxic
B13-9076	32.91632	-117.36379	Canyon	533.0	La Jolla Canyon	58	NA	High
B13-9095	33.03868	-117.50238	Canyon	676.0	Carlsbad Canyon	52	NA	High
B13-9099	33.06344	-117.49002	Canyon	634.0	Carlsbad Canyon	86	NA	Low
B13-9105	33.08764	-117.35097	Shelf	73.0	North San Diego Shelf	97	NA	Nontoxic
B13-9106	33.09091	-117.43286	Canyon	484.0	Carlsbad Canyon	92	NA	Nontoxic
B13-9125	33.221016	-117.51148	Shelf	181.0	North San Diego Shelf	99	NA	Nontoxic
B13-9129	33.265584	-117.53345	Shelf	62.0	North San Diego Shelf	97	NA	Nontoxic
B13-9150	33.464034	-117.76190	Shelf	155.0	Orange Shelf	100	NA	Nontoxic
B13-9151	33.464168	-118.06584	Canyon	582.0	San Gabriel Canyon	98	NA	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B13-9160	33.504268	-117.91602	Canyon	493.0	Newport Canyon	99	NA	Nontoxic
B13-9170	33.515133	-118.07760	Canyon	484.0	San Gabriel Canyon	94	NA	Nontoxic
B13-9171	33.520951	-117.77025	Shelf	16.0	Orange Shelf	102	NA	Nontoxic
B13-9178	33.553682	-117.92879	Canyon	359.0	Newport Canyon	96	NA	Nontoxic
B13-9193	33.58505	-118.11346	Canyon	154.0	San Gabriel Canyon	99	NA	Nontoxic
B13-9197	33.600301	-117.93322	Canyon	63.0	Newport Canyon	96	NA	Nontoxic
B13-9199	33.601949	-118.05646	Shelf	38.0	San Pedro Shelf	100	NA	Nontoxic
B13-9202	33.621	-118.19500	Shelf	42.0	San Pedro Shelf	103	NA	Nontoxic
B13-9204	33.627799	-117.98752	Shelf	13.0	San Pedro Shelf	101	NA	Nontoxic
B13-9205	33.63057	-118.26751	Canyon	200.0	San Pedro Valley	97	NA	Nontoxic
B13-9210	33.6391	-118.34243	Canyon	700.0	San Pedro Valley	94	NA	Nontoxic
B13-9211	33.64003	-118.33711	Canyon	710.0	San Pedro Valley	80	NA	Moderate
B13-9214	33.6434	-118.07874	Shelf	26.0	San Pedro Shelf	98	NA	Nontoxic
B13-9217	33.6481	-118.14900	Shelf	31.0	San Pedro Shelf	99	NA	Nontoxic
B13-9225	33.68738	-118.52052	Canyon	820.0	Redondo Canyon	90	NA	Low
B13-9229	33.6952	-118.29600	Shelf	27.0	Palos Verdes Shelf	104	NA	Nontoxic
B13-9246	33.73593	-118.52812	Canyon	730.0	Redondo Canyon	85	NA	Low
B13-9250	33.7604	-118.54171	Canyon	660.0	Redondo Canyon	88	NA	Low
B13-9251	33.7671	-118.46000	Shelf	133.0	Santa Monica Bay	101	NA	Nontoxic
B13-9266	33.860133	-118.44778	Shelf	60.0	Santa Monica Bay	91	NA	Nontoxic
B13-9277	33.918483	-118.56313	Canyon	176.0	Santa Monica Canyon	95	NA	Nontoxic
B13-9281	33.921533	-118.65020	Canyon	448.0	Santa Monica Canyon	93	NA	Nontoxic
B13-9285	33.935267	-118.55220	Canyon	100.0	Santa Monica Canyon	91	NA	Low
B13-9290	33.94136	-118.84450	Canyon	719.0	Dume Canyon	97	NA	Nontoxic
B13-9292	33.943783	-118.51978	Shelf	48.0	Santa Monica Bay	98	NA	Nontoxic
B13-9295	33.95007	-118.83696	Canyon	690.0	Dume Canyon	89	NA	Nontoxic
B13-9305	33.966217	-118.81142	Canyon	564.0	Dume Canyon	89	NA	Low

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B13-9307	33.96886	-119.07025	Canyon	755.0	Mugu Canyon	(104)*	NA	NA
B13-9338	34.0213	-119.22674	Canyon	595.0	Hueneme Canyon	(107)*	NA	NA
B13-9341	34.0233	-118.59348	Shelf	23.0	Santa Monica Bay	88	NA	Low
B13-9347	34.03989	-119.23463	Canyon	550.0	Hueneme Canyon	(110)*	NA	NA
B13-9349	34.04245	-119.08589	Canyon	562.0	Mugu Canyon	(107)*	NA	NA
B13-9350	34.04413	-119.05558	Shelf	205.0	Hueneme to Dume	(101)*	NA	NA
B13-9359	34.06644	-119.13415	Shelf	88.0	Hueneme to Dume	91	NA	Low
B13-9369	34.08348	-119.09496	Canyon	160.0	Mugu Canyon	100	NA	Nontoxic
B13-9371	34.09077	-119.23904	Canyon	341.0	Hueneme Canyon	94	NA	Nontoxic
B13-9383	34.12488	-119.19248	Shelf	15.0	Hueneme to Dume	97	NA	Nontoxic
B13-9397	34.17863	-119.34714	Shelf	26.0	East Santa Barbara Channel	91	NA	Low
B13-9416	34.2303	-119.68726	Shelf	138.0	East Santa Barbara Channel	100	NA	Nontoxic
B13-9417	34.23287	-119.70663	Shelf	159.0	East Santa Barbara Channel	103	NA	Nontoxic
B13-9434	34.28368	-119.35453	Shelf	18.0	East Santa Barbara Channel	94	NA	Nontoxic
B13-9440	34.30786	-119.71283	Shelf	139.0	East Santa Barbara Channel	98	NA	Nontoxic
B13-9464	34.39477	-120.33174	Shelf	184.0	West Santa Barbara Channel	97	NA	Nontoxic
B13-9466	34.396139	-119.66200	Shelf	24.0	East Santa Barbara Channel	97	NA	Nontoxic
B13-9467	34.398397	-119.86485	Shelf	29.0	Campus Point	98	NA	Nontoxic
B13-9470	34.400981	-119.83279	Shelf	29.0	East Santa Barbara Channel	99	NA	Nontoxic

NA= Not analyzed

*= Test batch did not meet test acceptability criteria

#= Station was targeted and not randomly selected. Therefore it was not used in the area calculations (i.e., regional assessment) associated with its stratum. The station was used in the descriptive results calculations.

APPENDIX B: STATION CROSS REFERENCE

Cross reference of station IDs for station sampled in Bight'13 and in at least one previous Bight survey.

Bight'13	Bight'08	Bight'03	Bight'98
8002	6001	4695	-
8008	-	-	-
8013	6025	4052	-
8014	6027	4212	-
8017	-	-	-
8018	-	-	-
8020	-	-	-
8028	6039	-	-
8029	6040	4148	-
8030	6041	-	-
8031	6042	-	-
8033	6659	-	-
8036	6045	-	-
8038	6046	-	-
8040	6047	-	-
8043	-	-	-
8045	6054	-	2262
8049	6660	-	-
8050	6661	-	-
8052	6071	4116	-
8053	6072	-	-
8056	6075	4084	-
8058	-	-	-
8060	6080	-	2242
8064	6085	-	-
8065	-	-	-
8066	6087	-	-
8068	6093	4028	-
8069	6094	-	-
8073	-	-	-
8074	6115	-	-
8075	6116	-	-
8076	6119	-	-
8077	6120	-	-
8078	-	-	-
8085	6128	-	2441
8087	6129	-	2252

Bight'13	Bight'08	Bight'03	Bight'98
8090	6685	-	-
8093	-	-	-
8095	6133	-	-
8096	6134	-	-
8098	6136	-	-
8099	6688	-	-
8100	6140	-	2251
8102	6145	-	2226
8105	-	-	-
8106	-	-	-
8108	6151	-	-
8109	6152	-	2436
8111	6154	4108	-
8112	6155	-	2263
8113	6157	4140	-
8116	6159	4076	-
8117	6161	-	2222
8118	-	-	-
8121	6171	-	-
8122	6172	4092	-
8123	6173	-	2434
8124	-	-	-
8127	6177	4284	-
8128	-	-	-
8129	6181	4264	-
8134	-	-	-
8136	6192	4033	-
8145	-	-	-
8146	6204	4204	-
8151	6211	-	2425
8152	6212	4020	-
8156	6216	-	2423
8157	-	-	-
8159	6217	4228	-
8160	6219	-	-
8163	6223	-	-
8169	-	-	-
8176	-	-	-
8179	-	-	-
8180	-	-	-

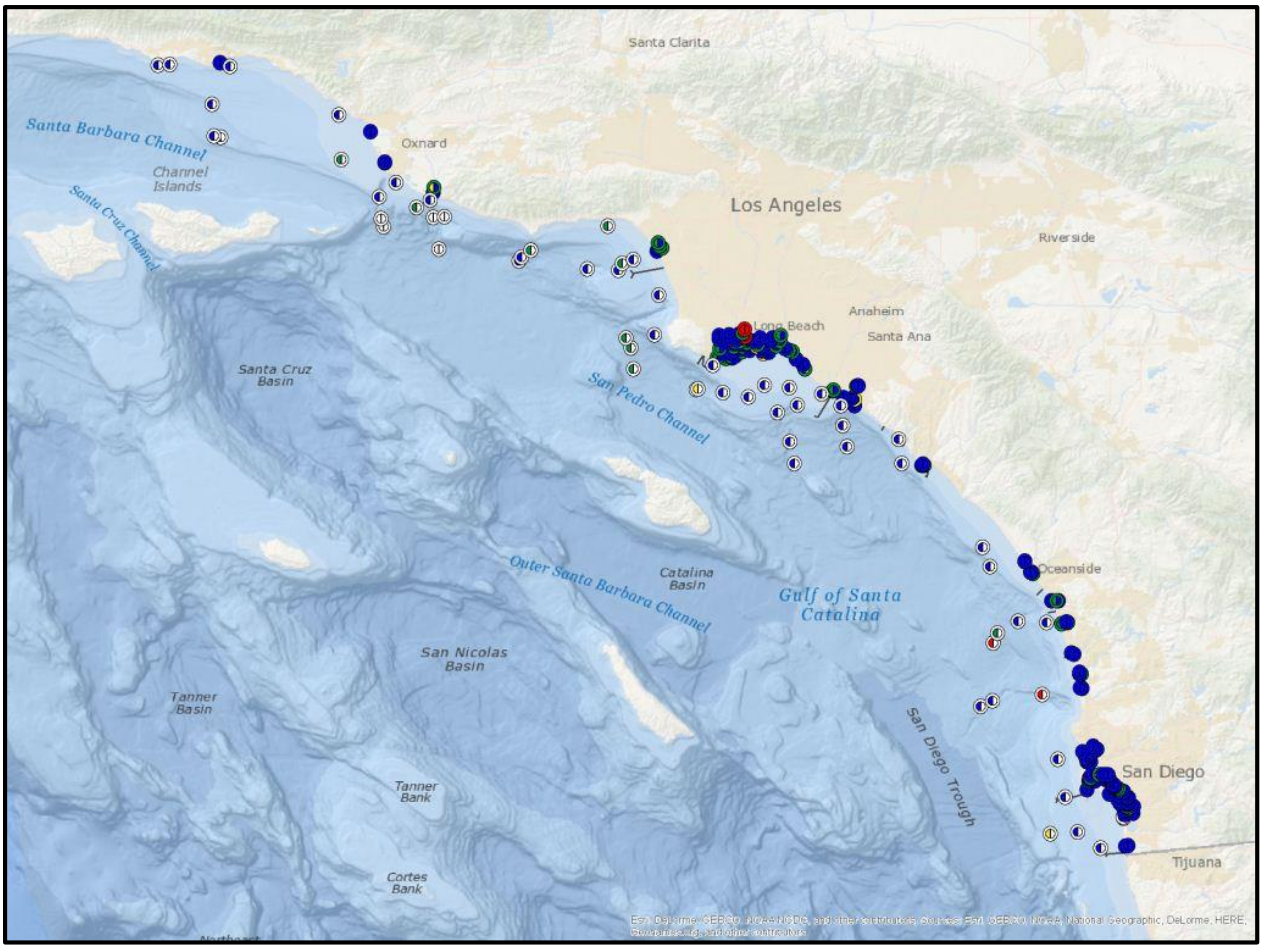
Bight'13	Bight'08	Bight'03	Bight'98
8187	-	-	-
8188	-	-	-
8189	-	-	-
8200	-	-	-
8202	-	-	-
8205	-	-	-
8218	6269	4049	-
8219	6270	4087	-
8222	6271	4304	-
8233	6288	-	-
8236	6291	-	-
8239	6294	-	-
8248	6303	4209	-
8250	-	-	-
8253	-	-	-
8259	6320	-	-
8263	6325	-	-
8265	6327	-	-
8267	6328	-	-
8269	-	-	-
8273	6343	4065	-
8274	-	-	-
8275	-	-	-
8279	-	-	-
8280	6350	-	2136
8286	-	-	-
8287	6355	4072	-
8290	6362	4017	-
8291	-	-	-
8292	6363	4075	-
8295	-	-	-
8298	-	-	-
8301	-	-	-
8302	6386	4178	-
8304	6387	-	2162
8306	-	-	-
8308	-	-	-
8310	-	-	-
8315	-	-	-
8316	6402	-	2182

Bight'13	Bight'08	Bight'03	Bight'98
8318	6404	4242	-
8319	-	-	-
8321	-	-	-
8322	-	-	-
8325	6411	4274	-
8326	6413	-	2298
8328	-	-	-
8333	6419	4162	-
8338	-	-	-
8340	-	-	-
8346	-	-	-
8347	6435	-	2179
8349	-	-	-
8350	6437	-	2156
8351	-	-	-
8353	-	-	-
8355	6444	-	2157
8356	-	-	-
8358	6448	4098	-
8360	6450	4146	-
8363	-	-	-
8365	-	-	-
8367	-	-	-
8371	6463	-	2432
8372	-	-	-
8374	6466	4210	-
8375	-	-	-
8378	6468	4194	-
8382	-	-	-
8383	6472	4018	-
8384	-	-	-
8388	6478	-	2152
8389	-	-	-
8390	-	-	-
8391	-	-	-
8394	6485	4118	-
8396	6487	4266	-
8397	6489	4010	-
8399	-	-	-
8401	-	-	-

Bight'13	Bight'08	Bight'03	Bight'98
8403	6500	4142	-
8407	6513	4085	-
8409	6518	-	2448
8411	6520	4053	-
8413	-	-	-
8417	6530	-	2443
8419	-	-	-
8421	-	-	-
8425	6549	-	2130
8426	-	-	-
8430	-	-	-
8431	-	-	-
8500	-	-	-
9007	7002	4000	-
9011	7008	4068	-
9012	7009	-	2419
9025	-	-	-
9037	-	-	-
9069	-	-	-
9071	-	-	-
9076	-	-	-
9095	-	-	-
9099	-	-	-
9105	7122	4048	-
9106	-	-	-
9125	7158	4144	-
9129	7166	4080	-
9150	7208	4110	-
9151	-	-	-
9160	-	-	-
9170	-	-	-
9171	7231	-	2304
9178	-	-	-
9193	-	-	-
9197	-	-	-
9199	7269	-	2208
9202	7287	4026	-
9204	7293	-	2325
9205	-	-	-
9210	-	-	-

Bight'13	Bight'08	Bight'03	Bight'98
9211	-	-	-
9214	7300	4058	-
9217	7301	-	2396
9225	-	-	-
9229	7321	4042	-
9246	-	-	-
9250	-	-	-
9251	7395	4038	-
9266	7417	4006	-
9277	-	-	-
9281	-	-	-
9285	-	-	-
9290	-	-	-
9292	7461	-	2192
9295	-	-	-
9305	-	-	-
9307	-	-	-
9338	-	-	-
9341	7517	-	2382
9347	-	-	-
9349	-	-	-
9350	7528	4133	-
9359	7542	4093	-
9369	-	-	-
9371	-	-	-
9383	7596	4003	-
9397	7629	-	2376
9416	7652	4067	-
9417	7654	4103	-
9434	7681	4043	-
9440	7696	4023	-
9464	7727	4111	-
9466	7728	4047	-
9467	7735	-	2359
9470	7741	-	2301

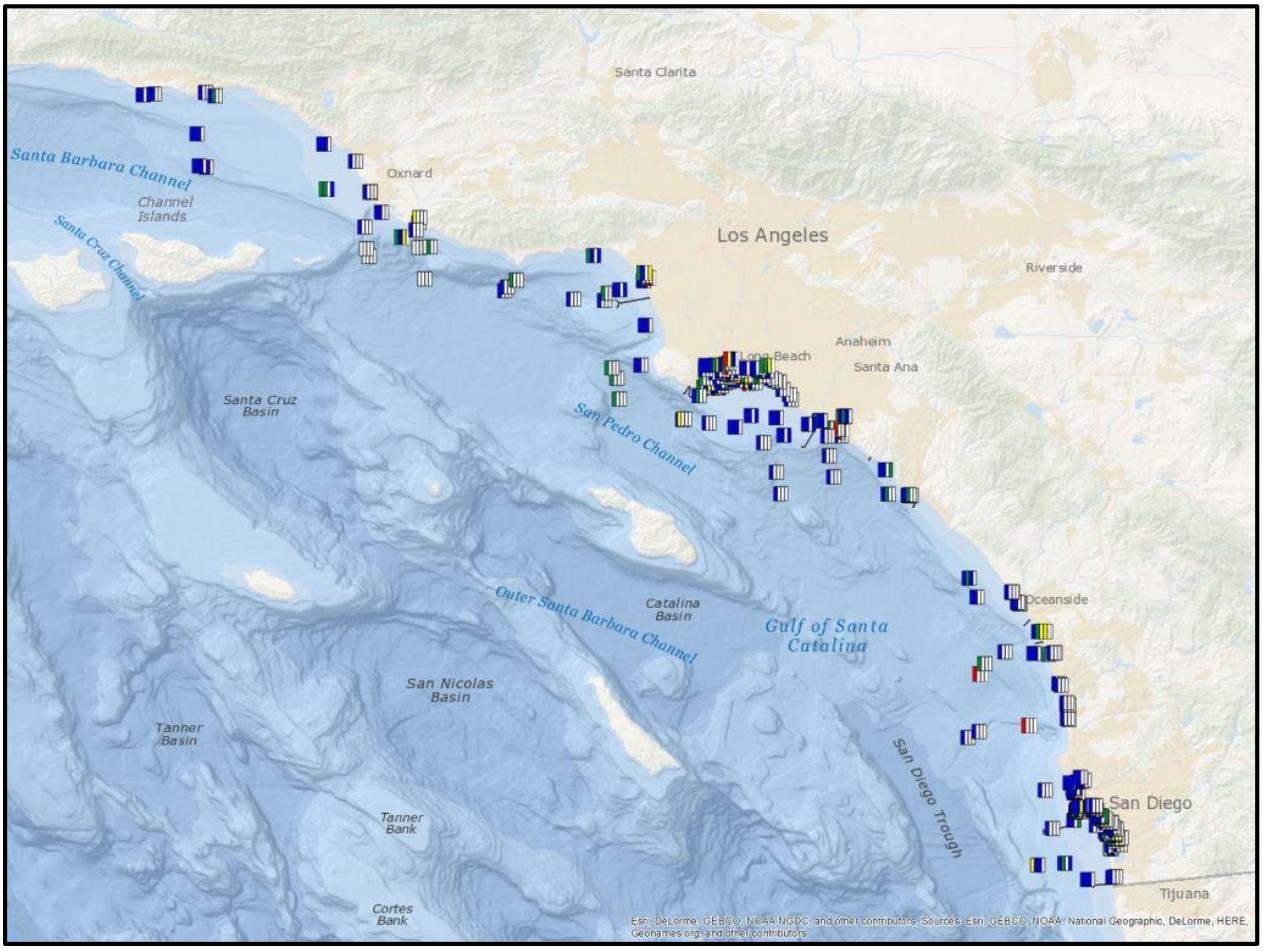
APPENDIX C: INTERACTIVE MAP OF BIGHT'13 AND BIGHT'08 TOXICITY RESULTS



The link below is for a scalable map of the Bight containing symbols representing SQO categorization of the amphipod, mussel, and integrated results from Bight'13 and Bight'08. Note that the results from each survey are on different layers that cannot be properly viewed simultaneously. Click on the layer icon in the upper left of the map and a table of the possible layers to view will open; make sure either Bight'13 or Bight'08 are chosen, not both. The Bight'08 layer contains symbols for all stations sampled in Bight'13, even if they were not sampled in Bight'08. The information icon contains a legend for the symbols. At the bottom center of the map is an upward arrow icon. Clicking on this icon opens a table of information for all of the stations. This table can be sorted by any of the columns by clicking in the column header. Clicking on any station in the table zooms and centers the map to that station. Clicking any station symbol on the map opens a table containing the toxicity information for Bight'13 and Bight'08. The table also contains the station information from Bight'13 (e.g. latitude and longitude).

Bight'13 Sediment Toxicity Report – Appendix C

APPENDIX D: INTERACTIVE MAP OF AMPHIPOD TOXICITY RESULTS FOR BIGHT'13 AND RESAMPLED STATIONS FROM PREVIOUS SURVEYS



The link below is for a scalable map of the Bight containing symbols representing SQO categorization of the amphipod test results from Bight'98, Bight'03, Bight'08, and Bight'13. The information icon contains a legend for the symbols. At the bottom center of the map in an upward arrow icon. Clicking on this icon opens a table of information for all of the stations. This table can be sorted by any of the columns by clicking in the column header. Clicking on any station in the table zooms and centers the map to that station. Clicking any station symbol on the map opens a table containing the toxicity information for all of the surveys represented. The table also contains the station information from Bight'13 (e.g. latitude and longitude).

Bight'13 Sediment Toxicity Report – Appendix D

APPENDIX E: DETAILS OF SPLIT SAMPLE COMPARABILITY ASSESSMENT METHODS

Comparability of the laboratories for the split samples was based on three factors: the percentage difference from the mean for each sample, a comparison of the toxicity category for each sample, and results from the reference toxicant test.

For the percentage difference from the mean the following procedure was used:

1. The data was pooled from all labs, treating each sample separately.

2. Removed outlier laboratory's data (if any) for each sample, which was not included in the grand mean (Grubb's test).

3. Calculate grand mean.

4. Assigned points to each laboratory based on the percentage difference between their mean and the grand mean (Table 1).

5. Sum the points assigned from each sample.

Given that there were two samples for comparison, the maximum attainable score for this evaluation factor was 6.

Table 1. Summary of scoring system for percent survival or normal alive data and toxicity category.

% Survival or Normal-alive (absolute difference from grand mean)		**Toxicity Category Agreement**	
Result	Pts	Result	Pts
0 – 10 %	3	Same cat.	1.5
>10 – 20 %	2	1 cat. difference	1.0
>20 – 30 %	1	2 cat. difference	0.5
> 30 %	0	3 cat. difference	0

The second comparison factor was based on the sediment toxicity category. For each sample, the grand mean was used to place the sample into a toxicity category based on California Sediment Quality Objectives thresholds (Table 2). The results for each laboratory were also assigned to a category. The category from the grand mean and for the individual samples was then compared. The number of categories difference was then used to assign point values (Table 1). For example, if the grand mean placed the sample in the nontoxic category and an individual laboratory was in the moderate toxicity category, then the difference would be 2 categories and 0.5 points would be assigned. Since there were two samples, the maximum points awarded for this category was 3.

Table 2. Threshold values for sediment toxicity test response.

Test species/endpoint	Statistical Significance	Nontoxic (%)	Low Toxicity (% Control)	Moderate Toxicity (% Control)	High Toxicity (% Control)
E. estuarius	Significant	90 to 100	82 to 89	59 to 81	< 59
Survival	Not Sig.	82 to 100	59 to 81		< 59
M. galloprovincialis	Significant	80 to 100	77 to 79	42 to 76	< 42
Normal Development	Not Sig.	77 to 79	42 to 76		< 42

The final factor to be considered was the reference toxicant. The evaluation method involved the following steps:

1. Collected ammonia reference toxicant data from all laboratories for both *Eohaustorius* and *Mytilus* tests (historical data). Data was formatted as mg/L un-ionized ammonia.

2. Calculated the standard deviation (SD) for all of the historical EC_{50}/LC_{50} data for each species.

3. Pooled intercalibration reference toxicant EC_{50}/LC_{50} data from all labs.

4. Removed outlier laboratory's data for each sample, which was not included in the grand mean (Grubb's test).

5. Calculated grand mean.

6. Calculated the difference from the grand mean for each laboratory.

7. Compared the difference from the grand mean to the standard deviation from the historical data and assign points as shown in Table 3.

As an example, we will say that the SD for all historical data for one of the methods is 0.1. The mean value for the labs participating in the intercalibration we will say is 0.124 mg/L un-ionized ammonia. If Lab A found the LC50 to be 0.263, then the difference would be 0.139 which is greater than 1 SD, but less than 2, so would therefore get a score of 2 points. The maximum achievable score for the reference toxicant evaluation factor was 3.

Table 3. Summary of scoring system for duplicate sample and reference toxicant results.

Reference Tox. (deviation from grand mean)	
Result	**Pts**
Within 1 SD	3
Within 2 SD	2
Within 3 SD	1
>3 SD	0

For integration of the three comparison factors, the points were summed for each laboratory. The "grading" system for the total score is shown in Table 4.

Table 4. Scoring system for sum of all factors

Description	% of maximum possible score	Number of points
Very High comparability	90	12-11
High comparability	80	10.5-9.5
Moderate comparability	70	9.0-8.0
Low comparability	<70	<8.0

APPENDIX F: ALTERNATIVE TOXICITY TEST METHOD COMPARISON USING *NEANTHES ARENACEODENTATA*

Christina Pottios, Sanitation Districts of Los Angeles County

Introduction

The sediment quality objectives (SQO) program was adopted in 2008 by the California State Water Resources Control Board for enclosed bays and estuaries. The SQO program includes a sediment quality assessment framework based on a multiple line of evidence (LOE) approach, integrating sediment toxicity, benthic community condition, and sediment chemistry. Sediment toxicity assessment under the SQO assessment framework requires, at a minimum, the use of one short-term acute survival and one sub-lethal sediment toxicity test method. Table 1 identifies the acceptable SQO toxicity test methods. Although only one acute and one sub-lethal toxicity test is required, multiple tests can be conducted. The data from each method are compared to a series of thresholds to categorize the results as Nontoxic, Low Toxicity, Moderate Toxicity, or High Toxicity. The average of all test method categories is classified into a final Toxicity LOE result (SWRCB 2009). The methods described in California's SQO policy provide the framework for interpreting sediment toxicity results in this study (Bay et al. 2014).

Table 1: Acceptable SQO Sediment Toxicity Test Methods (SWRCB 2009)

Test Organism	Exposure Type	Duration	Endpoint
Acute Sediment Toxicity Test Methods			
Eohaustorius estuarius	Whole Sediment	10 days	Survival
Leptocheirus plumulosus	Whole Sediment	10 days	Survival
Rhepoxynius abronius	Whole Sediment	10 days	Survival
Sub-lethal Sediment Toxicity Test Methods			
Neanthes arenaceodentata	Whole Sediment	28 days	Growth
Mytilus galloprovincialis	Sediment-Water Interface	48 hours	Embryo Development

Most recent sediment quality assessments have used only two of the recommended SQO test methods: the *Eohaustorius estuarius* (EE) amphipod acute 10-day survival test and the 48-hour embryo development test using *Mytilus galloprovincialis* (MG) at the sediment-water interface. One of the recommendations resulting from the Bight'08 regional survey was to include other SQO recommended toxicity test methods in future surveys (Bay et al. 2011). In addition to the MG sub-lethal toxicity test method, the 28-day *Neanthes arenaceodentata* (NA) growth test using whole sediment is also an approved SQO sub-lethal sediment toxicity test. The NA growth test has not been used to measure toxicity in Southern California in previous regional surveys, thus the performance of this test relative to other SQO toxicity methods is not well documented. While there are many technical differences in running these two methods, little is known about the differences of the individual test method toxicity category result, the benefit of running multiple sub-lethal tests, or the relative variability between replicates for each of the methods.

Methods

Study Design

Toxicity testing was conducted with the Bight'13 survey on a subset of stations and utilized the same EE and MG testing effort used to assess the main survey questions. Seventeen sediment testing locations were chosen, representing diverse environmental conditions as well as areas of special interest from groups participating in Bight'13. For each of the seventeen sediment samples collected, the EE acute toxicity test method and the sub-lethal methods using NA and MG were conducted concurrently. Acute EE sediment toxicity tests and MG sub-lethal tests were conducted by either the Sanitation Districts of Los Angeles County (LACSD), the City of San Diego Public Utilities Department (CSD), or the City of Los Angeles Sanitation (CLAEMD). All of the NA sub-lethal sediment toxicity tests were conducted by LACSD using methods in accordance with those specified for the SQO program.

For the NA tests, a total of three batches of samples were tested with each batch conducted approximately one month apart. The first batch of samples was collected from five sampling locations within the San Diego shelf. The second batch of tests consisted of samples collected from the Long Beach region and included samples from Alamitos Bay Marina, neighboring estuary locations, and the San Gabriel River. The third batch of tests was comprised of six samples collected in the Los Angeles area from Marina del Rey and Ballona Creek Estuary. An additional sample from the Los Angeles River Estuary was added to this batch. Table 2 identifies samples and testing dates for each of the test batches.

Table 2: Sample Identification

Batch	Test Initiation Date	Sample Locations Tested	Sample Description
1	July 18, 2013	B13-9012, B13-9037, B13-9025, CSD-E26, CSD-E14	San Diego Shelf
2	August 22, 2013	B13-8394, B13-8372, B13-8383, B13-8389, B13-8353, B13-8378	Los Alamitos Estuary, Alamitos Bay Marina, San Gabriel River
3	September 26, 2013	B13-8417, B13-8413, B13-8409, B13-8411, B13-8407, B13-8391	Marina del Rey, Ballona Creek Estuary, Los Angeles River Estuary

Batch 1 included stations that were located on the mainland shelf and thus not targeted for testing using MG as part of the Bight'13 survey. Two of the stations were not part of the Bight'13 survey design (CSD-E26 and CSD-E14), but were included due to special interest from CSD. So that comparisons between all three test methods could be made for these non-embayment stations, CSD ran concurrent MG tests and LACSD ran concurrent EE tests for these sites. The samples selected in batch 2 and 3 were all from embayment stations and were from areas of interest since previous Bight surveys had indicated Low to High Toxicity within these regions.

Neanthes Sediment Toxicity Test

Testing procedures for the NA tests were based on a combination of published protocol (ASTM 2002) and guidelines that have been recommended for the California SQO program (Bay et al. 2014). The major differences from the ASTM version were: 1) the utilization of less than seven-day-old, post-emergent juveniles instead of two to three-week-old worms, 2) the reduction of the exposure chamber volume from 1 L to 300 mL, 3) the reduction of the number of worms per chamber from five to one, and 4) the increase in the number of replicates per treatment from five to ten. To evaluate the relative sensitivity of the test organisms, a water-only reference toxicant test was performed with each test batch using ammonium chloride.

Samples were collected in accordance with the Bight 2013 Field Operations Manual (Bight'13 Field Sampling and Logistics Committee 2013). Upon collection, sediment was placed into 1 L high density polyethylene (HDPE) containers and stored in the dark at 4°C. All tests were initiated within two weeks of sample collection. Control sediment consisted of sediment from a station in LA/LB harbor that had been used routinely to assess the acceptability of this test. Prior to testing, sediments were thoroughly homogenized and sieved through a 1.0 mm mesh screen to remove organisms and debris using only the water available in the sample.

All NA tests were performed in 300 mL tall-form beakers containing approximately 75 mL of homogenized sediment and approximately 125 mL of 30 ± 2 ‰ filtered seawater. Sediment, water, and aeration were added to the beakers 24 hours prior to the addition of animals. Each sample consisted of 10 replicates and each replicate contained one organism. Organisms were purchased from Aquatic Toxicology Support in Bremerton, Washington and held at testing conditions for 1 day prior to being introduced to sediment samples.

On initiation day, an additional five organisms were randomly pre-selected for initial weight measurements. These organisms were rinsed with de-ionized water, placed on a pre-weighed pan and dried in an oven at 60°C for 24 hours. After 24 hours in the drying oven, the pans were removed, allowed to cool in a desiccator, and then weighed to obtain an initial weight for growth calculations.

During testing, temperature was maintained at 20 ± 2°C and light levels were kept at 500 to 1000 lux with a 12 hour light, 12 hour dark photoperiod. Test maintenance included daily observations to ensure proper aeration and the documentation of any unusual animal behavior or abnormality. At test initiation, prior to each water change and at test termination water quality parameters including pH, dissolved oxygen (DO), temperature, salinity, and ammonia of the overlying water were analyzed. The same water quality analyses were performed on pore water at test initiation and termination. Approximately 60 mL of overlying water was exchanged from each beaker once per week. Each replicate was fed twice per week a combination of Tetramarin® and alfalfa.

At test termination, the sediment from the chambers was sieved through a 425 µm mesh sieve and the number of surviving organisms was recorded. Surviving animals in each replicate were then rinsed with de-ionized water, put on pre-weighed pans, and placed in a drying oven at 60°C for 24 hours. After 24 hours in the drying oven the pans were removed, allowed to cool in a desiccator, and weighed to obtain the individual dry weight for each replicate to the nearest 0.1 mg.

Neanthes Data Analysis

Data were analyzed by comparing the growth rate in the test sediments to that in the control. The growth rate was calculated using the guidance provided by (ASTM 2002). Growth rate is calculated based on the change in weight of surviving worms. The following equation was used:

Eq 1: $$G = \frac{DW_t - DW_i}{T}$$

Where:

G = estimated individual growth rate, mg dry weight/day,
DW_t = mean estimated individual dry weight at the termination of the experiment, mg
DW_i = mean estimated individual dry weight at the initiation of the experiment, mg
T = exposure time, days.

Method Comparability

For each test method, statistical comparisons between the control and test samples were conducted using an unequal variance t two sample test. To calculate the magnitude of the response, the data were control normalized where appropriate. Individual toxicity categories of Nontoxic, Low Toxicity, Moderate Toxicity, or High Toxicity were assigned for each test method using SQO guidance as seen in Table 3. Categories were determined using a combination of statistical significance and the magnitude of the response.

Table 3: Sediment toxicity categorization values (SWRCB 2009)

Test Species/Endpoint	Statistical Significance	Nontoxic (%)	Low Toxicity (% of Control)	Moderate Toxicity (% of Control)	High Toxicity (% of Control)
EE Survival	Significant	90 to 100	82 to 89	59 to 81	<59
EE Survival	Not Significant	82 to 100	59 to 81		<59
NA Growth	Significant	90 to 100*	68 to 90	46 to 67	<46
NA Growth	Not Significant	68 to 100	46 to 67		<46
MG Percent Normal-Alive	Significant	80 to 100	77 to 79	42 to 76	<42
MG Percent Normal- Alive	Not Significant	77 to 79	42 to 76		<42

*Expressed as a percent of the control

For the final toxicity LOE category, the averages of all possible combinations of individual toxicity categories for the acute and sub-lethal test methods were determined: EE + MG, EE + NA, and EE + MG + NA. If averages fell midway between categories, the final LOE category was rounded to the next higher category. The coefficient of variation (%CV) was calculated to compare replicate variability for each test method.

Results

Test acceptability criteria were met for each NA test batch. The criteria included a mean control survival of at least 80%, and measurable positive growth in the controls. The statistics for the survival endpoint

were only used for test acceptability criteria, and were not used for method comparability. In addition, all water quality parameters were within acceptable limits. The LC50 for each of the concurrently run reference toxicant tests fell within two standard deviations from the mean of the previous tests in the control chart.

Batch 1: San Diego, CA

For the samples collect from the San Diego Shelf, all of the NA test results were significantly different from the control except for the sample collected from site E14. Conversely, none of the tests using EE and MG were significantly different from the control, except the MG test for 9012 which had complete mortality. Figure 1 illustrates these findings.

Figure 2 presents the toxicity categories associated with each of the test methods for batch 1. All of the EE acute methods resulted in a Nontoxic response. For the sub-lethal tests, site 9012 had the largest disagreement between the methods. The MG test had complete mortality and was Highly Toxic; whereas, the NA test resulted in Low Toxicity. For sites 9037, 9025, and E26, there was relative agreement (one toxicity category apart) between the sub-lethal tests resulting in Nontoxic responses for the MG tests and Low Toxicity for the NA tests. The acute and both sub-lethal test methods agreed for site E14 showing Nontoxic responses across the board.

For the final toxicity LOE category, the combination of all three test methods (EE + MG + NA) showed the lowest combined toxicity category for each of the sites. Figure 3 shows the final toxicity LOE categories for batch 1. Each of the different combinations for the final LOE category for all stations was relatively similar (within one toxic category). For site 9012, the integration of EE + MG showed the highest final toxicity LOE of Moderately Toxic; whereas, the integration of all three test methods and the integration of the EE + NA showed Low Toxicity. For sites 9037, 9025, and E26, the integration of all three test methods and the integration of the EE + MG agreed and resulted in Nontoxic final toxicity LOE scores. For these sites, the integration of EE + NA showed Low Toxicity. For site E14, no matter how the methods were combined, a final LOE score of Nontoxic was determined.

Figure 1. Batch 1 Percent of Control

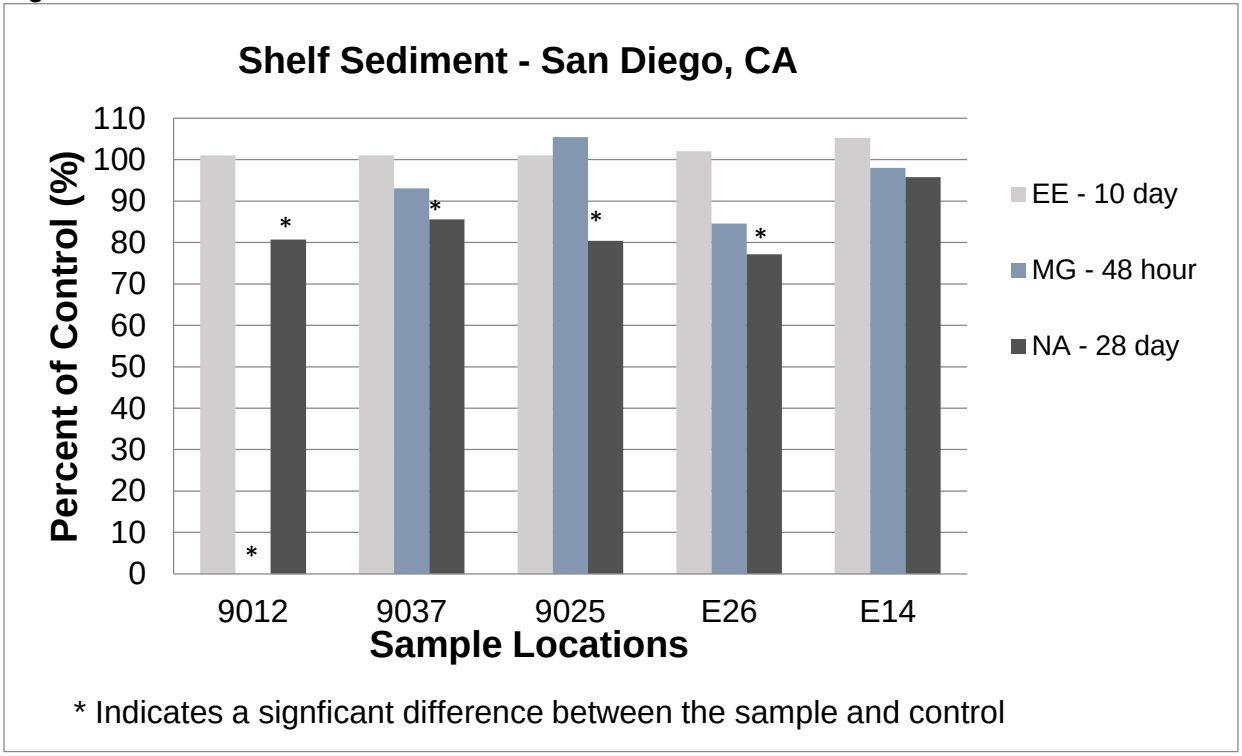


Figure 2. Batch 1 Test Method Toxicity Category

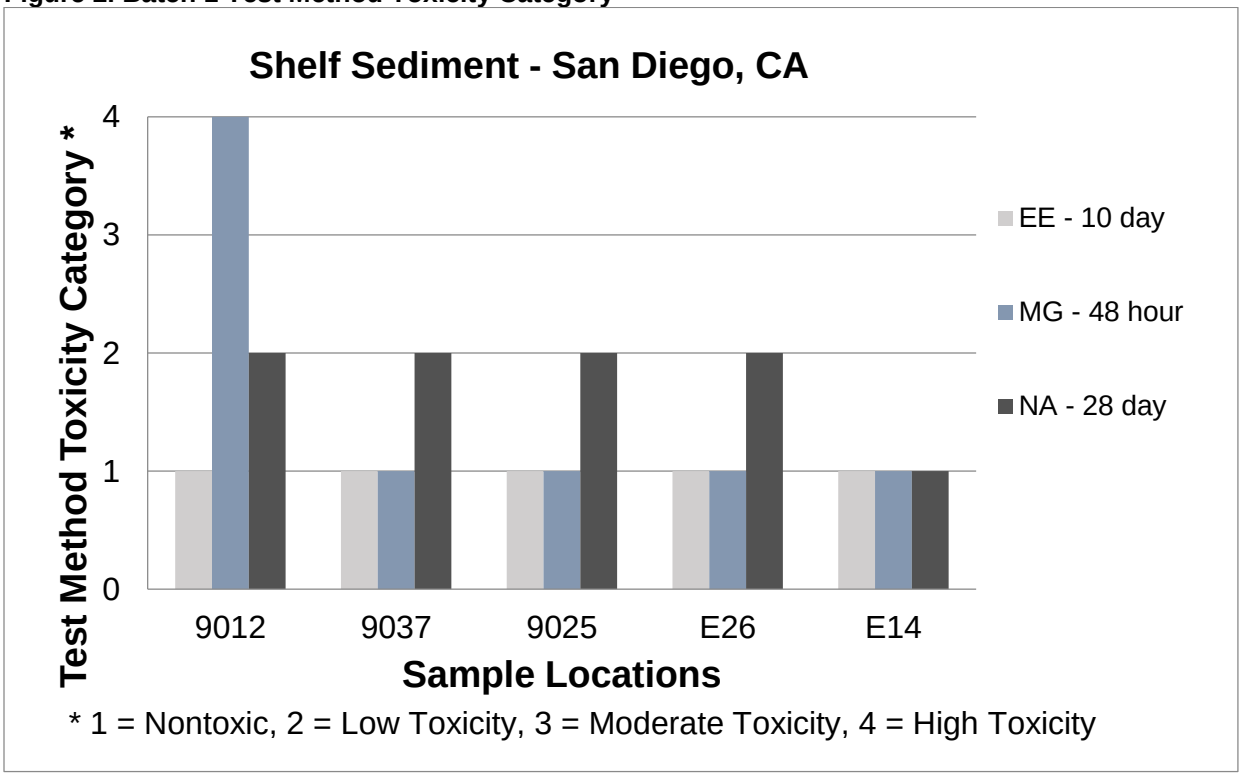
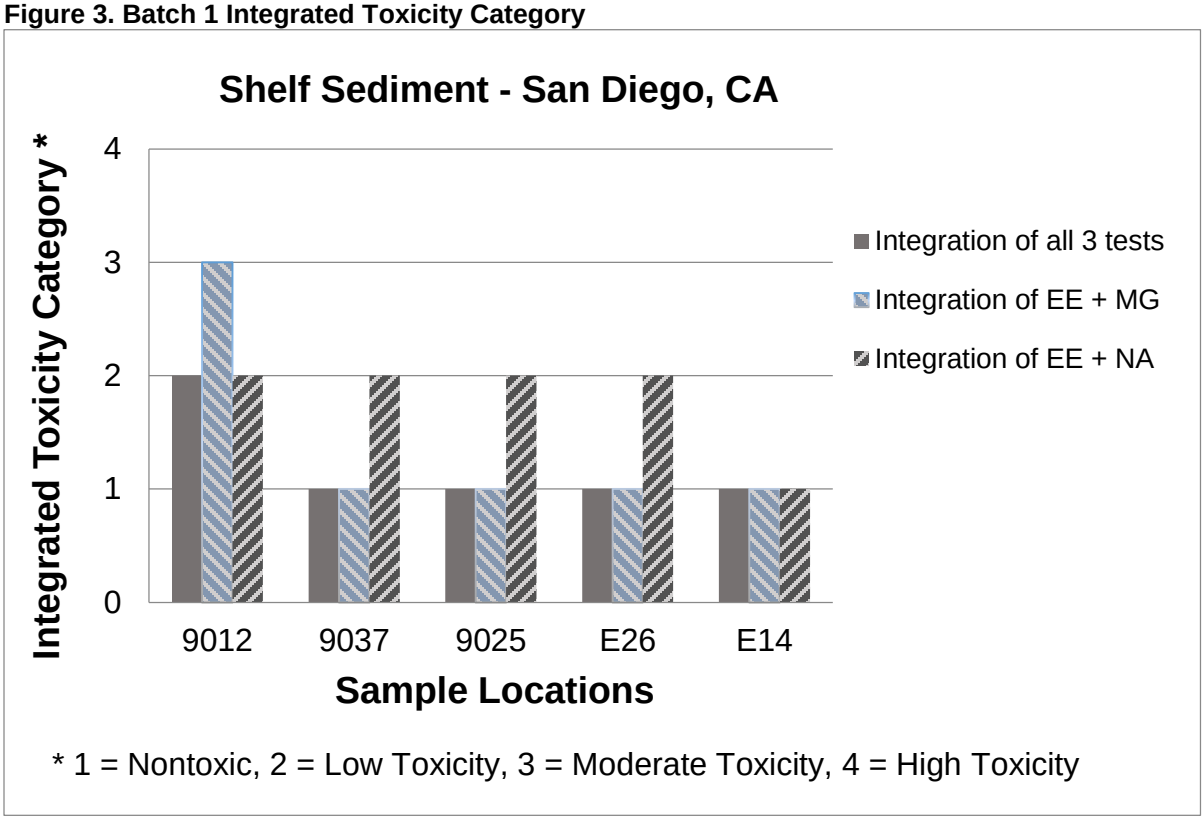


Figure 3. Batch 1 Integrated Toxicity Category



Batch 2: Long Beach, CA

Figure 4 illustrates the percent of control and statistical significance for each testing method and location for batch 2. Site 8394 was significantly different from the control for the EE method. Site 8389 was significantly different from the control for the NA method. Sites 8353 and 8378 were both significantly different from the control for the EE and MG method, but were not significantly different for the NA method.

For batch 2, there was relative agreement at all locations between the acute and the two sub-lethal methods (within one toxic category). For all locations, the MG method resulted in a Nontoxic category. For stations 8394, 8353, and 8378, the acute method had a Low Toxicity response, and both of the sub-lethal methods agreed and showed a Nontoxic response. For stations 8372 and 8383, the acute and each of the sub-lethal tests agreed and resulted in a Nontoxic category. For site 8389, the acute and MG method had a Nontoxic response, whereas, the NA method exhibited Low Toxicity. Figure 5 shows the test method category results for each of the samples collected in batch 2.

As demonstrated in Figure 6, the combination of all three test methods for each of the stations resulted in a Nontoxic response. In addition, the different combinations for the final LOE category for each location were relatively similar (within one toxic category). The results for stations 8394, 8353, and 8378 were all similar. For these locations, the integration using all three methods had a final Nontoxic LOE category; whereas, the integration of the acute and either of the sub-lethal endpoints (MG or NA) showed a Low Toxicity final LOE category. For stations 8372 and 8383, each of the three possible combinations resulted in a final Nontoxic LOE category. For station 8389, the integration of the EE + NA exhibited a Low Toxicity final LOE category; whereas, the integration of all three methods and the integration of EE + MG resulted in a Nontoxic final LOE category.

Figure 4. Batch 2 Percent of Control

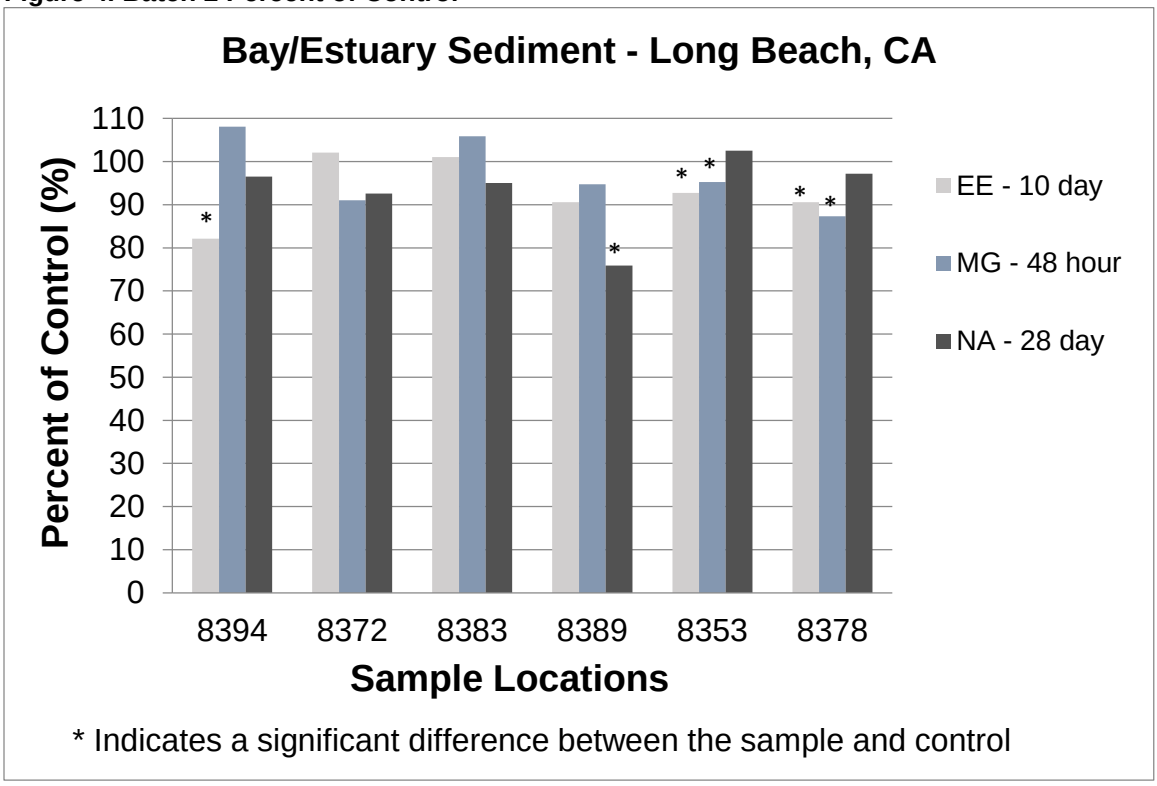


Figure 5. Batch 2 Test Method Toxicity Category

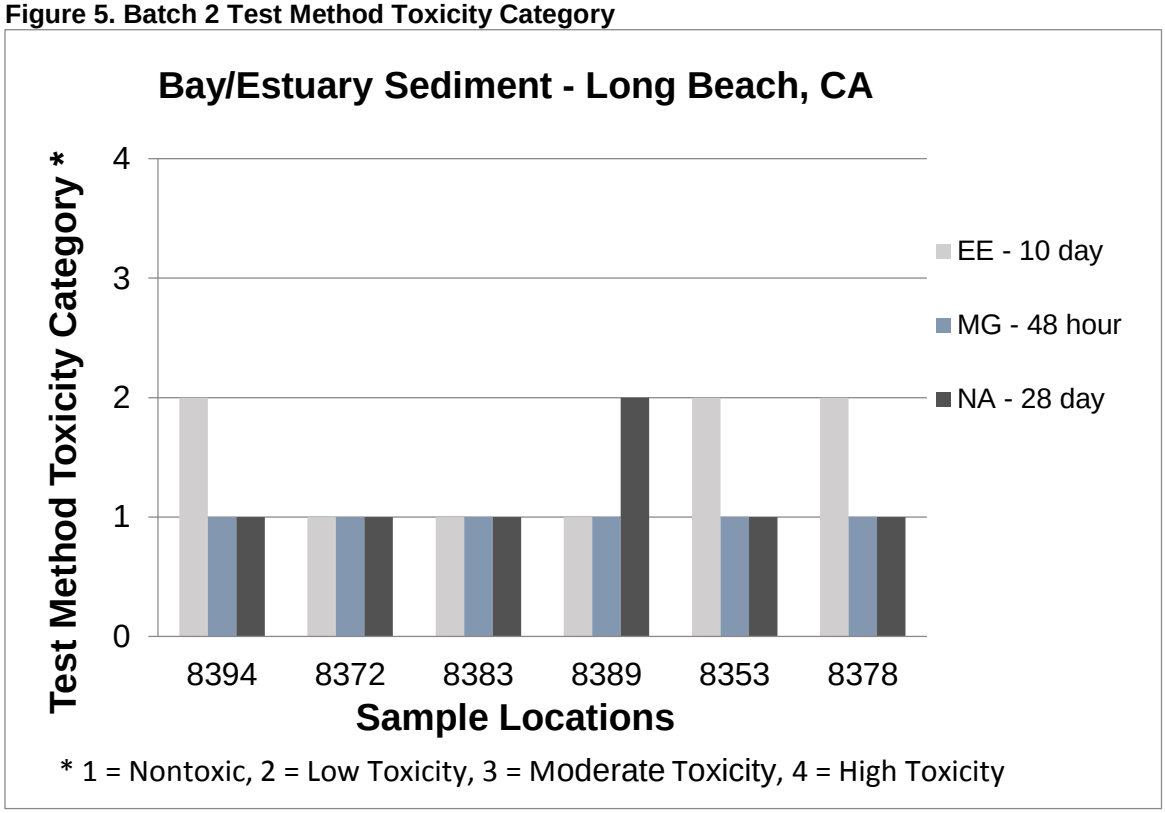
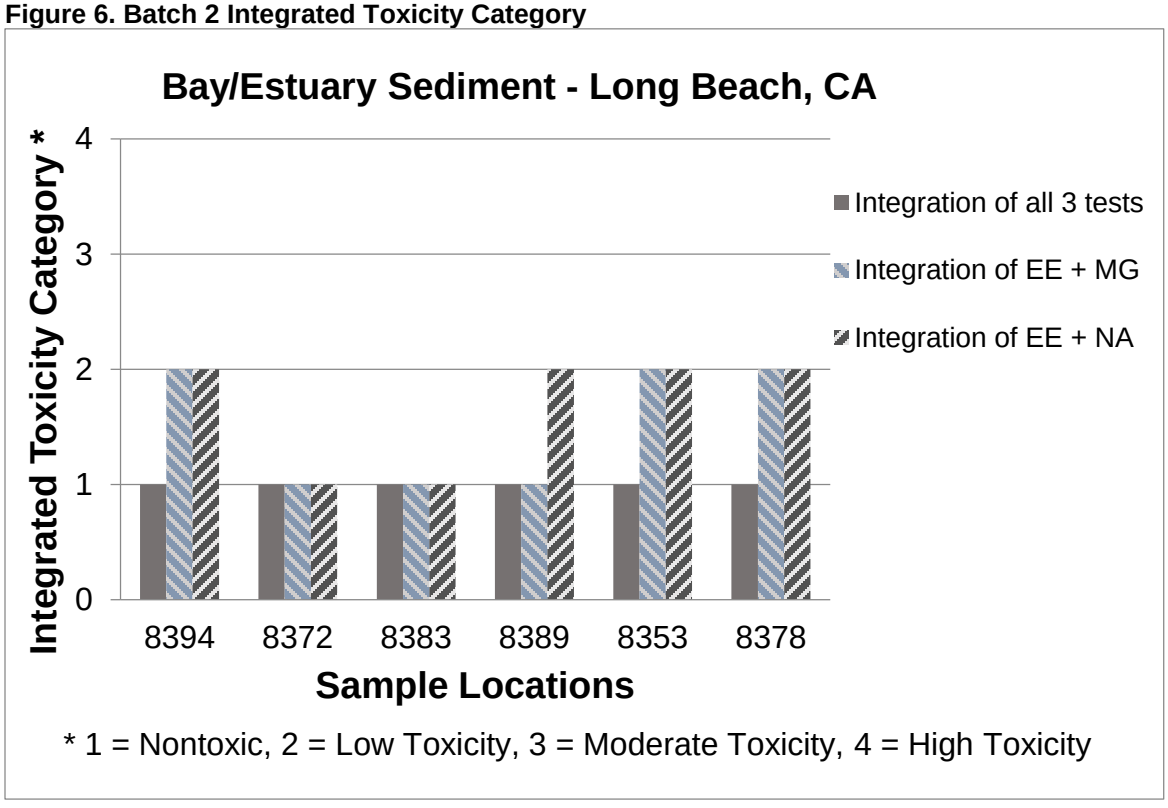


Figure 6. Batch 2 Integrated Toxicity Category



Batch 3: Los Angeles, CA

For batch 3, all NA tests resulted in a significantly different response from the control. Additionally, stations 8417, 8411, and 8391 were significantly different from the control for the EE method. Stations 8413, and 8391 were significantly different from the control for the MG method. Note that all three methods were significantly different from the control for stations 8391. Figure 7 displays the percent of control for each method and stations in batch 3.

The greatest number of Moderately Toxic and Highly Toxic test results was observed for the samples collected from the Los Angeles area and consequently, the largest discrepancy between individual test method toxicity categories was observed. Figure 8 exhibits the test method categories for each of the stations for batch 3. For stations 8417 and 8411, the EE method exhibited Low Toxicity, while MG was Nontoxic, and NA resulted in Moderate Toxicity. For station 8413, EE showed no toxicity, but MG resulted in Low Toxicity, and NA showed Moderate Toxicity. For stations 8409 and 8407, EE and MG methods both were Nontoxic; whereas, the NA method showed Moderate Toxicity. For station 8391, collected in the Los Angeles River, the EE and MG methods both were determined to be Highly Toxic; whereas, NA showed Low Toxicity.

Although there were inconsistencies between individual test method toxicity categories, when the methods were combined, they remained relatively similar (within one toxicity category). For stations 8417 and 8411, the integration of all three methods and the integrations of EE + MG resulted in a final LOE category of Low Toxicity; whereas the integration of EE + NA exhibited a final LOE category of Moderate Toxicity. For stations 8413, all combinations of methods showed a final LOE category of Low Toxicity. For stations 8409 and 8407, the integration of all three methods and the integrations of EE + NA resulted in a final LOE category of Low Toxicity; whereas the integration of EE + MG resulted in a final LOE category of Nontoxic. For station 8391, the integration of all three methods and EE + NA both exhibited a final LOE category of Moderately Toxic and the integration of EE + MG resulted in a final LOE category of Highly Toxic. Figure 9 illustrates the integrated toxicity categories for each of the stations for batch 3.

Figure 7. Batch 3 Percent of Control

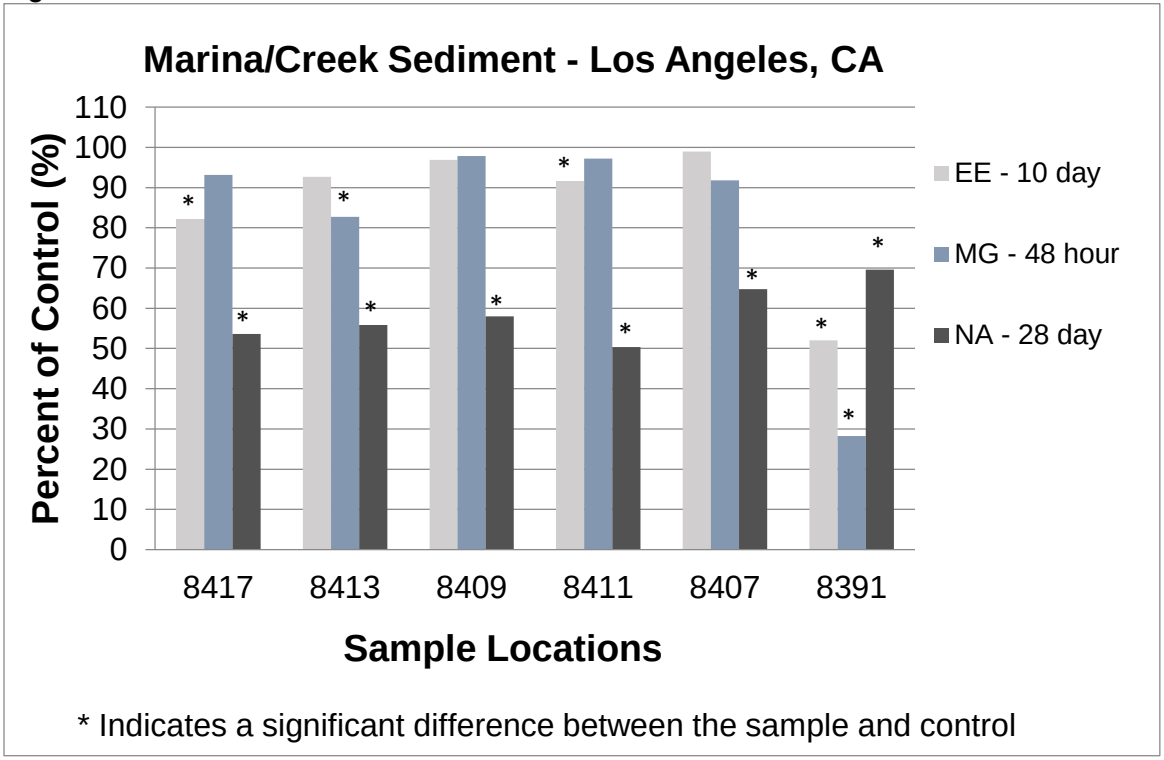


Figure 8. Batch 3 Test Method Toxicity Category

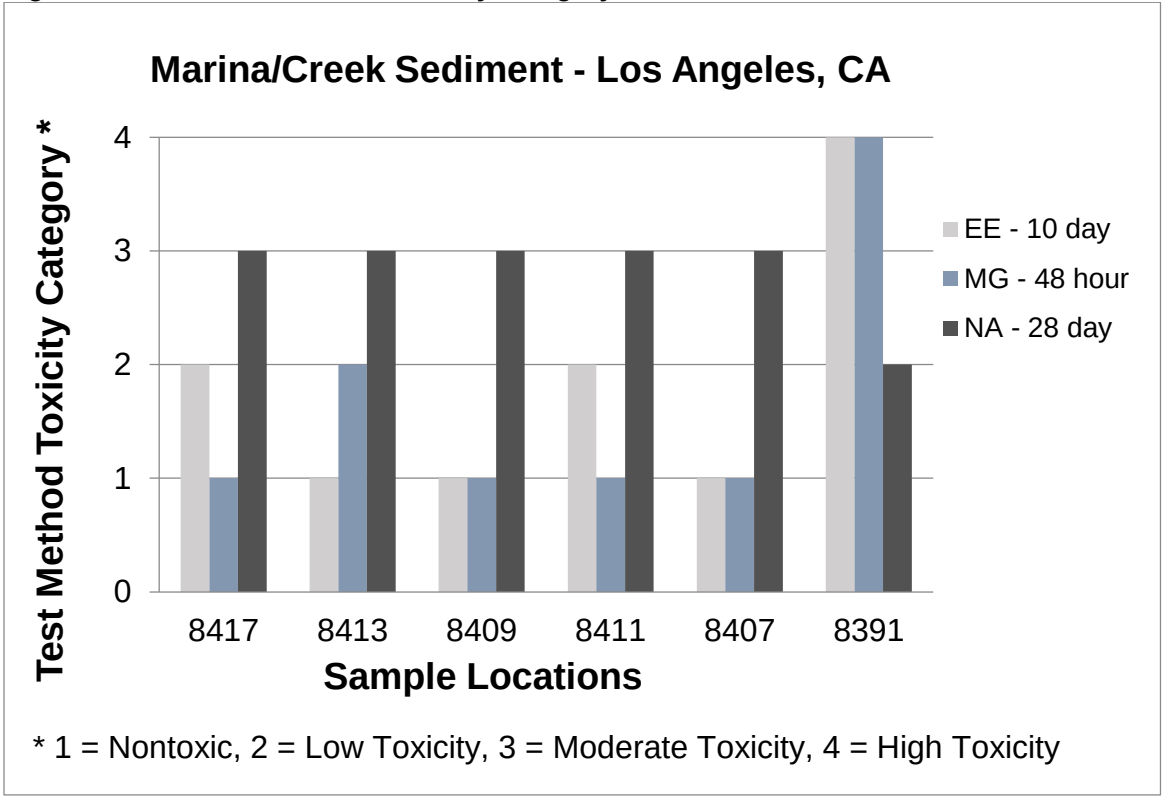
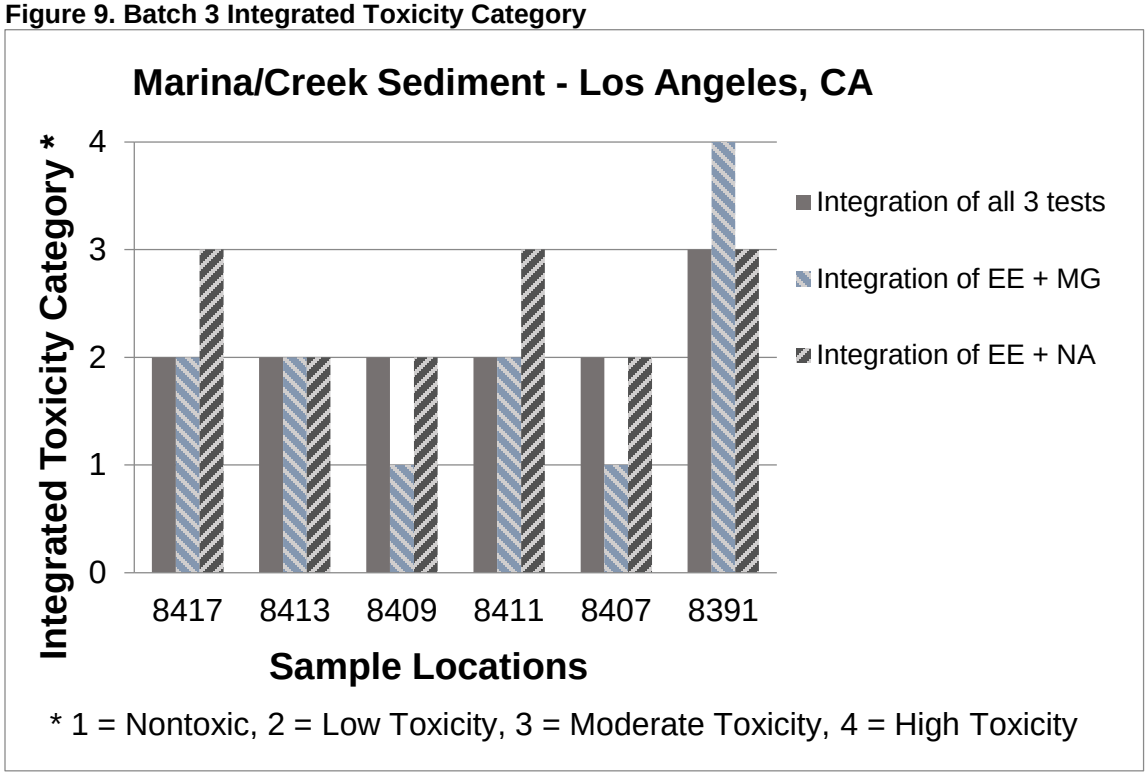


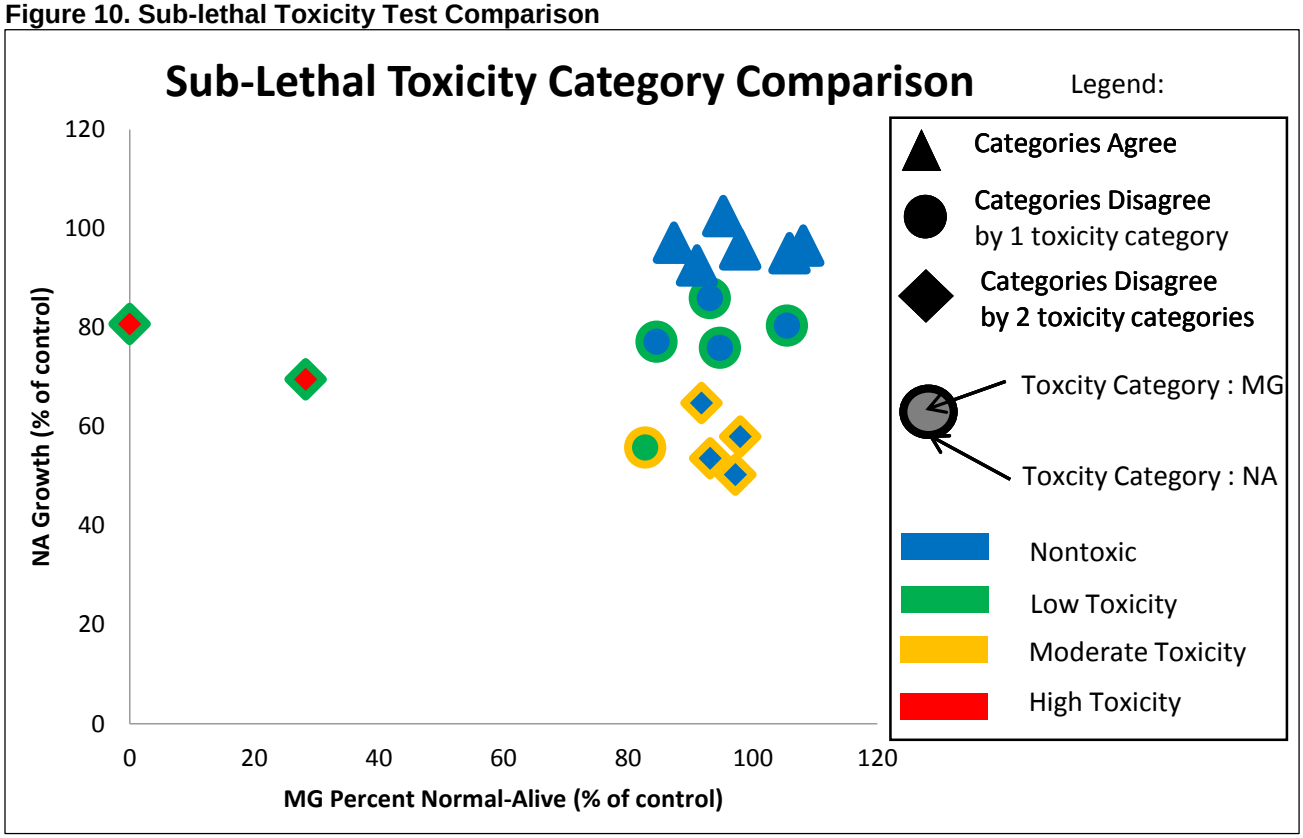
Figure 9. Batch 3 Integrated Toxicity Category



Sub-lethal Toxicity Test Category Comparisons

In general, the toxicity categorization was similar between the two sub-lethal methods, either agreeing or disagreeing by only one toxicity category; however, 6 out of the 17 stations resulted in a two category difference. Figure 10 displays the comparison between the sub-lethal test results. Overall, the MG method resulted in more Nontoxic outcomes than the NA method. A total of 14 locations resulted in a Nontoxic category for MG and of these stations, six agreed with a Nontoxic category for the NA method. Additionally, four disagreed by one toxic category and resulted in Low Toxicity, and four disagreed by two toxic categories and resulted in Moderate Toxicity for the NA method. For one station, MG exhibited Low Toxicity while the concurrent NA method resulted in a Moderately Toxic category. For two stations, the MG method resulted in High Toxicity while the concurrent NA method resulted in Low Toxicity.

Figure 10. Sub-lethal Toxicity Test Comparison

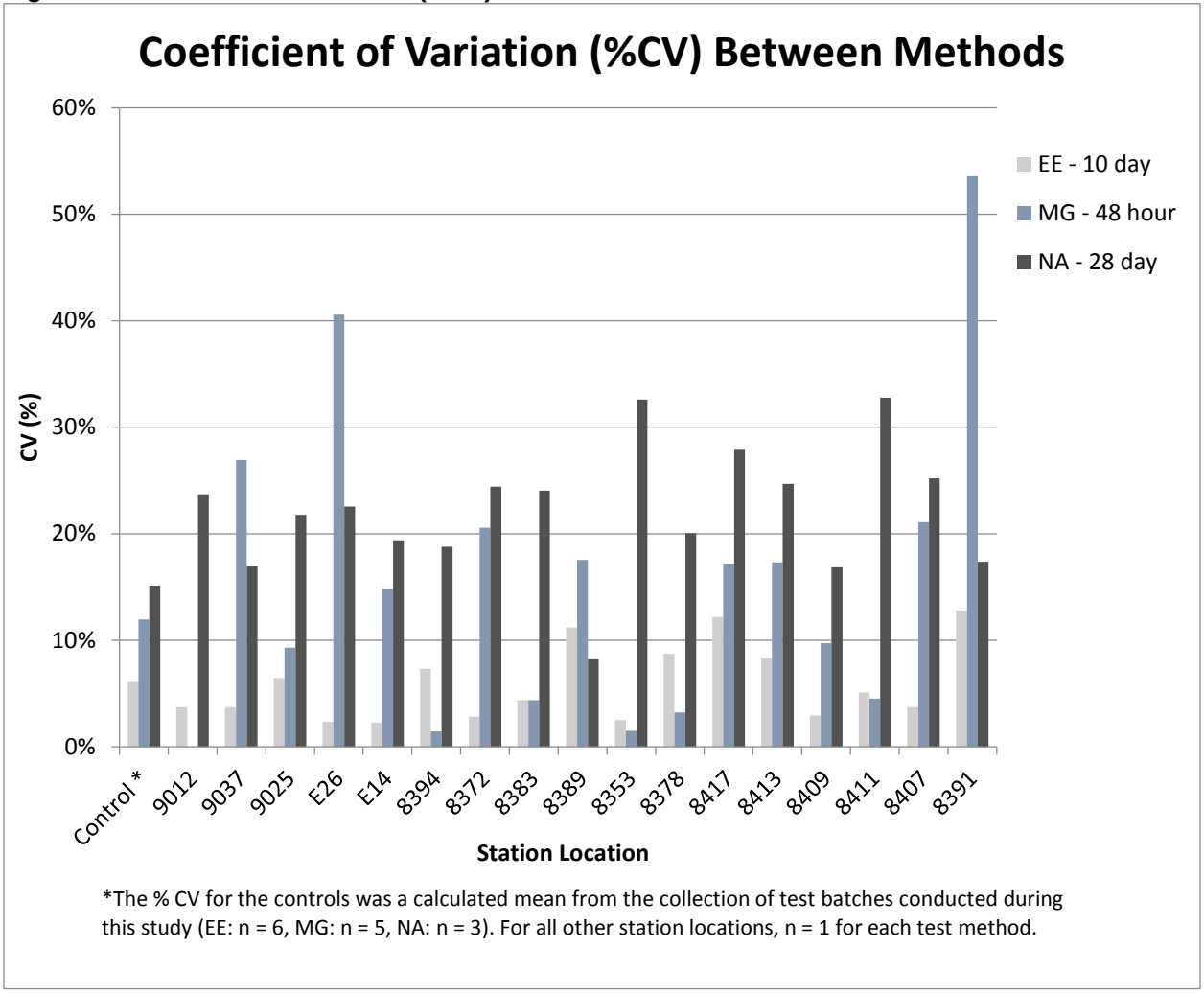


Test Precision

The coefficient of variation (%CV) is a measure of the relative variation of a distribution or set of data, defined as the standard deviation divided by the mean. It can be used as a measure of precision among replicates for each treatment in toxicity tests (USEPA 2000). Although there is no set limit for %CV for any of these methods, a smaller %CV generally indicates tests with lower variability and thus a greater chance of detecting differences between samples.

Since losses can occur in sub-lethal tests that are not necessarily inherent in acute tests (e.g. losses transferring embryos, factors affecting growth) higher variability is generally intrinsic to the sub-lethal test methods. In general, the NA method had a consistently higher %CV at each test location and the EE method had a consistently lower %CV at each test location (Figure 11). The %CV for the NA method was higher than the MG method 76% of the time and higher than the EE method 94% of the time. The MG method was higher than the EE method 76% of the time. The average %CV for the MG method was 16.2%, for the NA method was 21.8%, and for the EE method was 5.9%. For station 9012, a %CV for the MG method could not be calculated due to complete mortality.

Figure 11. Coefficient of Variation (%CV) Between Methods



Conclusions

The results of this study showed that when comparing the two sub-lethal test methods, the NA method generally resulted in a higher toxicity category than the MG method, however differences between the two methods were small. When all three methods were integrated (EE, MG, and NA), generally there was a lower final toxicity LOE category than when only two methods were combined (EE plus either MG or NA). The average %CV between replicates for the NA method was slightly higher than the MG method showing that the MG method is a marginally less variable test; however the %CV for the MG test had extreme highs on various samples possibly related to the nature of the test procedures. Specifically, organisms used for the MG test are microscopic and there is potential for losses transferring embryos. Not surprisingly, the EE test had the lowest %CV, hence the lowest variability. This was most likely due to the fundamental nature of the lower variability found in acute tests compared to chronic tests.

When the final LOE category using all three methods resulted in a nontoxic response, the individual sub-lethal test method toxicity categories varied no more than one toxic category away from one another (e.g. either Nontoxic or a Low Toxicity category). However, the Los Angeles area, where toxicity was more persistent, conducting both sub-lethal tests provides a more robust overview of toxicity assessment, as both tests provide unique data. In other words, when the final LOE category using all three methods

resulted in a higher toxic response, the individual sub-lethal test method toxicity categories were more variable and generally fluctuated two toxic categories from one another (e.g. Nontoxic versus Moderately Toxic).

Test method selection should be based on both environmental relevance and practical concern (USEPA 1994). Environmental conditions such as the physicochemical makeup of the sediment and the abundance and bioavailability of certain chemicals and how test organisms respond to these conditions have a large impact on test results. In addition, the practicality for conducting the test for a particular laboratory should also play into the decision making process. Laboratories must look at the cost-effectiveness for running one or both of the test sub-lethal methods. In addition, the overall ease of testing such as the availability of testing organisms, the ease of training laboratory staff, and the duration of the test method should all be evaluated during the decision process.

Future regional monitoring studies, such as Bight 2018, should continue to utilize multiple sub-lethal toxicity tests, especially if there is any uncertainty in toxicity. Additionally, future special studies should include a comparison between the three acute toxicity test methods. Most recent sediment quality assessments have only used the EE acute 10-day survival test; however, there are two other acceptable SQO toxicity test species (*Leptocheirus plumulosus* and *Rhepoxynius abronius*) and little is known about their relative sensitivities or the benefit of running multiple acute tests.

One factor that needs to be emphasized is that the sediment toxicity LOE is just one of three lines of evidence necessary for sediment assessment under SQO guidelines. The benthic community condition and the sediment chemistry results must also be included to make a final sediment quality assessment. All of the Bight'13 samples had concurrent benthic community and chemistry analyses. Further analysis should be done to evaluate the integration with the other LOEs in order to provide a more confident assessment of sediment quality.

References

American Society for Testing and Materials ASTM. 2002. E 1611 Standard guide for conducting sediment toxicity tests with Polychaetous Annelids. pp. 987-1012, Annual book of ASTM standards, Vol. 11.05. American Society for Testing and Materials. West Conshohocken, PA.

Bay, S.M., D.J. Greenstein, M. Jacobe, C. Barton, K. Sakamoto, D. Young, K.J. Ritter, and K.C. Schiff. 2011. Southern California Bight 2008 regional monitoring program: I. Sediment toxicity. . Southern California Coastal Water Research Project. Costa Mesa, CA.

Bay, S.M., D.J. Greenstein, J.A. Ranasinghe, D.W. Diehl, and A.E. Fetscher. 2014. Sediment quality assessment technical support manual. Technical Report Number 777. Southern California Coastal Water Research Project. Costa Mesa, CA.

Bight'13 Field Sampling and Logistics Committee. 2013. Southern California Bight 2013 regional marine monitoring survey (Bight'13). Southern California Coastal Water Research Project. Costa Mesa, CA.

State Water Resources Control Board SWRCB. 2009. Water quality control plan for enclosed bays and estuaries; Part I: Sediment quality. State Water Resources Control Board. Sacramento, CA.

U.S. Environmental Protection Agency USEPA. 1994. Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA/600/R-94/025. Office of Research and Development, U.S. Environmental Protection Agency. Narragansett, RI.

U.S. Environmental Protection Agency USEPA. 2000. Understanding and accounting for method variability in whole effulent toxicity applications under the National Pollutants Discharge Elimination System Program. EPA-833/R-00/003. Office of Water. Washington, D.C.