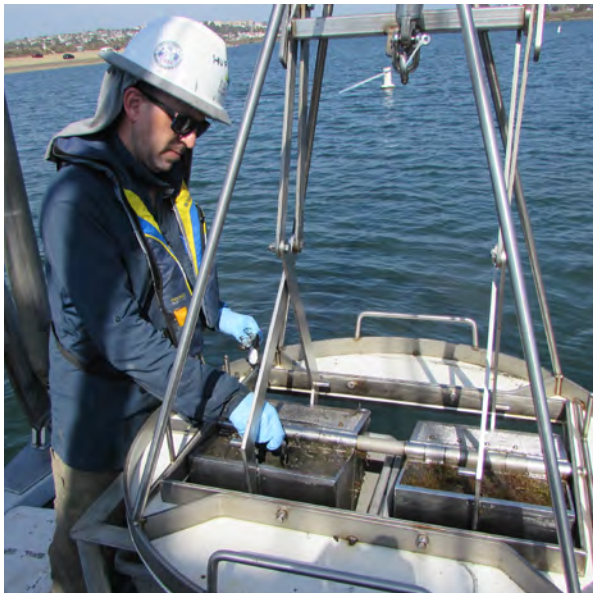
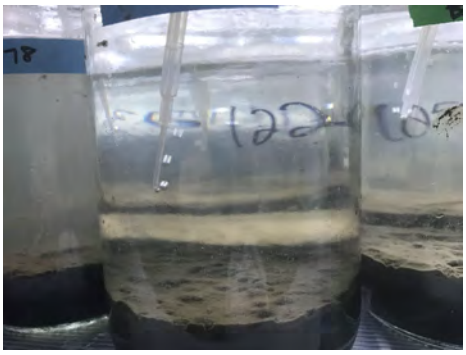
BIGHT '18

Sediment Toxicity





Southern California Bight
2018 Regional Monitoring Program
Volume I

SCCWRP Technical Report 1117

Southern California Bight 2018 Regional Monitoring Program: Volume I. Sediment Toxicity

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FOREWORD

The 2018 Southern California Bight Regional Monitoring Survey (Bight ’18) is part of a collaborative effort to provide a large-scale, integrated assessment of the Southern California Bight (SCB). The Bight ’18 survey is a continuation of previous regional monitoring surveys conducted on a five-year cycle since 1994. This collaboration represents the joint efforts of 46 organizations. Bight ’18 is organized into five elements: 1) Sediment Quality (formerly Contaminant Impact Assessment/Coastal Ecology), 2) Microbiology, 3) Ocean Acidification, 4) Harmful Algal Blooms, and 5) Trash. This assessment report presents the sediment toxicity results, which is one component of the Sediment Quality element. Copies of this and other Bight ’18 reports, as well as work plans and quality assurance plans, are available for download at www.sccwrp.org.

ACKNOWLEDGEMENTS

This report came to fruition because of the hard work and commitment of many individuals and organizations 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 '18 Toxicology Committee for their assistance with study design, sample analysis, data analysis, and report review. We also thank the Bight '18 Sediment Quality Planning Committee for their guidance and support of sediment toxicity testing in regional monitoring. This study would not have been possible without the knowledge and skill of the field sampling personnel from the following organizations: City of San Diego, Orange County Sanitation District, Sanitation Districts of Los Angeles County, City of Los Angeles- Environmental Monitoring Division, City of Los Angeles-Watershed Protection District, Southern California Coastal Water Research Project, Anchor QEA, Aquatic Bioassay and Consulting Laboratories, Weston Solutions, Wood Environment and Infrastructure Solutions, Inc., NOAA, MBC Aquatic Sciences, and Naval Information Warfare Center. We also wish to thank Abel Santana (SCCWRP) for assistance with map preparation.

Toxicity testing was provided by ten laboratories: Aquatic Bioassay and Consulting Laboratories, City of Los Angeles, City of San Diego, EcoAnalysts, Inc., Enthalpy Analytical (formerly Nautilus Environmental), Naval Information Warfare Center Pacific, NOAA, Orange County Sanitation District, Sanitation Districts of Los Angeles County, and Wood Environment and Infrastructure Solutions, Inc.

EXECUTIVE SUMMARY

Sediment toxicity, in combination with sediment chemistry and benthic infauna, is a critical component for assessing overall sediment quality. However, sediment toxicity monitoring is typically focused around specific areas where sediment quality is expected to be impacted, such as urbanized coastal environments and areas near regulated discharges. The Southern California Bight (SCB) Regional Marine Monitoring Survey is a comprehensive, collaborative effort to characterize overall sediment quality – including sediment toxicity - for the entire 3,700 km^2 region. The objective of the Bight '18 sediment toxicity study was to answer three questions:

1) What is the extent and magnitude of sediment toxicity in the SCB?
2) How does the extent and magnitude of sediment toxicity compare among specific habitats of interest?
3) How does the extent and magnitude compare to previous regional surveys?

Sediment was collected from 261 stations for toxicity testing. Stations were sampled between July 1 and September 30, 2018, and were located between Point Conception, California, and the United States-Mexico border. The sampling scheme was based on a stratified random design to ensure spatial representativeness and minimize bias. For toxicity testing, a total of seven strata were sampled over two general regions: offshore and embayments. The offshore strata included Channel Islands, and Shelf (Inner, Mid, and Outer) stations. Embayments included four strata from previous surveys (Bays, Ports, Marinas, and Estuaries), and one new stratum (Brackish Estuaries). Surface sediments (upper 2 cm for offshore and upper 5 cm for embayments) were collected at each station by Van Veen grab and tested for toxicity.

Two toxicity tests were used to assess sediment condition. All stations were tested with the 10-day amphipod survival test using *Eohaustorius estuarius*. Embayment stations were also tested with the mussel embryo sediment-water interface test using *Mytilus galloprovincialis*. The amphipod test has been used since Bight '98 and the mussel test was added to embayment stations in Bight '08. These two toxicity tests are approved methods described in California's sediment quality objectives (SQO) policy for bays and estuaries. Their results can be integrated and used to provide an overall assessment of sediment toxicity as part of the sediment quality triad approach (toxicity, chemistry, and benthic community).

Similar to the five previous surveys dating back to 1994, Bight '18 included a rigorous quality control and assurance program to ensure laboratory comparability and competency. This program included a pre-survey interlaboratory calibration exercise, standardized test methods, laboratory audits, and split sample analysis. All samples tested passed the test acceptability criteria for both the amphipod and mussel tests. For the mussel test, nine samples were tested outside of the acceptable holding time range (> 28 days) and were excluded from the dataset. All other data was deemed acceptable.

Sediment toxicity test results were evaluated using the California Sediment Quality Objective (SQO) program's classification system and thresholds to characterize sediment into one of four categories. Nontoxic represents samples with results in the same range as acceptable controls. Low Toxicity represents samples with a minor toxic response that is typically very small relative to, and frequently not significantly different from, controls. Moderate and High Toxicity represent a substantial toxic response relative to controls as defined by unique thresholds for

each test. For making a final assessment of overall condition, stations were ‘Not Toxic’ if they were in the Nontoxic or Low Toxicity categories; stations were ‘Toxic’ if they were in the Moderate or High Toxicity categories. These categorical responses were estimated for the amphipod and the mussel tests individually, and then combined into a single unified assessment.

Overall, toxicity across the SCB was low, with the amphipod test results showing 99.8% of the area was not toxic (categorized as Nontoxic or Low Toxicity). One hundred percent of the offshore strata area was not toxic, whereas the embayments showed 90.1% of the area as not toxic. Within the embayments, the greatest extent of toxicity was observed in the new stratum, Brackish Estuaries (41.7% area toxic), followed by Estuaries (38.2% area toxic) and Marinas (29.9% area toxic). The mussel test results indicated 96.6% of embayment area was not toxic, 0.5% area was toxic, and 2.9% area was uncharacterized due to the nine excluded stations. Similar to the amphipod test results, the Brackish Estuaries (10% area), Estuaries (0.05% area), and Marinas (2% area) had the greatest extent of toxicity. For both toxicity tests, 100% of the area in the Ports was not toxic.

Once the amphipod and mussel toxicity scores were integrated, the Brackish Estuaries, Estuaries, and Marinas had the highest incidence of toxicity. Brackish Estuaries had the greatest extent of sediment toxicity with 40% area toxic, 40% area not toxic, and 20% area uncharacterized. It is important to note that the Brackish Estuaries had the smallest sample size (n=12 for amphipod test and n=10 for mussel test), which leads to less confidence in the areal extent estimates compared to the other strata which had larger sample sizes (n=30). The reduced sample size was a reflection of the dynamic extent of these habitats, where spatial extent can vary based on changes of freshwater inputs (2017-18 was a drought year).

The temporal changes in extent and magnitude were assessed by comparing the Bight ’18 survey to the extent and magnitude of sediment toxicity from previous Bight surveys conducted over the past twenty years. Based on the amphipod test, the extent and magnitude of sediment toxicity in the offshore strata of SCB has remained low. The combined embayment strata, which has always had a greater extent and magnitude of toxicity than the offshore strata, has been decreasing since 1998. The extent of sediment toxicity in the combined embayment strata generally remained the same from 2013 to 2018. However, there was a slight decrease in the magnitude of sediment toxicity (from High to Moderate). These changes in amphipod toxicity were not consistent between the various embayment strata. There was an increase in the extent of sediment toxicity from Marina and Estuary Strata in 2018 compared to 2013. The mussel test and integrated SQO results showed a decrease in extent and magnitude of toxicity in total embayments from 2013 to 2018.

As part of the Bight design, up to half of the sites are comprised of revisited sites (as opposed to new random sites) from previous regional surveys enabling a more detailed examination of trends. In Bight ’18, 78 of the sites were revisited for the amphipod test and 56 were revisited for the mussel test and integrated SQO score. The subset of revisited sites produced similar results to all sites combined, suggesting additional confidence in the Bight-wide assessment of stable or decreasing extent and magnitude sediment toxicity.

This report summarizes the toxicity test results and puts these results into perspective using the SQO assessment framework. These outcomes are not meant to provide an overarching analysis of sediment quality, but instead comprise one of three analyses (chemistry, toxicity, and benthic

community) in the sediment quality triad. The chemistry and benthic community lines of evidence will be reported separately in stand-alone Bight ’18 reports. All three lines of evidence using the complete SQO triad approach will be combined in a final, inclusive report providing an integrated assessment of sediment condition in the SCB.

This report provides four recommendations based on the results from the Bight ’18 survey. First is to further investigate sediment toxicity, particularly where toxicity increased between 2013 and 2018, by conducting additional sampling including toxicity identification evaluations (TIEs). Second, the large extent and magnitude of sediment toxicity in the new stratum, Brackish Estuaries, should be confirmed. The reduced sample size stemmed from an inadequate sampling frame, which did not extend far enough into the freshwater reaches of the estuaries. Thus, improvements in the sampling frame will be necessary if this stratum is meant to be resampled in future regional surveys. Third, additional test species should be evaluated to ensure an adequate supply of test organisms are available during the next survey. Fourth, because sediment compositing prior to chemistry and toxicity subsampling was conducted for the first time in Bight ’18, relationships between sediment toxicity and sediment chemistry should be conducted once the chemistry data becomes available.

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I. INTRODUCTION

The Southern California Bight (SCB) is an important ecological resource, providing economic, cultural and recreational services to large populations living along the coast. However, it is also subject to significant pollutant inputs due to a highly urbanized coastal environment. Historically, monitoring for sediment quality had been focused on areas nearest to regulated discharges associated with National Pollutant Discharge Elimination System (NPDES) permits, providing a potentially biased perspective (Schiff et al. 2002). Beginning in 1994 and conducted every five years since, nearly 100 regulated, regulatory, non-governmental and academic organizations have joined forces to implement the SCB Regional Marine Monitoring Program (the Bight Program), a probabilistic survey intended to assess regional condition of SCB habitats to provide much needed context for NPDES and Total Maximum Daily Load (TMDL) monitoring (Schiff et al. 2019).

Beginning with the 2008 survey, the Sediment Quality component of the Bight Program evaluates potential impacts on marine benthic communities through multiple lines of evidence: sediment chemistry, biological assemblages, and sediment toxicity, compatible with the California Sediment Quality Objectives Program (SQO). Using the standardized SQO assessment methods allows for quantitative comparison of the Bight Sediment Quality results to other regions of the state and provides a mechanism to detect and monitor changes through time.

Sediment toxicity, which is the focus of this report, is a key component of the overall assessment of sediment quality using the SQO tool. While chemical measurements provide much needed information on magnitude of contamination by specific toxicants, only a limited number of contaminants are analyzed in monitoring programs. Furthermore, this analysis cannot account for the interactive effects of multiple contaminants and does not account for bioavailability. Toxicity testing complements chemical measurements by providing a measure that integrates the effects of all bioavailable contaminants present at a site. However, toxicity testing also has drawbacks, including a limited selection of testing species and an uncertain connection between results obtained in the laboratory as compared to *in situ* conditions. Thus, use of toxicity testing together with chemical measurements and benthic community assemblages in the SQO framework provides a more robust measure of sediment quality.

The sediment toxicity portion of Bight ’18 was designed to address three questions:

1) What is the extent and magnitude of sediment toxicity in the SCB?
2) How does the extent and magnitude of sediment toxicity compare among specific habitats of interest?
3) How does the extent and magnitude compare to previous regional surveys?

The probabilistic design of the Bight Program allows for characterization of the breadth and depth of variability in sediment toxicity for multiple habitats and the region overall, providing much needed context for local NPDES monitoring. Furthermore, because toxicity was evaluated in seven habitats, or strata, during Bight ’18, relative habitat quality between habitats can also be described. Two strata representing the offshore region: Shelf and Channel Islands, and five embayment strata were assessed for sediment toxicity: Bays, Marinas, Ports, Estuaries (salinity > 27 ppt) and Brackish Estuaries (salinity < 27 ppt), the latter of which was assessed for the first time during this survey.

The strategy to revisit a subset of sites during each Bight survey allows for characterization of site-specific trends in sediment toxicity for the region. State and local agencies have made significant investments in improving water quality and treatment. Long-term monitoring, like the Bight Program, provides a means to document the impact of these management actions on regional sediment quality and the relative rate of those impacts.

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 2 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 the results of a special study comparing results of chronic exposure at the sediment water interface using the SQO mussel species and an alternate species, electronic maps of results, and a station-by-station summary of the toxicity results. Combined SQO scores for sites using the multiple lines of evidence: sediment toxicity, chemistry, and benthic community responses are not included in this report. Rather these results, and comparisons between indicators, will be addressed in the Bight ’18 Sediment Quality Synthesis Report.

II. METHODS

A. Sampling Design

There were 261 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, 2018. 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 seven strata: Shelf, Marinas, Ports, Bays, Estuaries, Channel Islands, and Brackish Estuaries. Enhancement of the sampling design was achieved through intensified sampling in targeted areas and by resampling of stations from previous surveys. Intensified sampling was applied within portions of San Diego Bay to encompass additional substrata (freshwater influenced, shallow harbor, and deep harbor). In order to assess temporal trends, approximately 50% of the Bight ’18 samples were new sites while 50% of the sample sites were previously sampled in previous Bight surveys. This was the first Bight survey in which samples were collected and tested from the Brackish Estuaries stratum. Toxicity has not been tested in the Channel Islands strata since Bight ’03.

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, Brackish Estuaries, and Marinas).

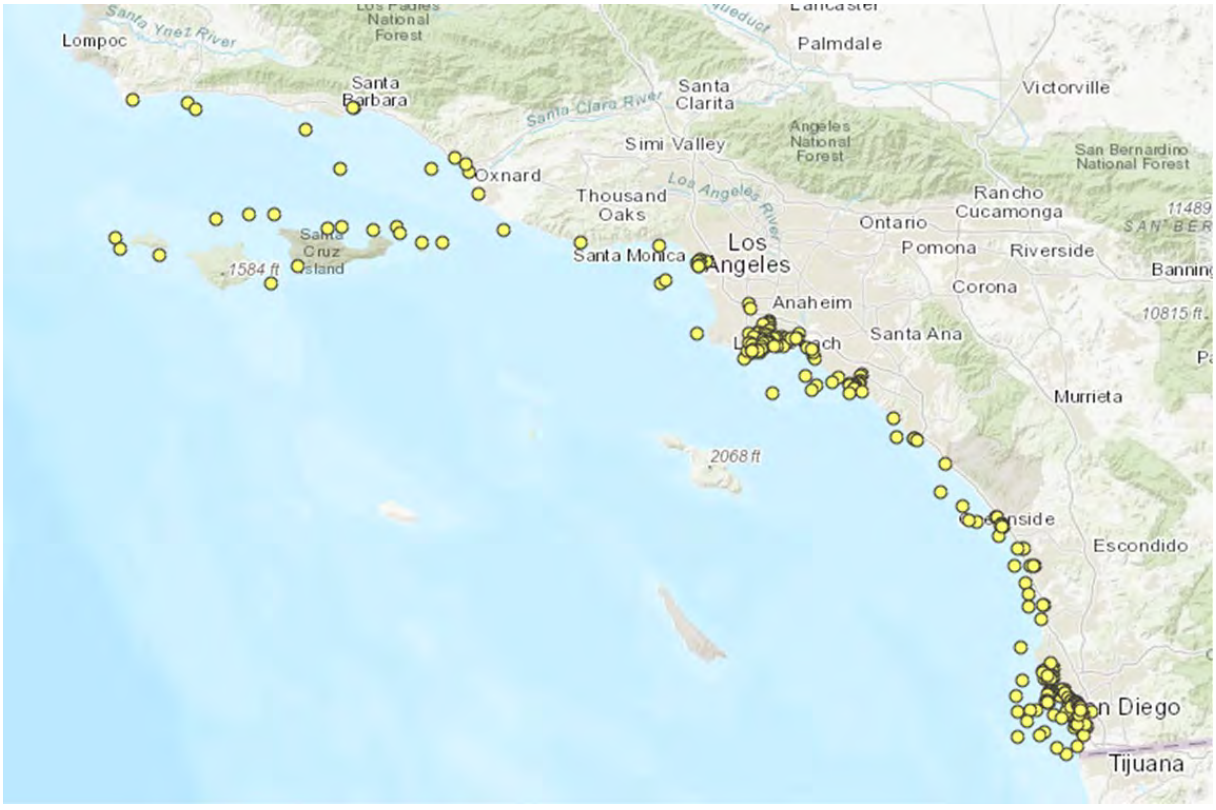

Figure II-1. Locations of all stations targeted for toxicity testing as part of the Bight ’18 project.

B. Field Methods

Sediment samples were collected with a 0.1 m^2 modified Van Veen grab. Up to 6.0 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. In most cases, multiple grabs were required to obtain enough sediment for toxicity testing and chemical analysis. If more than one grab was required, sediment from each grab was added to the Teflon bag and homogenized thoroughly using either a clean Teflon or plastic spoon, or by kneading the sample within the bag. After homogenization, sub-samples were aliquoted for chemical analysis and the remaining contents of the bag was saved for toxicity testing. Homogenization of sediments prior to subsampling for chemistry and toxicity was required for all embayment stations. For offshore sites, the contents of multiple grabs could be homogenized as was done for the embayment sites, or samples could be distributed directly to containers (HDPE jars) for toxicity and chemistry (glass jars) by placing approximately equal aliquots of sediment from the surface of a grab sample into each container type. Once collected, the samples were stored in the dark at 4°C in the laboratory for no longer than four weeks prior to testing.

C. Laboratory Methods

Whole Sediment Toxicity

The toxicity of whole sediment to amphipods was determined using a 10-day 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 days, but not longer than 10 days, 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 (≤ 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 equilibrated 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 at a minimum on day 0 and day 10. Pore water measurements of ammonia and salinity were made at sample receipt and day 0. Measurements of the pore water at sample receipt was used to determine if adjustments to testing procedures were

necessary due to high ammonia or low salinity. Since samples with low salinity were expected for this study, secondary controls were added to any test containing samples with salinity below 30 g/kg. For the secondary controls, salinity was dependent on the porewater salinity of samples in the test batch and in some cases multiple controls were needed (Table II-1). 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. The amphipods were exposed to the sediment samples for 10 days. 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. 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%. If control CV was greater than 11.9%, any samples with a mean ≥ 90% would be acceptable and not need to be retested, but samples with a mean < 90% would need to be retested.

Table II-1. Secondary control salinities based on sample porewater salinities.

Range of Sample Porewater Salinity	Appropriate Control Salinity (g/kg)
0-4	2 ± 2
5-9	7 ± 2
10-14	12 ± 2
15-19	17 ± 2
20-24	22 ± 2
25-29	27 ± 2
≥ 30	32 ± 2

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). In Bight ’08 and Bight ’13 the laboratories were required to test a sediment grain size control to account for this possibility. Since grain size was not identified as a primary factor for

observed toxicity during these past two programs, testing of a grain size control was voluntary in Bight ’18. This sample consisted of a 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 Wood Environment and Infrastructure Solutions, Inc. The sediment was placed into 20 L buckets, put into coolers with ice, and shipped to SCCWRP where it was held in the dark at 4°C. The sediment was homogenized and transferred to 1 L HDPE jars. On request SCCWRP provided samples of the grain size control sediment to the participating laboratories that wished to include this material for comparison.

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 adult animals were obtained from a variety of sources in southern California and Washington. Several laboratories have experienced difficulty getting mussels to spawn in the warmer summer months. Because of this, laboratories frequently rely on multiple animal sources in preparation for this toxicity test. In this survey, two laboratories performed a side-by-side toxicity test comparison with oyster and mussel embryos for the two split sample sediments to start the process of determining a potential alternate test species (Appendix F). The oyster was chosen as a candidate because its typical spawning period is complementary to that of mussels.

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 and overlying water equilibrated overnight before addition of a screen tube (Figure II-2). The screen tubes were made of polycarbonate tubing with a 25 to 30 μm Nylon mesh 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 vial 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 initiated in a controlled environment. Approximately 250 fertilized mussel eggs from a stock solution were added to the screen tube to begin the exposure. The same volume of embryo stock was also added to five replicate glass vials which were immediately preserved for determination of the initial number of embryos added. 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%.

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 methods similar to 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.

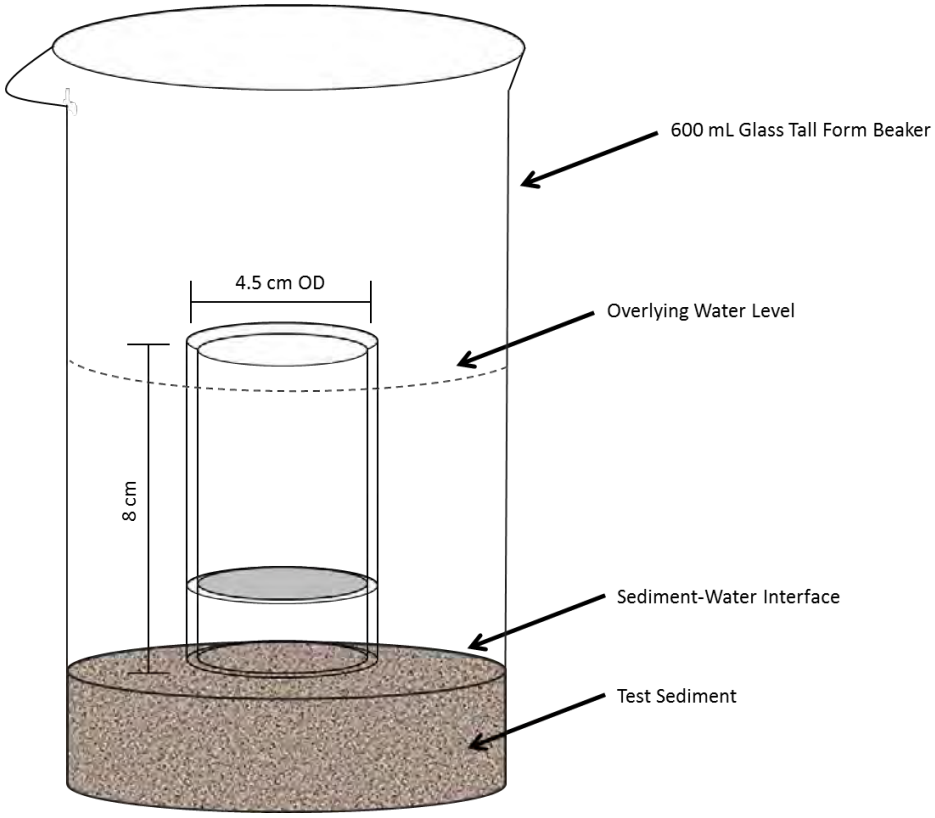

Figure II-2. Schematic diagram of sediment-water interface exposure system.

D. Data analysis

Data were analyzed using four methods: 1) calculation of the mean control-normalized response; 2) determination of the toxicity category using SQO thresholds; 3) assessment of the percent of stations within each stratum that was classified into each of the SQO toxicity categories; and 4) 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-2). 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-2 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 the expected results) are either identical or very similar to those used throughout the US 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-2. 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 falling into an SQO category). Using information provided by the sample design, these probability-based areal estimates account for 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 sample. The area weighted proportions were computed as a ratio of the sum of the area weights for all sites which fell within an SQO 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. For a complete description of the statistical tools used in this analysis as well as a download of scripts for probability-based estimation, go to http://archive.epa.gov/nheerl/arm/web/html/monit_intro.html website.

The representativeness of the few randomly selected Bight sample locations in some of the more spatially and physically diverse habitats (i.e., Bays and Estuaries) may have more uncertainty when extrapolating results over a larger area. To address this concern, an additional analysis that

evaluated the SQO scores as a percent of stations sampled was also included in the overall toxicity assessment.

Four previous toxicity surveys of the SCB have been conducted using similar methods, including a probabilistic sampling design and the amphipod toxicity test: the 1998, 2003, 2008, and 2013 Southern California Bight regional surveys (Bay et al. 2000, 2005, 2011, and 2014). The previous three surveys have also included the mussel embryo sediment-water interface test for the embayment strata. These historical datasets, in conjunction with the Bight ’18 results, allow for a temporal analysis of the region over the past 20 years. These comparisons can be made using the individual test methods as well as the integrated SQO category results for the embayments. Examination of temporal trends requires any differences in toxicity test criteria to be normalized. As such, during Bight ’13, the toxicity test results from the 1998 and 2003 surveys were reevaluated using the same SQO thresholds utilized during the 2008 and 2013 surveys. These normalized results were again used in this temporal evaluation. Additionally, the areas and groupings of strata have not been consistent over time. To minimize these differences, comparisons were made on a percent area basis and the Port and Bay strata were combined. The offshore stratum represents the shelf stations over all the surveys. The Brackish Estuaries were not included in the temporal comparison since that stratum was not previously sampled.

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 ’18 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, an acceptable level of sampling and testing success was established. 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 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 amphipod test, 95% of the samples were tested within 14 days of sample collection (Table III-1). All samples were tested with amphipods within 28 days. For the mussel test, 92% of the samples were tested within 14 days of collection, with an additional 3% of the samples tested within 28 days. There were nine mussel test samples representing about 4% of the total that were tested outside of the acceptable 28 days (Figure III-1). Results for these samples were excluded from analysis and will not appear in the database. The excluded samples fell within multiple strata and each had other samples located in relatively close proximity so that loss of this data should have a limited effect on the overall analysis.

Of the excluded samples, one was in Ports, six were in Estuaries, and two were in Brackish Estuaries. These samples were only excluded for the mussel embryo test. For Ports, 54 of the 55 stations were successfully tested. Of those tested, one had Low Toxicity and 53 were Nontoxic. In the Estuary stratum, 36 stations were assigned for both tests with 45 tested using the amphipod and 39 tested using the mussel embryos. This increase in station number was due to the post-sampling stratification of 9 of the Brackish Estuary stations to the Estuary strata. Even with the 6 excluded samples for the Estuary stratum, there was 108% test completeness for the mussel test. The Brackish Estuary stratum had the fewest number of stations tested due to the loss of stations to re-stratification as well as the 2 excluded samples. The potential impact of low sample size is described in more detail in the Results and Discussion sections of this report.

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

	Eohaustorius estuarius		*Mytilus galloprovincialis*	
Time Interval (days)	# Samples	Percent of Total	# Samples	Percent of Total
0-14 (acceptable)	266	95	193	92.3
15-28 (acceptable w/ qualifier)	14	5	7	3.4
> 28 (unacceptable)	0	0	9	4.3

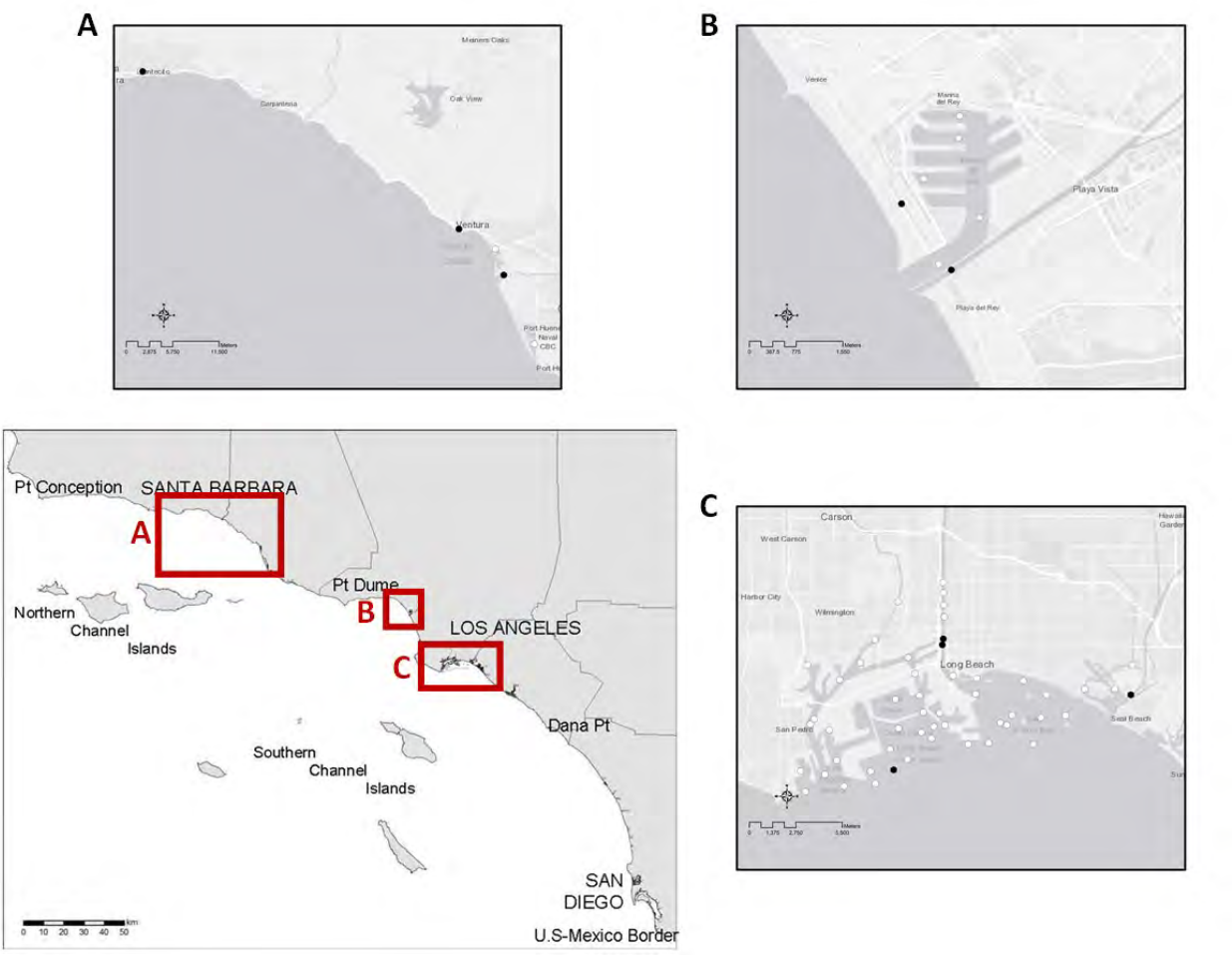

Figure III-1. Location of the nine samples tested outside the holding time limit for the mussel test (black dots). White dots indicate nearby stations which were tested within the holding time limit.

C. Organism Holding

All organisms were held in accordance with the protocols set forth in the Toxicology Laboratory Manual. One amphipod batch was held toward the longer end of the acceptable window. 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 40 amphipod and 23 mussel embryo reference toxicant batches. The EC_{50}/LC_{50} data were computed for the un-ionized ammonia measured at time zero using the CETIS™ statistical software package.

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. Most of the amphipod test results for batches having reported LC_{50} values fell within two historical standard deviations of the historical grand mean (Figure III-2). However, two amphipod test batches were above the historical + 2 standard deviation threshold. This threshold is solely informational and was not used to judge the acceptability of test data. Since both test batches also were above the individual laboratory's control charts, an internal review of the data was triggered. One amphipod test batch was 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 embryo reference toxicant test batches were within the historical two standard deviations, except for two tests by Lab 6 (Figure III-3). However, since both test batches also were above the individual laboratory's control charts, an internal review of the data was triggered. After reviewing the water quality data and laboratory records, no specific cause was determined, and the results were deemed acceptable. The out of bounds results for each laboratory appeared to be a singular event and not part of an increasing or decreasing organism sensitivity trend. Between the two toxicity test types, there were 39 samples tested alongside these reference toxicant tests. Of those, 28 were amphipod samples, with samples from Bays (8), Shelf (14), Marinas (2), and Ports (4); and 11 were mussel samples from Brackish Estuaries (4), Estuaries (2), and Marinas (5).

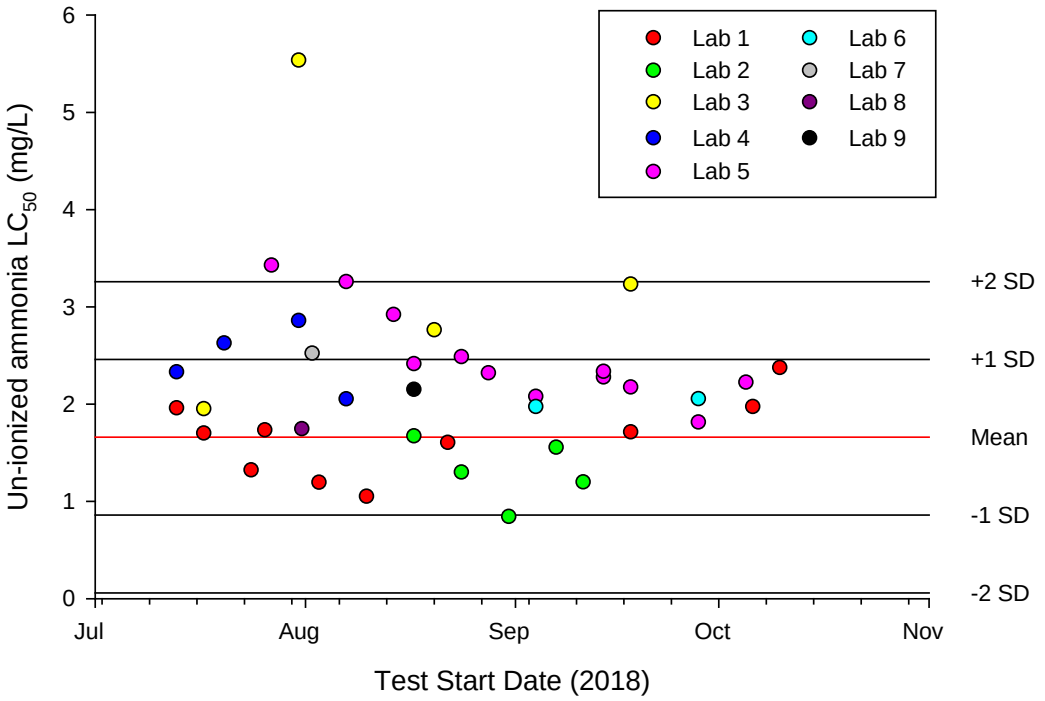

Figure III-2. Results of amphipod 96 hr reference toxicant tests with ammonia. The historical grand mean and standard deviations are plotted for reference.

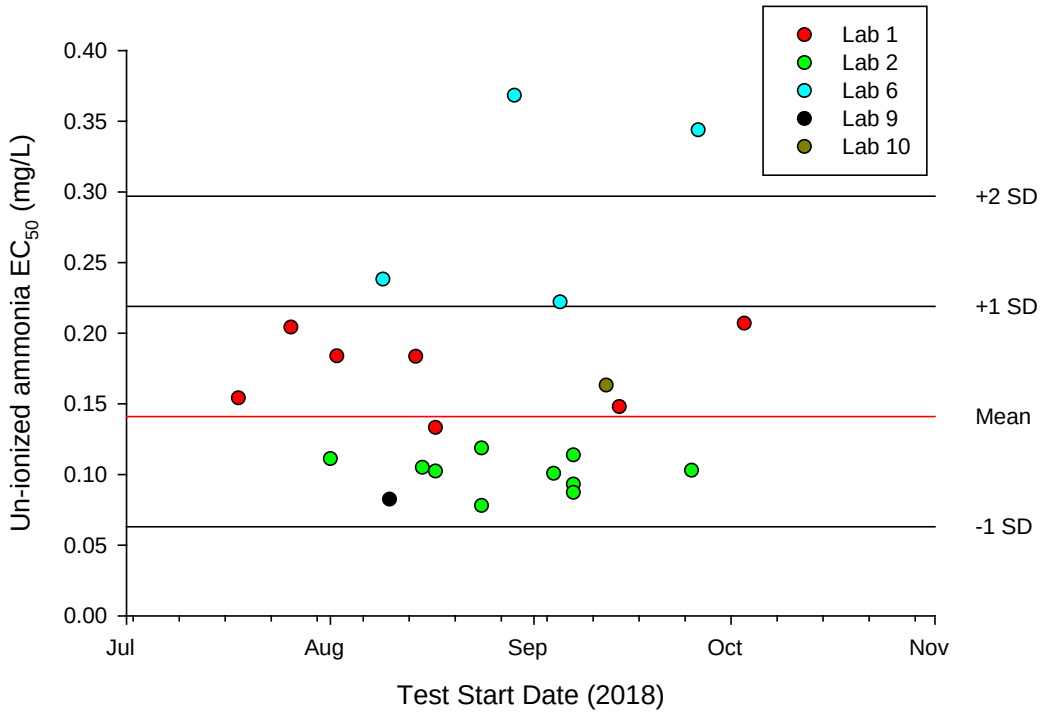

Figure III-3. Results of mussel embryo 48 hr reference toxicant tests with ammonia. The historical grand mean and standard deviations are plotted for reference.

E. Water Quality

There was 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. All data outside of the limits will be flagged in the database.

F. Test Performance

All amphipod test batches met the control criteria of 90% survival and a coefficient of variation of no more than 11.9%. 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 Brackish Estuaries (Table III-2). The target for this stratum was not met because nine of the stations turned out to not have a salinity below 27 g/kg which was the threshold for inclusion. This issue also occurred for the mussel test. All of these stations were instead placed into the Estuary stratum for both tests. 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*		
Time Interval (days)	Collected	Tested	Testing Success (%)	Collected	Tested	Testing Success (%)
Bays	43	43	100	43	43	100
Brackish Estuaries	12	12	100	10	8	80
Estuaries	45	45	100	45	39	87
Marinas	44	44	100	44	44	100
Ports	56	56	100	56	55	98
Channel Islands	15	15	100	0	0	--
Shelf	46	46	100	0	0	--
Total	261	261	100	198	189	95

G. Interlaboratory Study and Split Samples

Interlaboratory Study

Prior to the Bight ’18 survey period, an interlaboratory study was conducted for both test methods. This study was performed for both test species 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. Each laboratory tested four samples, one of which was a duplicate sample. Details for evaluating and scoring each category are described in Appendix E. The final score was broken down into four categories: Low, Moderate, High, and Very High comparability. A laboratory passed the intercalibration test if they received a score of Moderate or above. Ten laboratories participated in the amphipod intercalibration and six laboratories participated in the mussel embryo intercalibration. Laboratories which did not participate in the mussel embryo intercalibration were not given Bight samples for this test. All laboratories participating in the amphipod test were found to be comparable during the intercalibration exercise. One laboratory failed the initial intercalibration for the mussel test. This laboratory was allowed to repeat the test against a referee laboratory that had passed the first round. The laboratory passed on the second attempt. The final intercalibration results ranged from High to Very High comparability for the amphipod test and Moderate to Very High comparability for the mussel test (Table III-3).

Table III-3. Final laboratory intercalibration results.

Laboratory	*Eohaustorius estuarius*	*Mytilus galloprovincialis*
1	High	Very High
2	High	Very High
3	Very High	DNP
4	Very High	DNP
5	Very High	Moderate
6	Very High	DNP
7	Very High	Very High
8	Very High	DNP
9	Very High	Very High
10	Very High	High

DNP-Laboratory did not participate in this test

Split Samples

Split samples from stations B18-10115 and B18-10178 were tested by all laboratories. The results of these split samples were used to monitor interlaboratory variability. Due to the less controlled nature of the split samples compared to the interlaboratory study (e.g., expanded holding times), the outcomes for the split samples 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 '18 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 9. The ranges used for assessment were: 90% or greater of the points, very high comparability; 80-90%, high comparability; 70-80%, moderate comparability; and < 70%, low comparability.

For the amphipod testing, all the laboratories fell within an acceptable range of agreement based on the intercalibration scoring method. For station B18-10115, six classified the sample as Nontoxic, two as Low Toxicity and one in the Moderate category (Figure III-4). For B18-10178, there was somewhat less agreement with five of the laboratories identifying it as Nontoxic and two each in the Low and Moderate categories. While there were these differences in the SQO category identified, the overall range in survival percentages was acceptable; 79-99% for B18-10115 and 71-95% for B18-10178.

The testing of the split samples with the mussel test showed acceptable agreement between the laboratories for both stations (Figure III-4). All five participating laboratories found both samples to be Nontoxic. The remaining five laboratories which tested Bight '18 samples did not conduct the mussel test for the survey.

Eight of the nine laboratories were found to have either high or very high comparability for the amphipod tests of the split samples (Table III-4). Laboratory 2 was found to have moderate comparability. Four of the five laboratories had high or very high comparability for the mussel test with Laboratory 10 having moderate comparability (Table III-5).

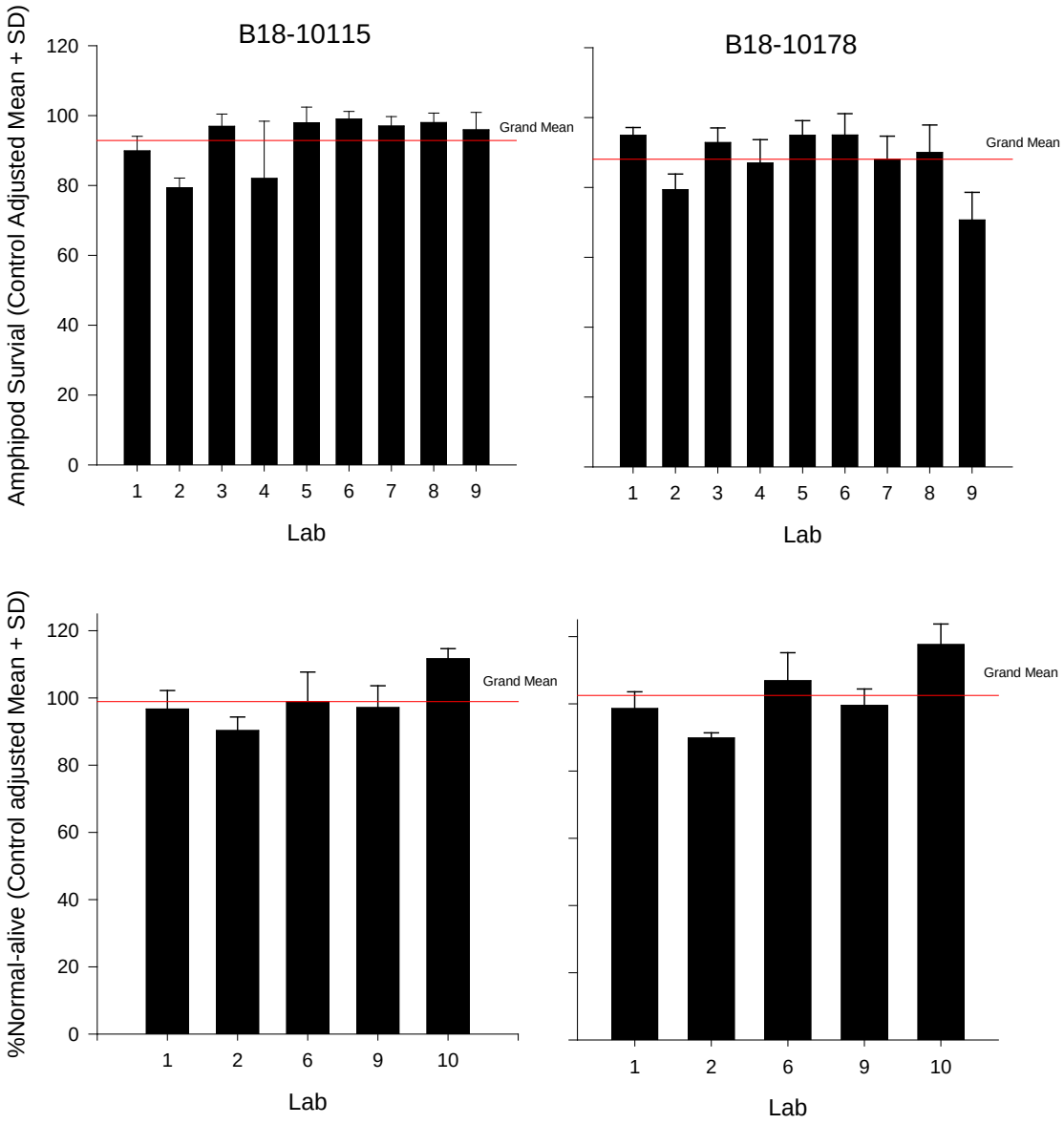

Figure III-4. Results of split sample testing of two Bight stations for the amphipod and mussel embryo tests.

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

Laboratory	10115 Difference[1]	10115 Category[2]	10178 Difference[1]	10178 Category[2]	Total	Comparability Category
1	3	1.0	3	1.0	8.0	High
2	2	0.5	3	1.0	6.5	Moderate
3	3	1.5	3	1.0	8.5	Very High
4	2	1.0	3	1.5	7.5	High
5	3	1.5	3	1.0	8.5	Very High
6	3	1.5	3	1.0	8.5	Very High
7	3	1.5	3	1.5	9.0	Very High
8	3	1.5	3	1.0	8.5	Very High
9	3	1.5	2	1.0	7.5	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.

Table III-5. Split sample assessment of each laboratory's comparability using the *Mytilus galloprovincialis* sediment toxicity test.

Laboratory	10115 Difference[1]	10115 Category[2]	10178 Difference[1]	10178 Category[2]	Total	Comparability Category
1	3	1.5	3	1.5	9.0	Very High
2	3	1.5	2	1.5	8.0	High
6	3	1.5	3	1.5	9.0	Very High
9	3	1.5	3	1.5	9.0	Very High
10	2	1.5	2	1.5	7.0	Moderate

[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.

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 protocols could be made to all laboratories. Very few deviations from the test protocols were observed. One laboratory did not sieve the sediment before conducting testing for mussel sediment-water interface method. The largest possible source of error that can occur from not sieving for this test is from native animals dying in the sediment and causing poor water quality. There was no indication from the water quality analysis that this occurred, but data from this test batch will be flagged and a comment included in the database.

IV. DESCRIPTIVE RESULTS

A. Frequency of Toxicity

Of the 261 stations tested with the amphipod survival test, 226 (87%) were in the Nontoxic and Low Toxicity categories and therefore considered to be not toxic (Table IV-1, Figure IV-1 and Figure IV-2). Of these, 17 stations (6% of all stations) fell into the Low Toxicity category. No toxicity (Moderate or High Toxicity) was observed in any of the offshore stations (Channel Islands and Shelf), nor in the Port stratum. The highest percentage of toxic stations was found in the Brackish Estuaries where 5 of the 12 (42%) stations were toxic. The next highest percentage of toxic stations was the Estuaries (40%), followed by Marinas (24%), and Bays (2%).

Of the 189 stations successfully tested using the mussel embryo test, 186 (98%) were found to be not toxic (Table IV-2, Figure IV-3). Toxicity was observed at one station each in Estuaries, Brackish Estuaries, and Marinas strata. There were also relatively few stations in the Low Toxicity (6%) category. Only one station was found to be in the High Toxicity category.

The Brackish Estuaries strata was new for Bight ’18 and proved to have the most stations exhibit toxicity to the amphipods but had a low prevalence of toxicity to the mussel embryo test (Tables IV-1 and IV-2). This stratum also had the lowest number of stations tested because the salinity of about one third of the targeted stations was higher than expected placing them into the Estuaries stratum.

After integration of the results from the two toxicity tests, 177 of the 189 (94%) embayment stations tested with both methods were found to be not toxic (Table IV-3, Figure IV-4). All stations found to be toxic were in the Moderate Toxicity category. There were no stations in the High Toxicity category after integration, which indicates that none of the stations fell into this category for both test species. The Bays and Ports strata had no stations found to be toxic. The Brackish Estuary stratum again had the highest prevalence of integrated toxicity with 4 out of 8 (50%) found to be toxic. In addition to the numerical analysis of toxicity frequency, these results were plotted as color-coded SQO categories overlaid on a map of the SCB (Figure IV-5). This provides additional perspective on the location of each of the stations and how they relate to surrounding locations. Although the integrated results do not show toxicity in the Offshore strata, all those stations were not toxic. A summary table of the toxicity test results and SQO categories for all stations tested in Bight ’18 is provided in Appendix A of this report.

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

Stratum	Nontoxic	Low Toxicity	Moderate Toxicity	High Toxicity
Bays	39	3	1	0
Brackish Estuaries	6	1	1	4
Estuaries	22	5	13	5
Marinas	29	5	10	1
Ports	54	1	0	0
Channel Islands	14	1	0	0
Shelf	45	1	0	0
Total Embayments	150	15	25	10
Total Bight	209	17	25	10

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
Bays	43	0	0	0
Brackish Estuaries	6	1	0	1
Estuaries	32	6	1	0
Marinas	41	3	1	0
Ports	53	1	0	0
Total Embayments	175	11	2	1

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
Bays	39	4	0	0
Brackish Estuaries	2	2	4	0
Estuaries	19	14	6	0
Marinas	26	17	2	0
Ports	52	2	0	0
Total Embayments	138	39	12	0

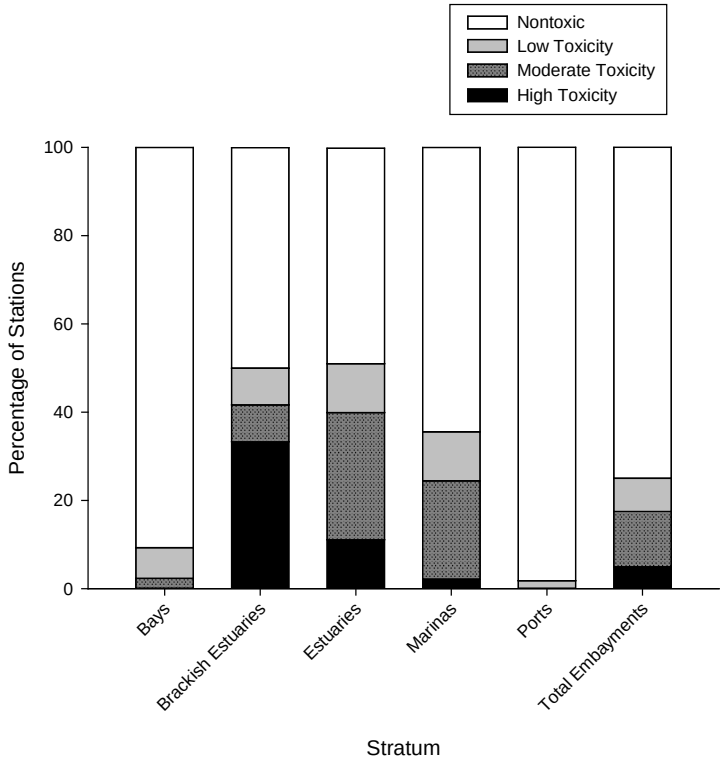

Figure IV-1. The percentage of stations in each sediment quality objective category for *Eohaustorius estuarius* survival test by embayment strata.

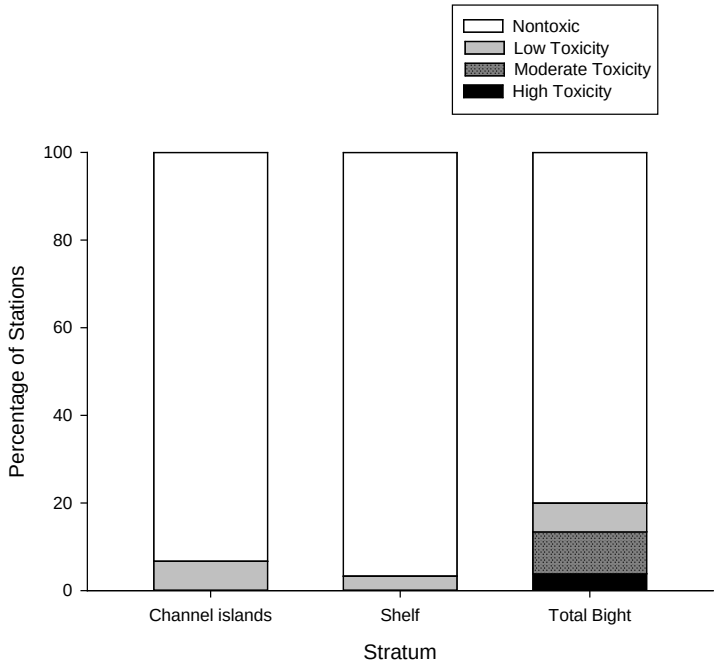

Figure IV-2. The percentage of stations in each sediment quality objective category for *Eohaustorius estuarius* survival test by offshore strata and the Bight as a whole.

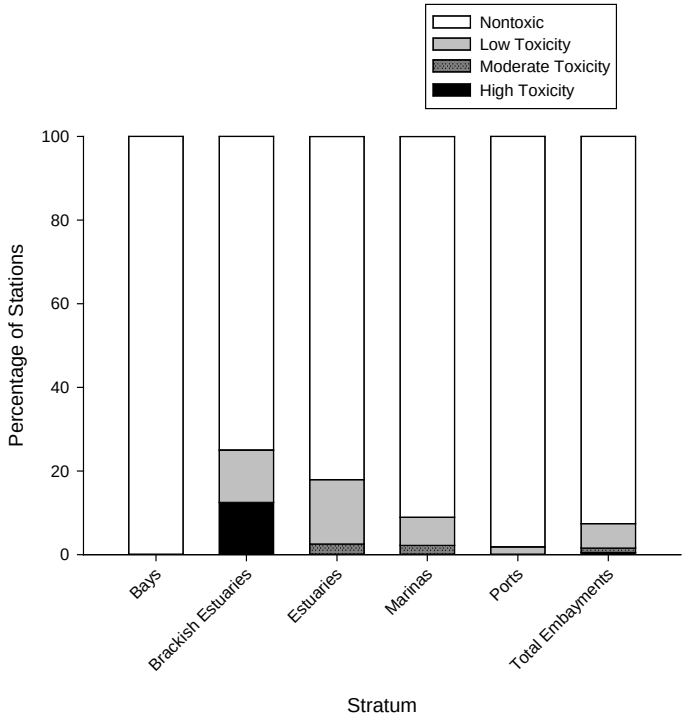

Figure IV-3. The percentage of stations in each sediment quality objective category for mussel embryo sediment-water interface test by embayment strata.

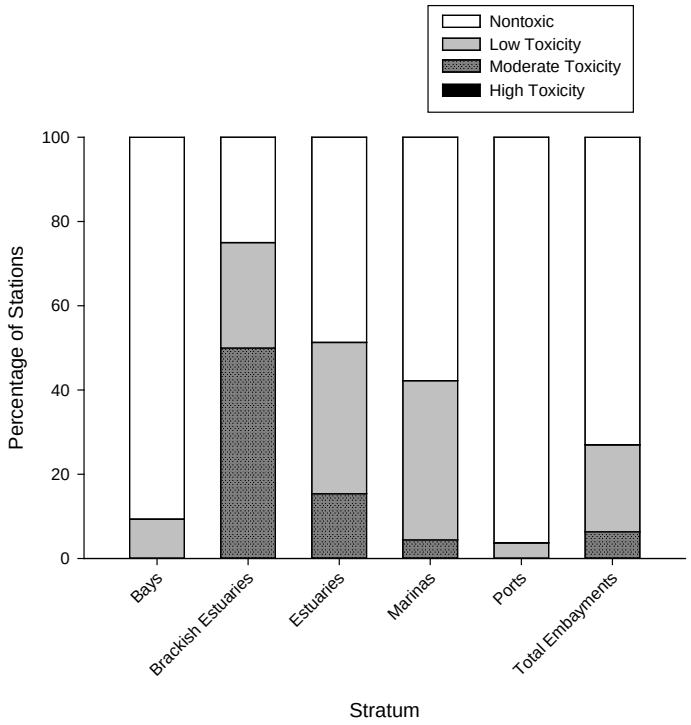

Figure IV-4. The percentage of stations in each sediment quality objective category after integration of the results from the amphipod and embryo sediment-water interface tests by embayment strata.

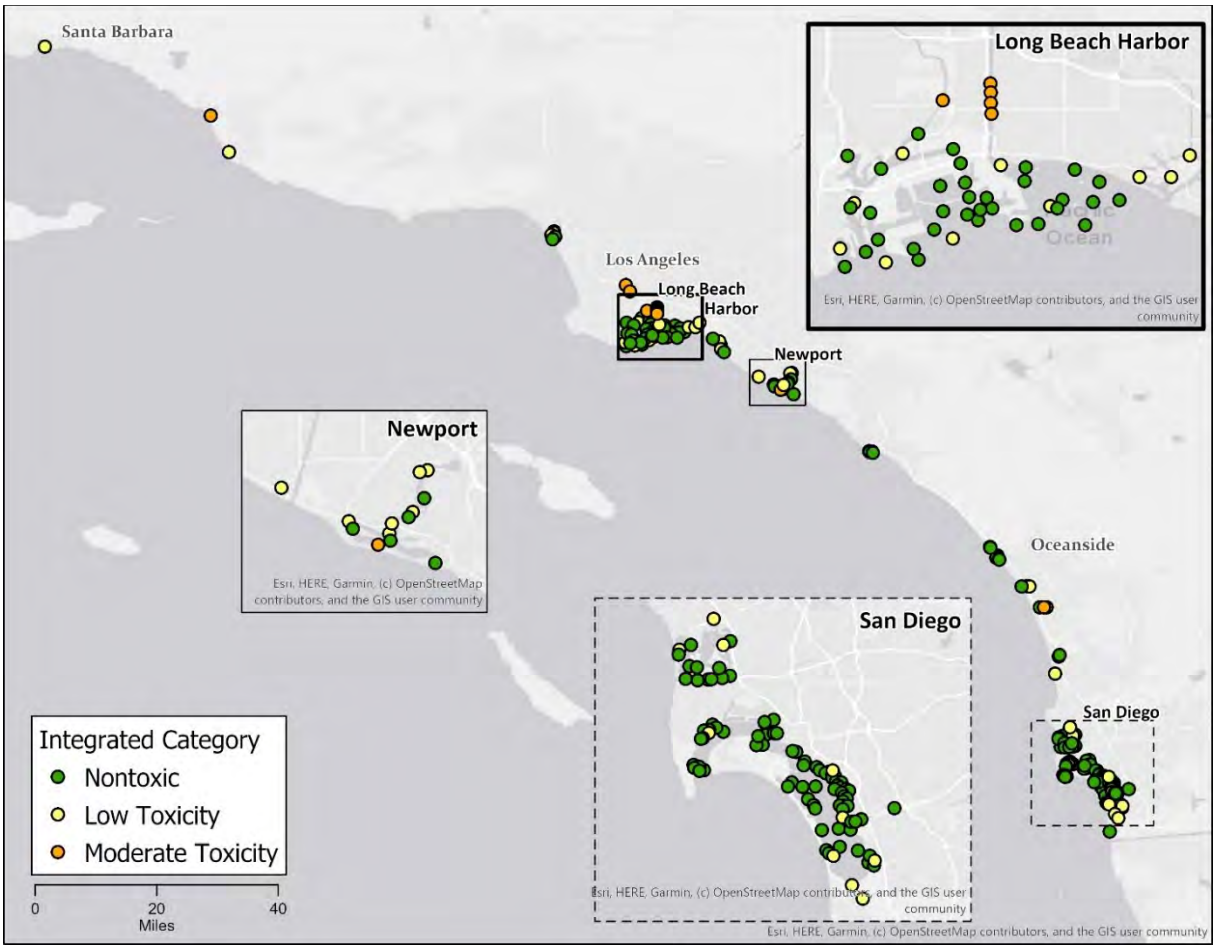

Figure IV-5. The relative location and corresponding integrated toxicity SQO category for each embayment station in the SCB.

B. Magnitude of Toxicity

The magnitude of toxicity for each stratum is described by the degree of control-adjusted response for each test method. The greatest magnitude of toxicity to the amphipod test was observed in the Brackish Estuaries where the mean survival in both the Moderate and High categories was the lowest (Table IV-4). There were only three stations for the mussel embryo test that fell into the Moderate or High Toxicity categories making any comparison between strata difficult. The lowest percentage normal-alive in the study was 1% in a Brackish Estuary station.

There was no agreement between the two test methods for samples that were found to be toxic. This is indicated by the lack of stations in the lower left quadrant of Figure IV-6. There were multiple stations where the amphipod test indicated toxicity, but the mussel embryo test did not (upper left quadrant), with 10 of those stations falling into the High Toxicity category (Table IV-1). There was only one sample location that had High Toxicity for the mussel embryo test but had a Nontoxic amphipod test result (Figure IV-6). Aside from this one observation, the results indicate that the amphipod test was more sensitive than the mussel embryo test. This same

relationship was also observed in Bight ’08 and ’13 and can be expected based on organism-specific sensitivities to different compounds.

Table IV-4. Summary data of mean control-adjusted survival of amphipods in each stratum for stations in the Moderate and High Toxicity categories.

	Moderate Toxicity			High Toxicity		
Stratum	Mean	Range	n	Mean	Range	n
Bays	82	na	1	na	na	0
Brackish Estuaries	73	na	1	7	0-26	4
Estuaries	77	59-81	13	21	1-58	5
Marinas	75	65-81	10	48	na	1
Ports	na	na	0	na	na	0
Channel Islands	na	na	0	na	na	0
Shelf	na	na	0	na	na	0
All Strata	76	59-82	25	18	0-58	10

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

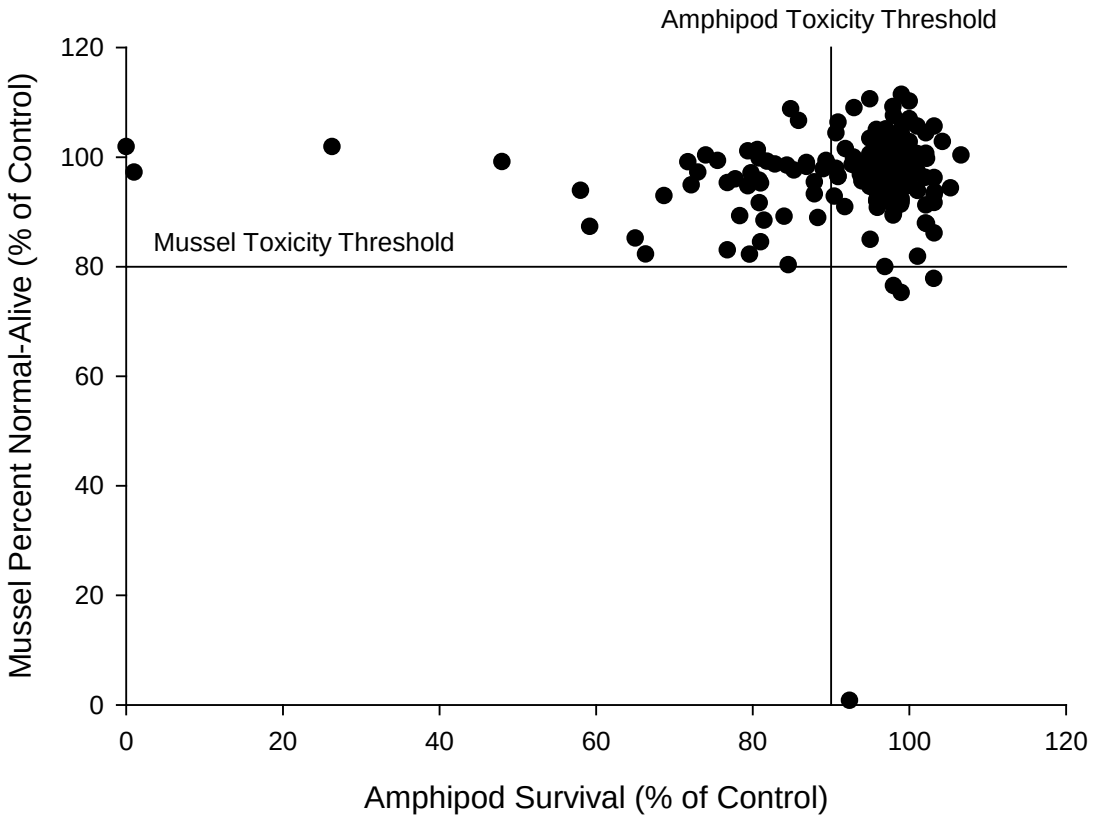

Figure IV-6. Comparison of the results between the amphipod and mussel embryo toxicity test methods. Note that samples falling below the thresholds indicated may not be identified as toxic because a statistical difference from the control is also necessary to indicate toxicity.

V. REGIONAL ASSESSMENT OF TOXICITY

A. Extent

The area data presented in this section should be evaluated with a few key points in mind. First, the stations excluded for the mussel test due to holding time issues leaves a total of 3.57 km^2 for which complete assessments cannot be made. This represents 2.86% of the total embayments and 0.02% of the SCB. About half of this area is accounted for by Estuaries with the other half split between Brackish Estuaries and Ports. Second, all area estimates within any toxicity category that are made up of only a few stations have high uncertainty around those estimates. This is a consideration for any statistical analysis with low sample size. The lower bounds of the 95% confidence limit for these estimates may include zero. Additionally, percent area allows for evaluation of the toxicity in each stratum as a whole and does not represent site-specific toxicity results.

The total area of the Bight surveyed in 2018 for sediment toxicity was 6118 km^2. The amphipod survival test indicated 96.3% (or 5892 km^2) of the total SCB was in the Nontoxic category (Table V-1 and Figure V-1). An additional 3.5% (or 213 km^2) was categorized as Low Toxicity, bringing the total area considered to be not toxic (Nontoxic and Low categories) to 99.8% (or 6105 km^2). The area classified as toxic (Moderate and High categories) was 0.2% (or 12 km^2) of the total SCB.

For the embayment strata (Bays, Brackish Estuaries, Estuaries, Marinas, and Ports), the total area was 126 km^2, of which 82.4% (or 104 km^2) was Nontoxic and 7.7% (or 9.7 km^2) was in the Low Toxicity category based on the amphipod test (Table V-1, Figure V-1). Therefore, the total area categorized as not toxic was 90.1% (or 114 km^2). The amphipod test identified 9.9% (or 13 km^2) of embayments as toxic. Within the embayments, the Estuaries and Brackish Estuaries strata had the largest percentage of area (38% and 42%, respectively) identified as toxic (Figure V-2). No calculated area within the Port stratum was categorized as toxic by the amphipods. The Offshore strata (Channel Islands and Inner-, Mid-, and Outer-shelf) were categorized as not toxic for 100% of the area (or 5883 km^2) by the amphipod test (Table V-1, Figure V-3).

The mussel embryo SWI test was only conducted in embayment strata samples. The embayment area found to be Nontoxic by the mussel embryo test was 94.4% (or 118 km^2) of the total area (Table V-2 and Figure V-4). An additional 2.2% (or 2.8 km^2) was categorized as Low Toxicity, bringing the total area identified as not toxic to 96.6% (or 121 km^2). The area identified as toxic was 0.5% (or 0.7 km^2). The entirety of the toxic percent area was due to results at three stations, one each from the Brackish Estuary, Estuary, and Marina strata, with the Brackish Estuary contributing the largest percent area at 0.4% (or 0.38 km^2). Due to holding time exceedances for samples from the Ports (1 station), Estuaries (6 stations), and Brackish Estuaries (2 stations), the toxicity for 2.9% of the embayment area (or 3.6 km^2) could not be categorized.

Results from the two toxicity tests were integrated only for the embayment strata, where both tests were performed. The area categorized as Nontoxic was reduced to 78.8% of the area (or 99 km^2; Table V-3 and Figure V-5). The integrated toxicity estimate of percent area is slightly reduced from the amphipod-only test results and greatly reduced from the mussel-only test results. This reduction is caused by the effect of averaging the category results and rounding up if the mean was between two categories. The Low toxicity category percent area (15.6% or 19.5

km^2) was greatly increased from the individual test results (2.2% and 7.7% for mussels and amphipods, respectively). The total percent area categorized as not toxic was 94.4% (or 118 km^2), which is similar to the mussel embryo test results and amphipod test results. The integrated area classified as toxic was 2.7% (or 3.4 km^2), which was lower than the results from the amphipod test alone but higher than the results from the mussel test. Due to the holding time exceedances, the toxicity of 2.9% (or 3.6 km^2) of the area could not be categorized.

When the data was integrated for the two tests, the largest area of toxic sediment was in the Brackish Estuary stratum with 1.5 km^2 representing 40% of that stratum. This is due to the low number of stations and high incidence of toxicity observed in the Brackish Estuary. The lowest toxicity was observed in the Bay and Port strata, with 100% of each area (70.4 km^2 and 25.9 km^2, respectively) identified as not toxic.

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
Bays	62.8	6.2	5.4	5.3	2.1	3.5	0	-
Brackish Estuaries	2.3	1.2	0.4	0.6	0.4	0.7	1.5	1.0
Estuaries	5.7	1.5	1.5	1.4	2.8	1.2	1.6	1.1
Marinas	8.1	1.6	1.1	1.0	3.6	1.6	0.4	0.7
Ports	24.7	2.3	1.3	1.7	0	-	0	-
Channel Islands	1946	237	139	237	0	-	0	-
Shelf	3843	755	64.9	108	0	-	0	-
Total Bight	5892	1144	213	262	8.9	4.4	3.5	1.6

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
Bays	70.4	1.1	0	-	0	-	0	-
Brackish Estuaries	2.3	1.1	0.4	0.6	0	-	0.4	0.7
Estuaries	8.6	1.6	1.1	0.8	0.006	0.01	0	-
Marinas	11.8	1.1	1.1	1.0	0.3	0.5	0	-
Ports	24.9	2.0	0.2	0.3	0	-	0	-
Total Bight	118	10.9	2.8	1.5	0.3	0.5	0.4	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
Bays	62.8	6.2	7.6	6.3	0	-	0	-
Brackish Estuaries	0.8	0.8	0.8	0.9	1.5	1.0	0	-
Estuaries	5.1	1.6	3.5	1.7	1.1	0.8	0	-
Marinas	7.2	1.6	5.3	1.7	0.7	0.9	0	-
Ports	22.7	3.0	2.1	2.1	0	-	0	-
Total Bight	98.5	11.4	19.5	6.8	3.4	1.5	0	-

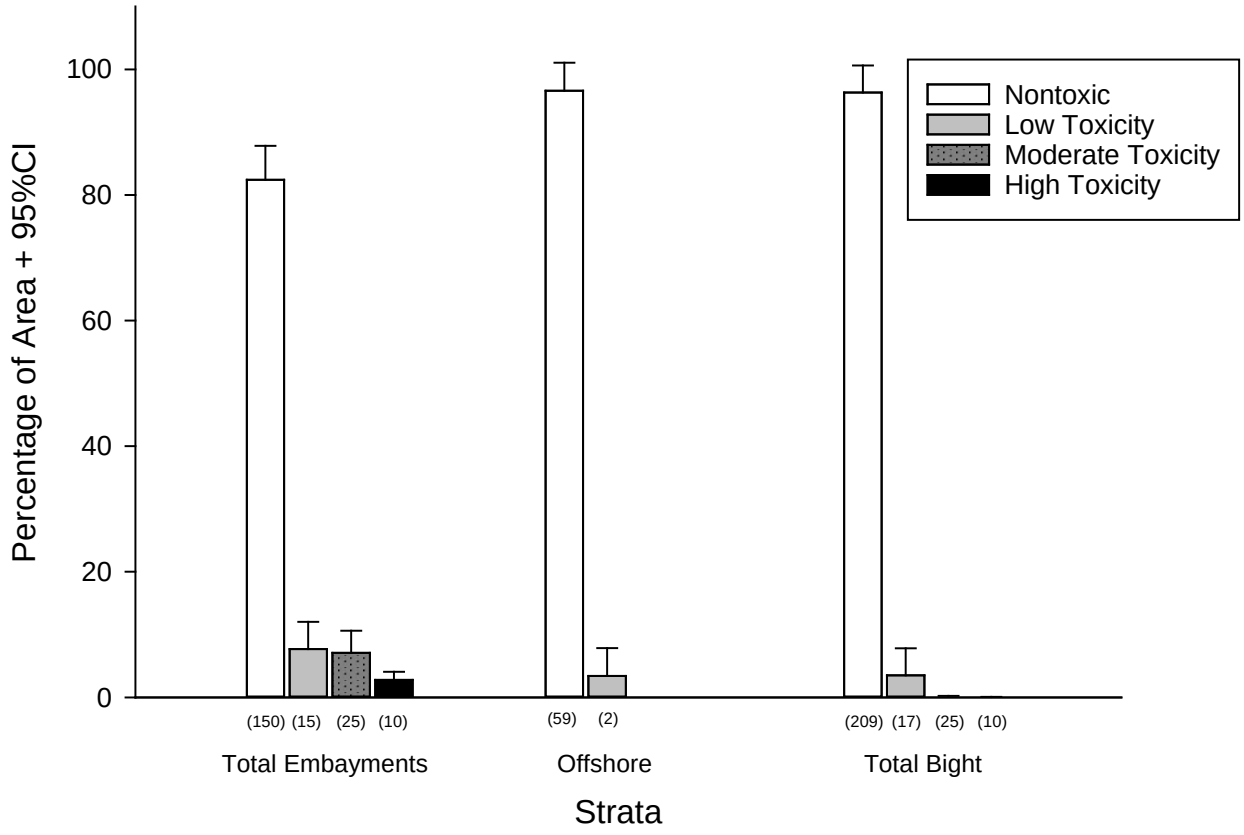

Figure V-1. Percentage of area falling into each of the sediment quality objective categories by major strata groups using the amphipod survival test. The number of stations representing the data (n) is listed for each bar.

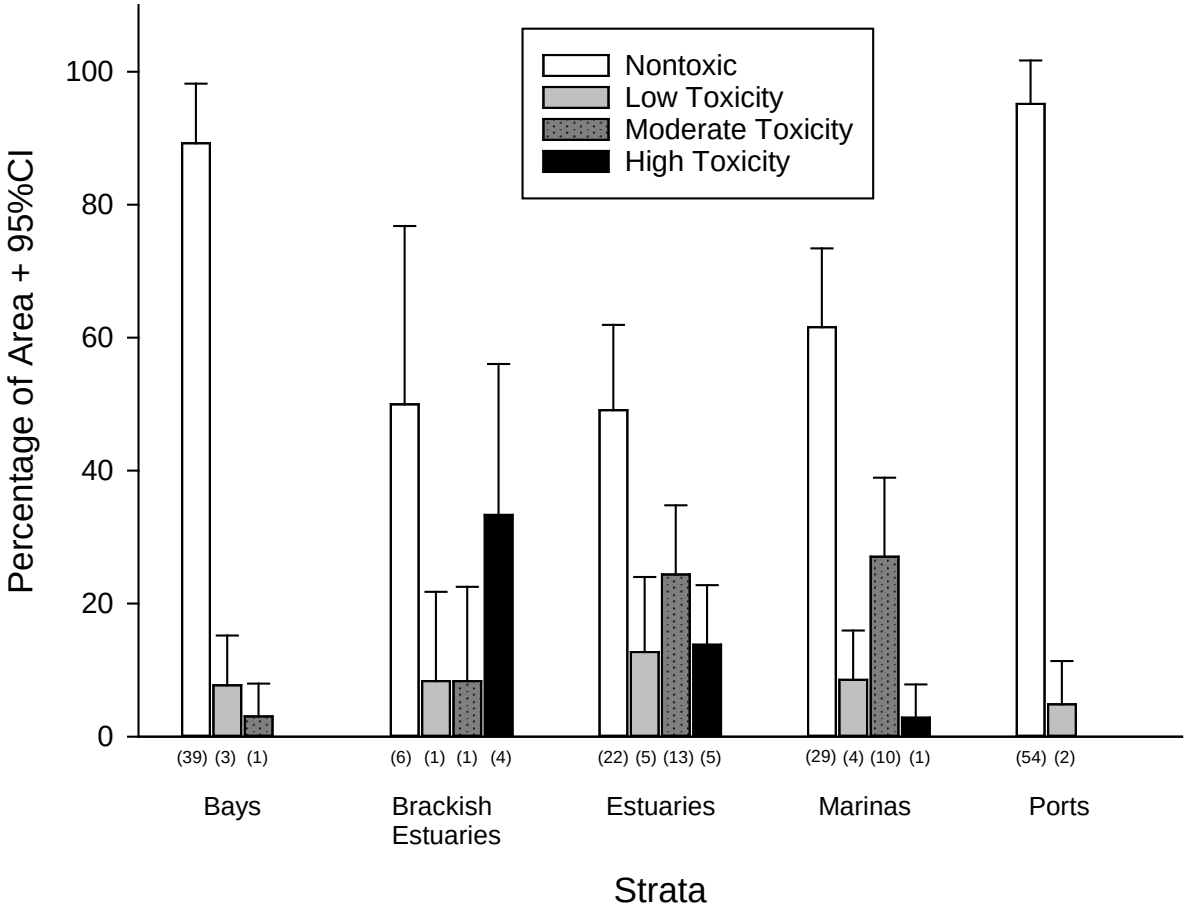

Figure V-2. Percentage of area falling into each of the sediment quality objective categories by embayment strata using the amphipod survival test. The number of stations representing the data (n) is listed for each bar.

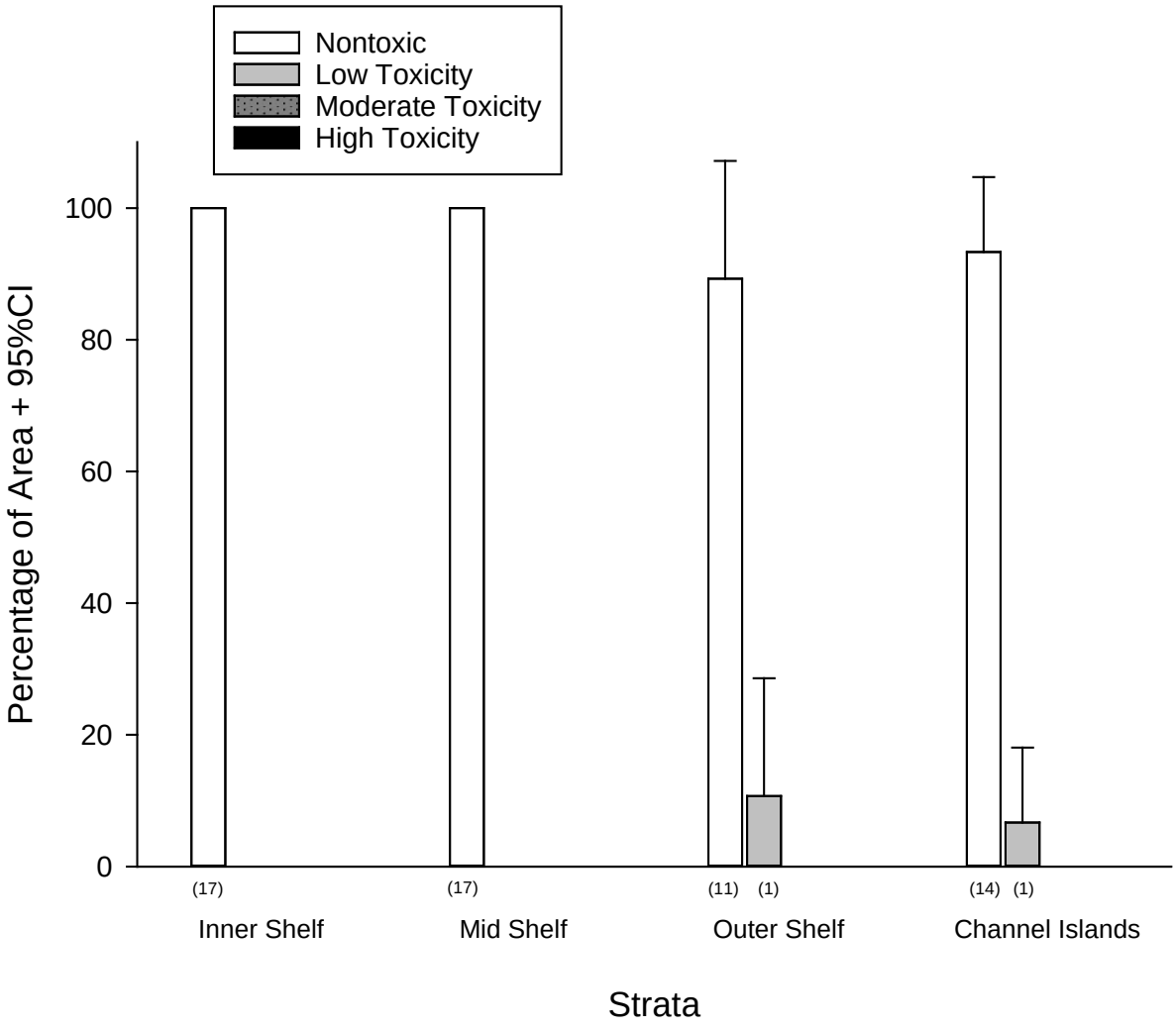

Figure V-3. Percentage of area falling into each of the sediment quality objective categories by offshore strata using the amphipod survival test. The number of stations representing the data (n) is listed for each bar.

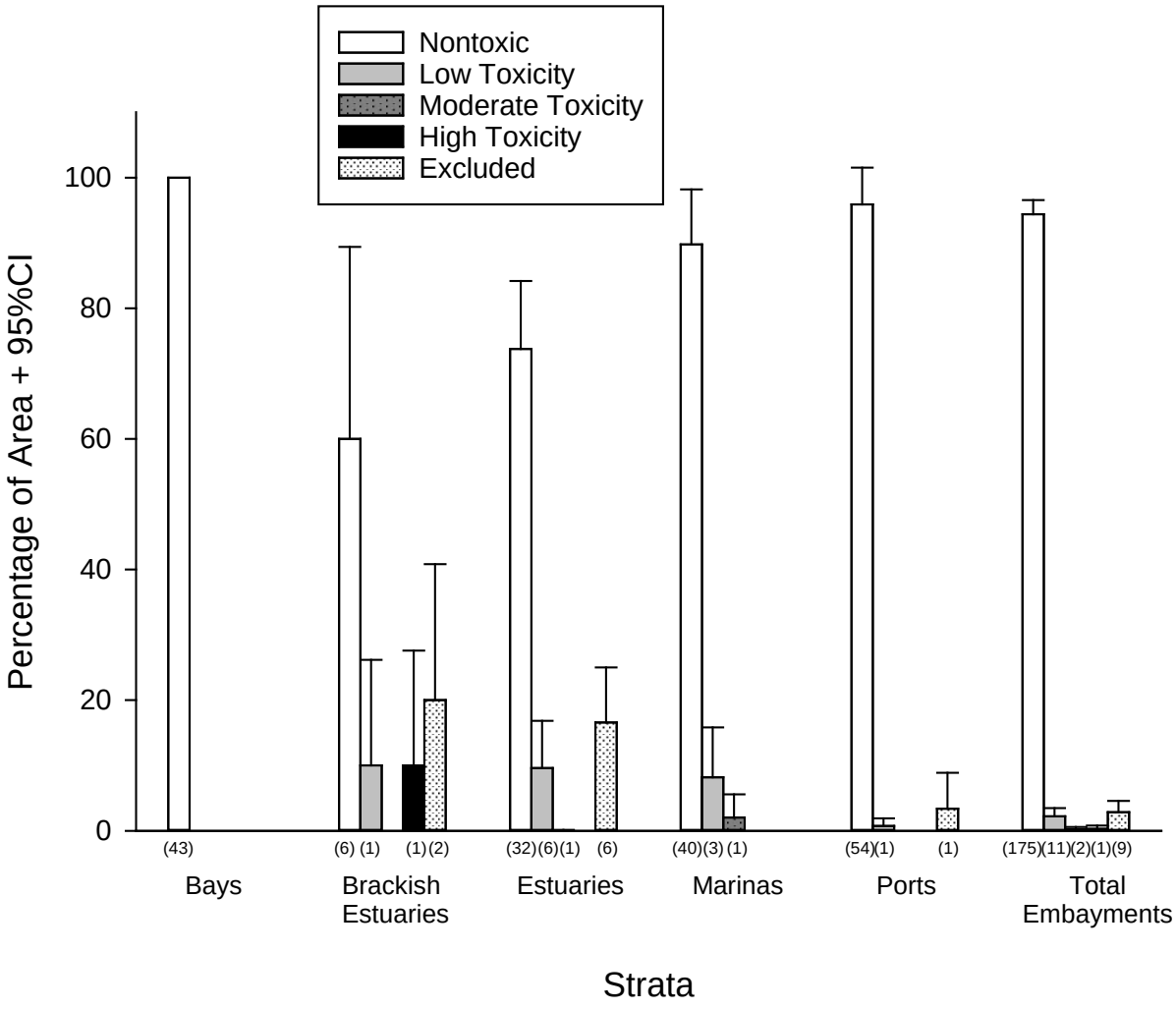

Figure V-4. Percentage of area falling into each of the sediment quality objective categories by strata using the mussel embryo sediment-water interface test. The number of stations representing the data (n) is listed for each bar.

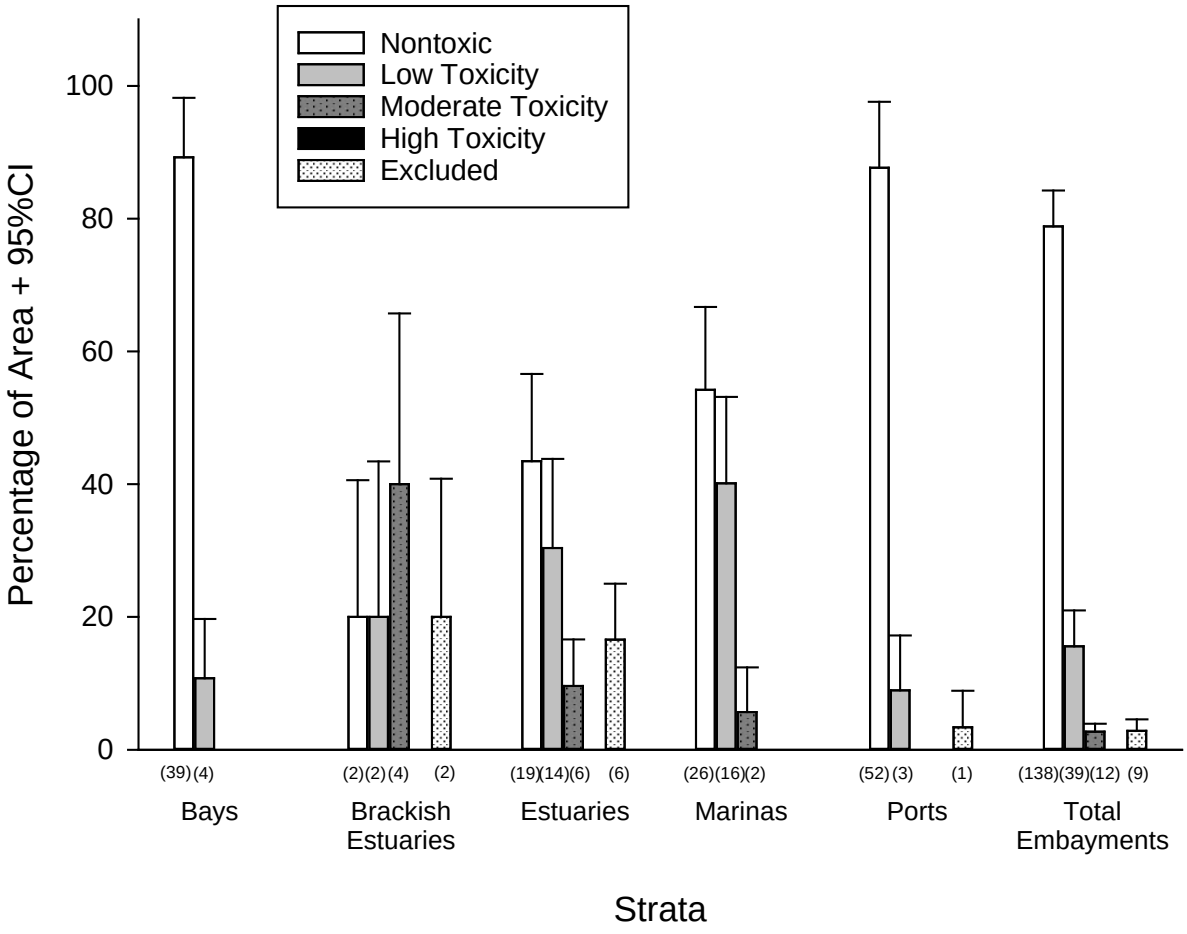

Figure V-5. Percentage of area falling into each of the sediment quality objective categories by strata when the results of the amphipod and mussel embryo tests are integrated. The number of stations representing the data (n) is listed for each bar.

B. Temporal Variation

Temporal variation was evaluated using survey data from Bight ’98 through Bight ’18. Overall, for Offshore, Total Embayments, and Total Bight amphipod test results, the trend towards decreasing toxicity identified in Bight ’08 and ’13 continued in this survey (Figure V-6). However, when the embayment strata were evaluated separately, the only strata to continue this decrease was the combination of Ports and Bays (Figure V-7). The percent area identified as toxic increased for Marinas and Estuaries in 2018 compared to the Bight 2008 and 2013 survey results and was similar to the results obtained in Bight ’98.

The embayment strata can be further evaluated by comparing the mussel test and integrated SQO score results from the past three surveys. The Bay, Port, Estuary, and Total embayment strata show a trend towards reduced toxicity for the mussel (Figure V-8) and integrated SQO (Figure V-9) test results, consistent with the Bight ’13 findings. The reduction in the percent area of Low, Moderate and High integrated toxicity for the Estuary stratum was minimal, with what appears to be a change of the High Toxicity percent area to Moderate Toxicity. The mussel test results indicate an increase in toxicity extent and magnitude for the Marina stratum, with an overall increase in total percent area as well as a Moderate Toxicity classification at a few stations. A comparison of mussel and integrated toxicity test results from the Bight ’08 to ’18 surveys indicate a notable reduction in toxicity across all embayment strata.

Looking at temporal comparisons for the amphipod test on individual stations provides some insight on localized changes over time. Appendix B provides a cross-talk table for the revisit station IDs from Bight ’18 and all prior surveys. Appendices C and D contain links to maps of the SCB illustrating results from Bight ’18 as well as comparisons to results from previous Bights for repeated stations. There are 78 revisit stations which have been sampled and tested with the amphipod survival test during at least two Bight surveys. Of those, 68 stations were sampled during the last three surveys and 64 stations were sampled in four of the last five surveys. None of the stations were found to be toxic (Moderate or High Toxicity) during all surveys (Figure V-10). The majority (67%) of stations were found to be not toxic (Nontoxic or Low Toxicity) for all surveys sampled. A station was considered to be “trending toxic” or “trending not toxic” if there was a change in the SQO category classification in one direction. If the station changed category with no set pattern, the trend was considered “Inconsistent”. With the addition of the Bight ’18 data, 12.6% of stations were trending not toxic, 5.1% were trending toxic, and 15.2% were inconsistent using the amphipod test.

For the mussel test and integrated toxicity test results, there were 56 stations which were sampled at least twice, and 52 of those were sampled during the last three surveys. The mussel test results found 84% of stations to be always not toxic, with the remaining 16% trending not toxic (Figure V-11). When the mussel and amphipod test results were integrated, the SQO results showed 87.5% of stations were always not toxic, 8.9% were trending not toxic, 1.8% were trending toxic, and 1.8% were inconsistent (Figure V-12).

The specific stations trending toxic for either of the two tests or the integrated score were primarily in Estuaries (4 stations) with one station in Marinas (Table V-4). The specific stations trending not toxic were located across several strata, including offshore and embayment stations. Across the three evaluations (amphipod, mussel, and integrated toxicity), there were 18 unique stations trending not toxic for at least one result. These stations were located in Bays: Los

Angeles/Long Beach (2), Mission Bay (1); Marinas: Alamitos Bay (1), Newport Bay (2), San Diego Bay (3); Ports: Los Angeles/Long Beach (1), San Diego (4); Estuaries: Los Alamitos Estuary (1), San Gabriel River (1); and Offshore: Outer shelf Hueneme to Dine (1) and Channel Islands (1).

Table V-4. Locations of revisit stations trending toxic over time.

Strata	Region	Station ID	Test
Marinas	Alamitos Bay	B18-10005	Amphipod
Estuaries	Upper Newport Bay	B18-10158	Amphipod
Estuaries	Upper Newport Bay	B18-10159	Amphipod
Estuaries	Aqua Hedionda Lagoon	B18-10168	Integrated
Estuaries	Aqua Hedionda Lagoon	B18-10169	Amphipod

In addition to this general comparison of categorical changes over time, Pearson's chi-squared test were also used to test the relationship between categorical variables, such as toxicity in relation to time. For this analysis, a 2 x 2 relationship was evaluated for toxicity category (toxic or not toxic) and change over time (initial survey versus final survey). The initial survey and final survey for each station differed by the exact year, but the comparison is made to evaluate the total change over time. In some instances, the statistical test could not be completed due to insufficient sample size ($n < 5$) for a specific combination. Significance was determined using an alpha = 0.05. The Total Bight, Offshore, Total Embayments, and individual strata were evaluated. For amphipod test results, the Total Bight, Total Embayments, Marinas, and Estuaries were the only strata with enough categorical replicates for this statistical analysis technique, all of which resulted in a non-significant trend in classification from the initial to final survey thus far. At this time comparisons for the mussel test and integrated SQO category results for all strata could not be evaluated due to low sample size.

The previous temporal trend analyses have been made using categorical results. Another point of comparison is the toxicity magnitude over time, represented by either percent survival or percent normal-alive for amphipods and mussel embryos, respectively. For this analysis, the non-parametric Wilcoxon Signed Rank test was used to evaluate the change in toxicity magnitude from the initial to final survey for which data was obtained. The non-parametric test was used because toxicity test results (percent survival or normal-alive) are not truly continuous data and, therefore, do not fit the assumptions of the parametric test. Significance was determined using an alpha = 0.05, and the two-tailed hypothesis was used to determine if any significant difference exists, independent of direction of change. Grouped (i.e., Total Embayments) and individual strata were evaluated for both amphipod and mussel test results. Integrated toxicity test results could not be evaluated using this method because that result is categorical. For the amphipod test results, the change in percent survival from the initial to final survey was significantly greater for the Total Bight, Total Embayment, Bay, and Port strata (Figure V-13). The average increase for each significant stratum was 6.9, 8.9, 8.1, and 12.4 percent survival, respectively. For the mussel test results, the change in percent normal-alive from the initial to final survey was significantly greater for the Total Embayment, Bay, Marina, and Port strata (Figure V-14). The average

increase for each significant stratum was 10.3, 10.4, 14.1, and 11.0 percent normal-alive, respectively. These results indicate some improvement in condition over time for some strata.

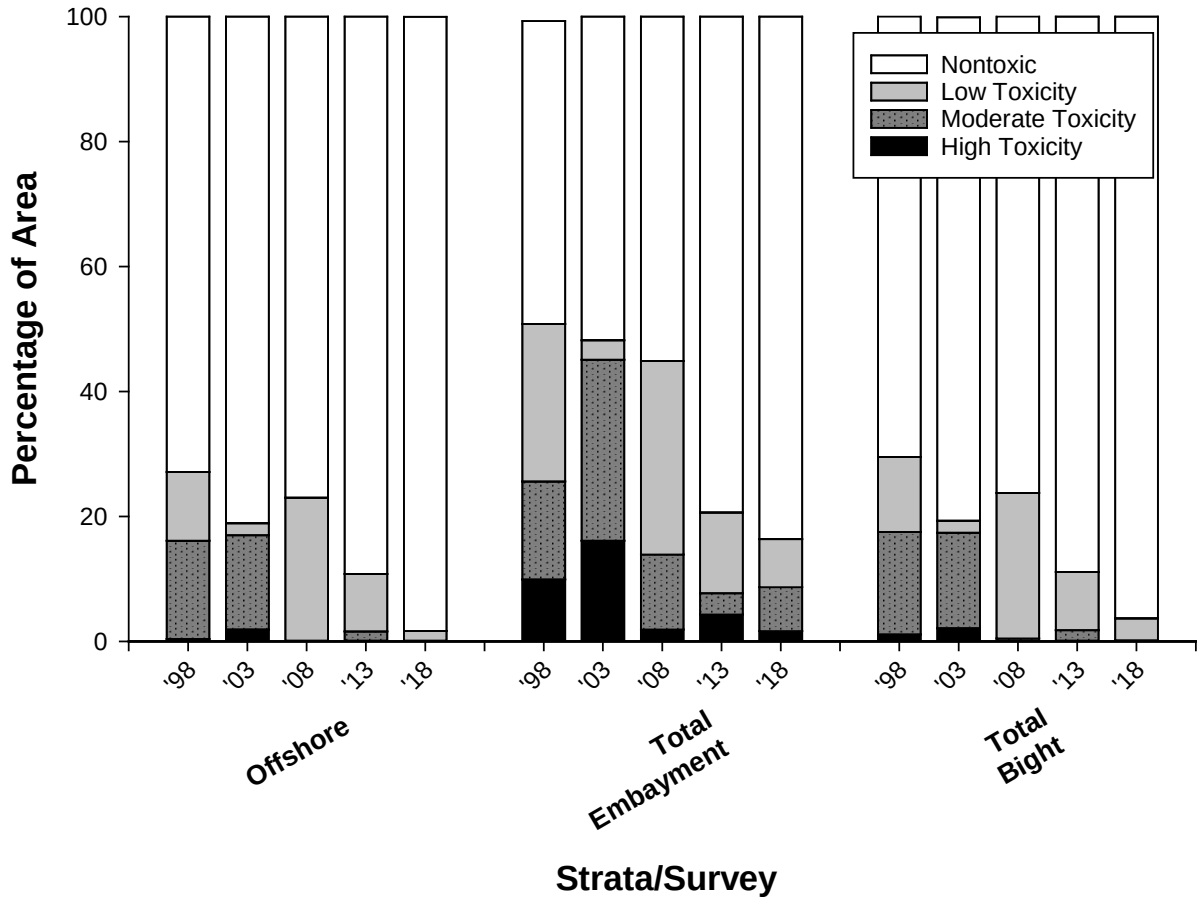

Figure V-6. Comparison of percentage areas by sediment quality objective categories for the amphipod test, shown by major strata groups over multiple surveys.

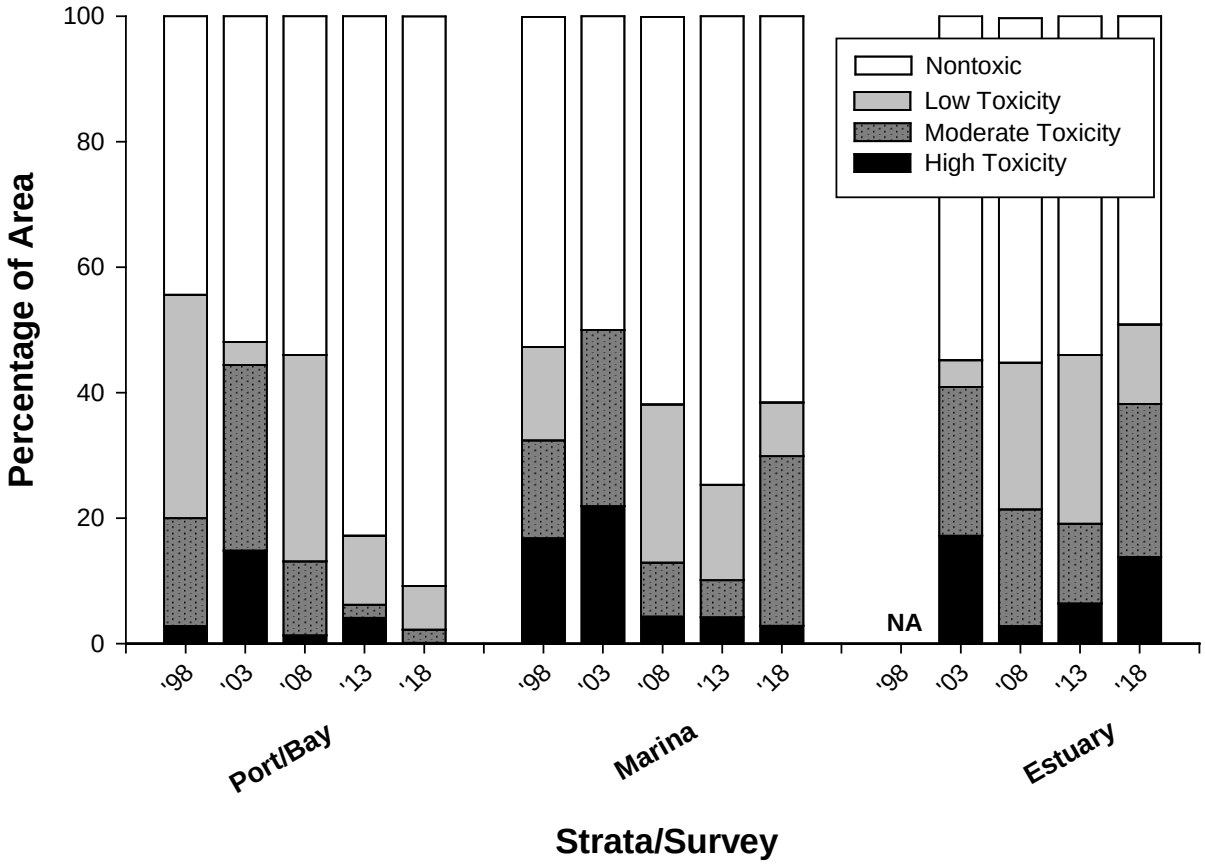

Figure V-7. Comparison of percentage areas by sediment quality objective categories for the amphipod test, shown by embayment strata over multiple surveys.

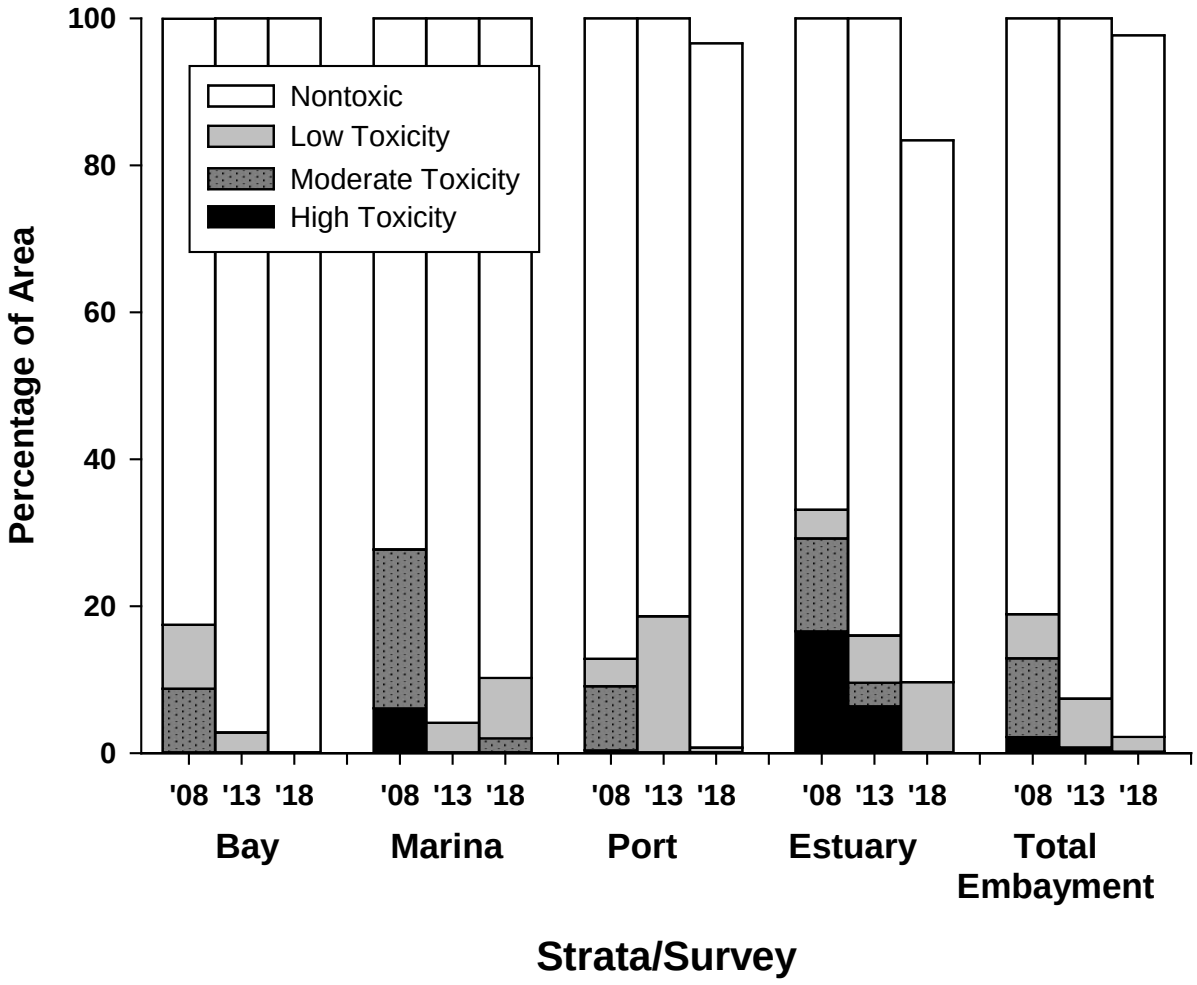

Figure V-8. Comparison of percentage areas by sediment quality objective categories for the mussel test, shown by strata over multiple surveys.

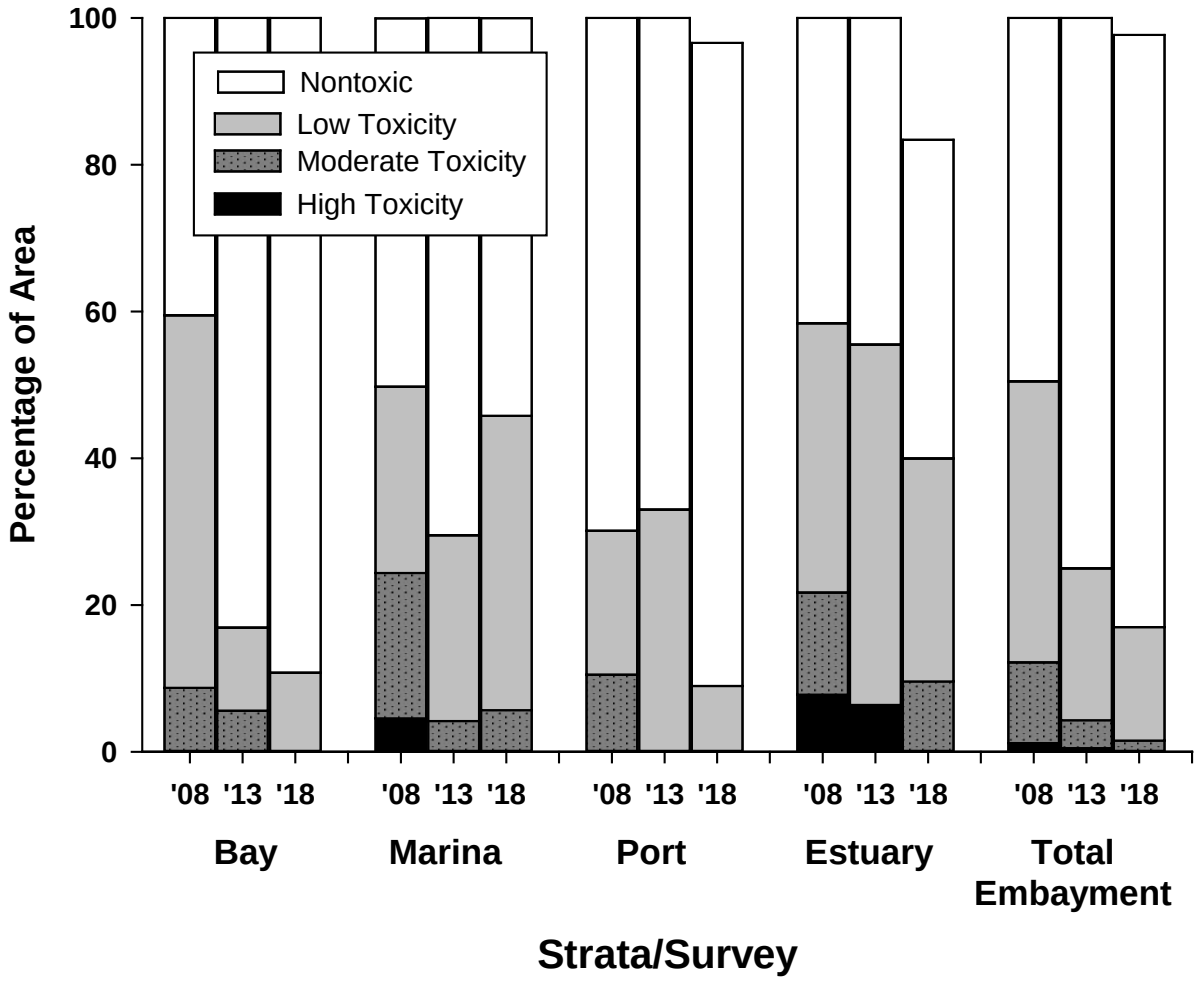

Figure V-9. Comparison of percentage areas by sediment quality objective categories for the integrated SQO score, shown by strata over multiple surveys.

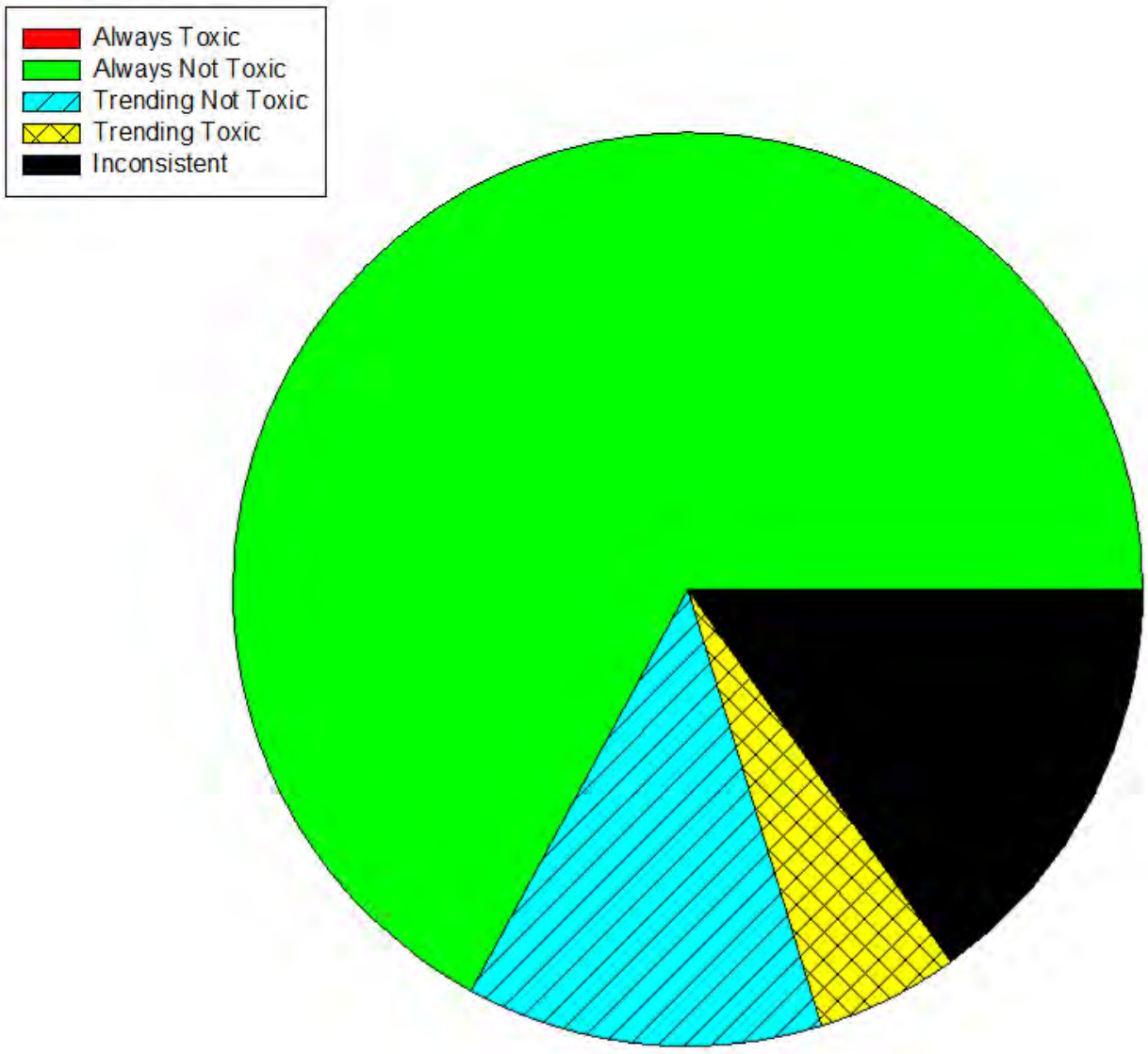

Figure V-10. Temporal trends for the amphipod survival test for individual revisit stations over four sampling periods: Bight ’18, Bight ’13, Bight ’08, and either Bight ’03 or Bight ’98. No stations were found to always be toxic.

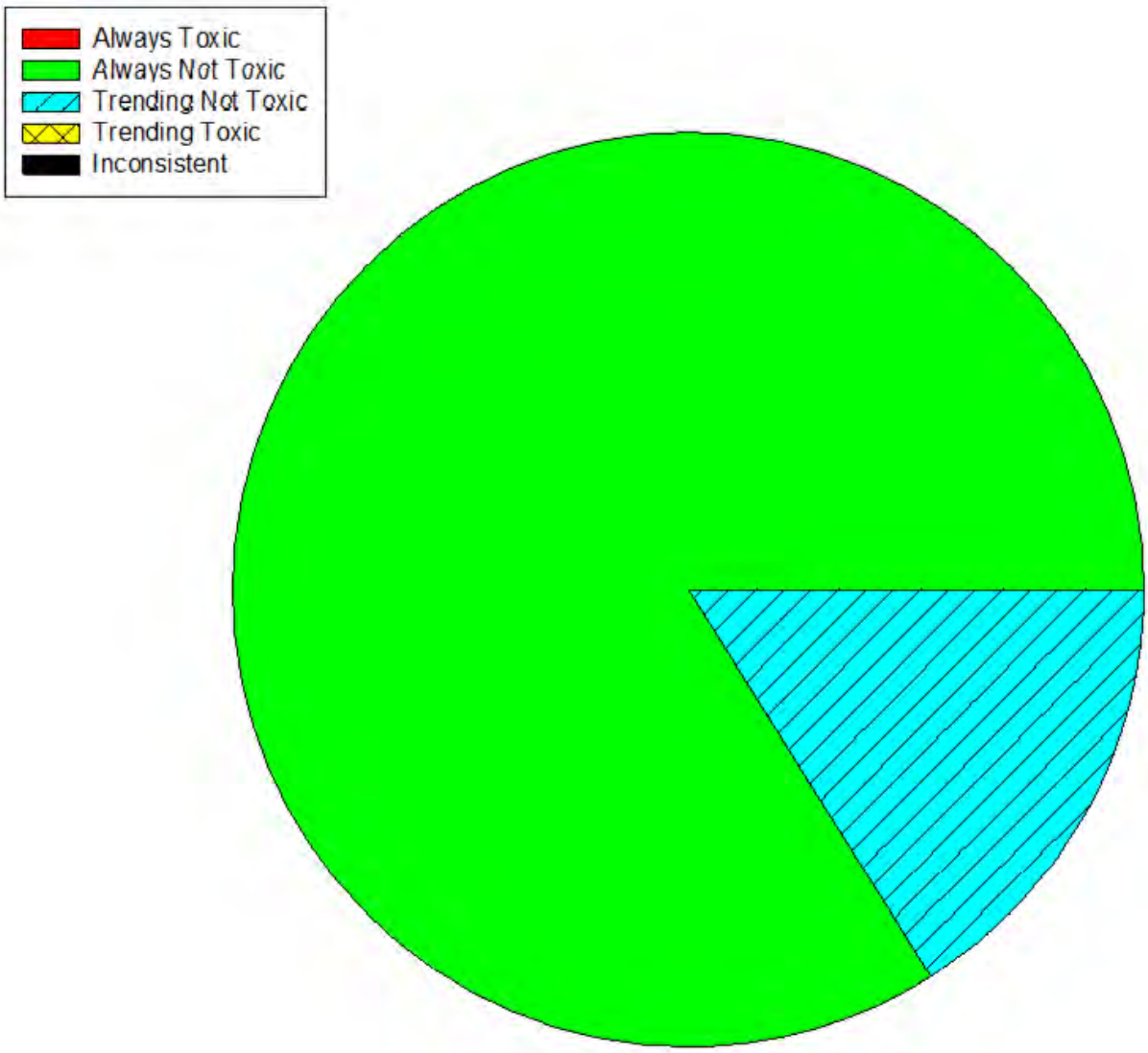

Figure V-11. Temporal trends for the mussel test for individual revisit stations over three sampling periods: Bight ’18, Bight ’13, and Bight ’08.

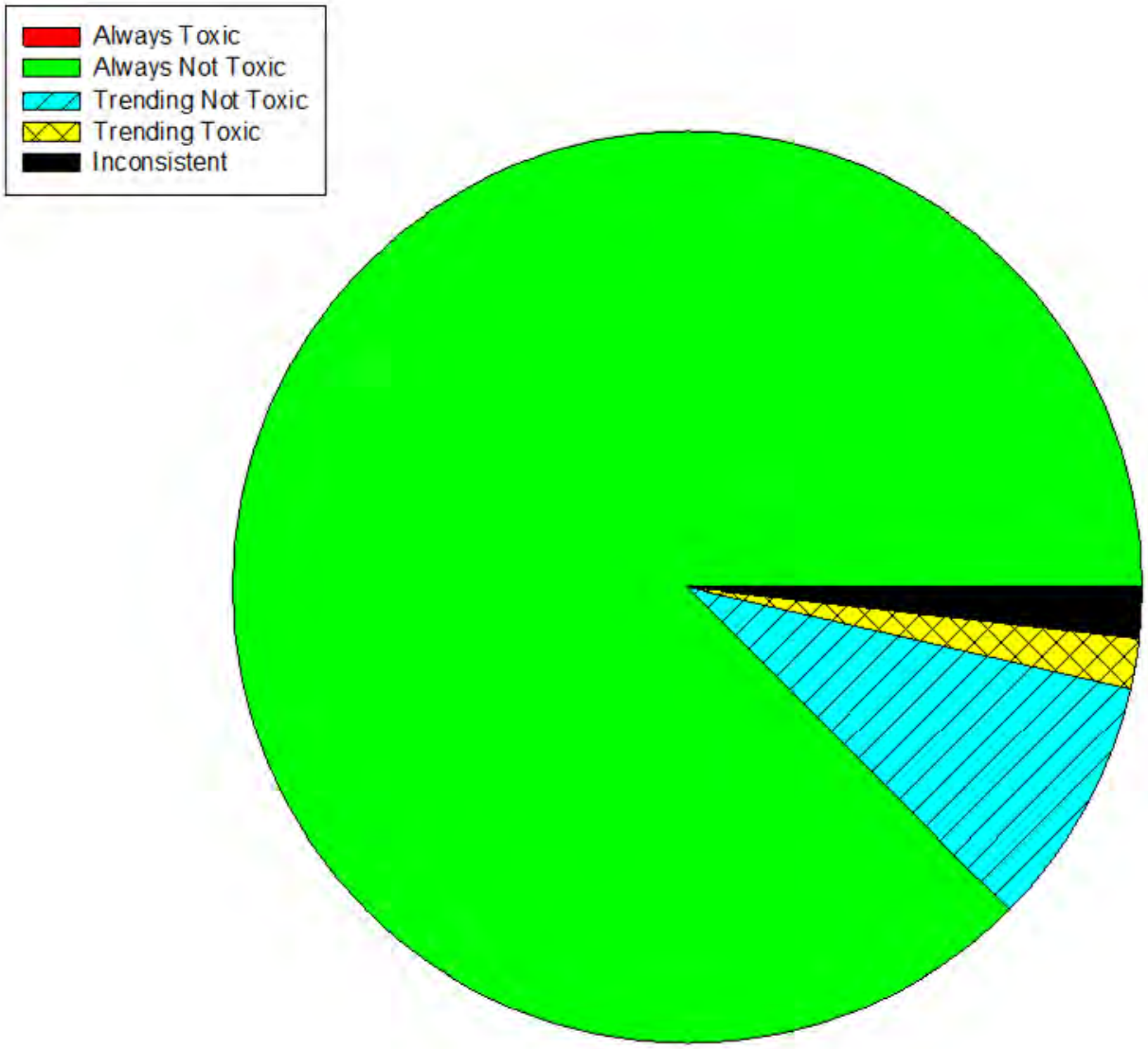

Figure V-12. Temporal trends for the integrated SQO score for individual revisit stations over three sampling periods: Bight ’18, Bight ’13, and Bight ’08.

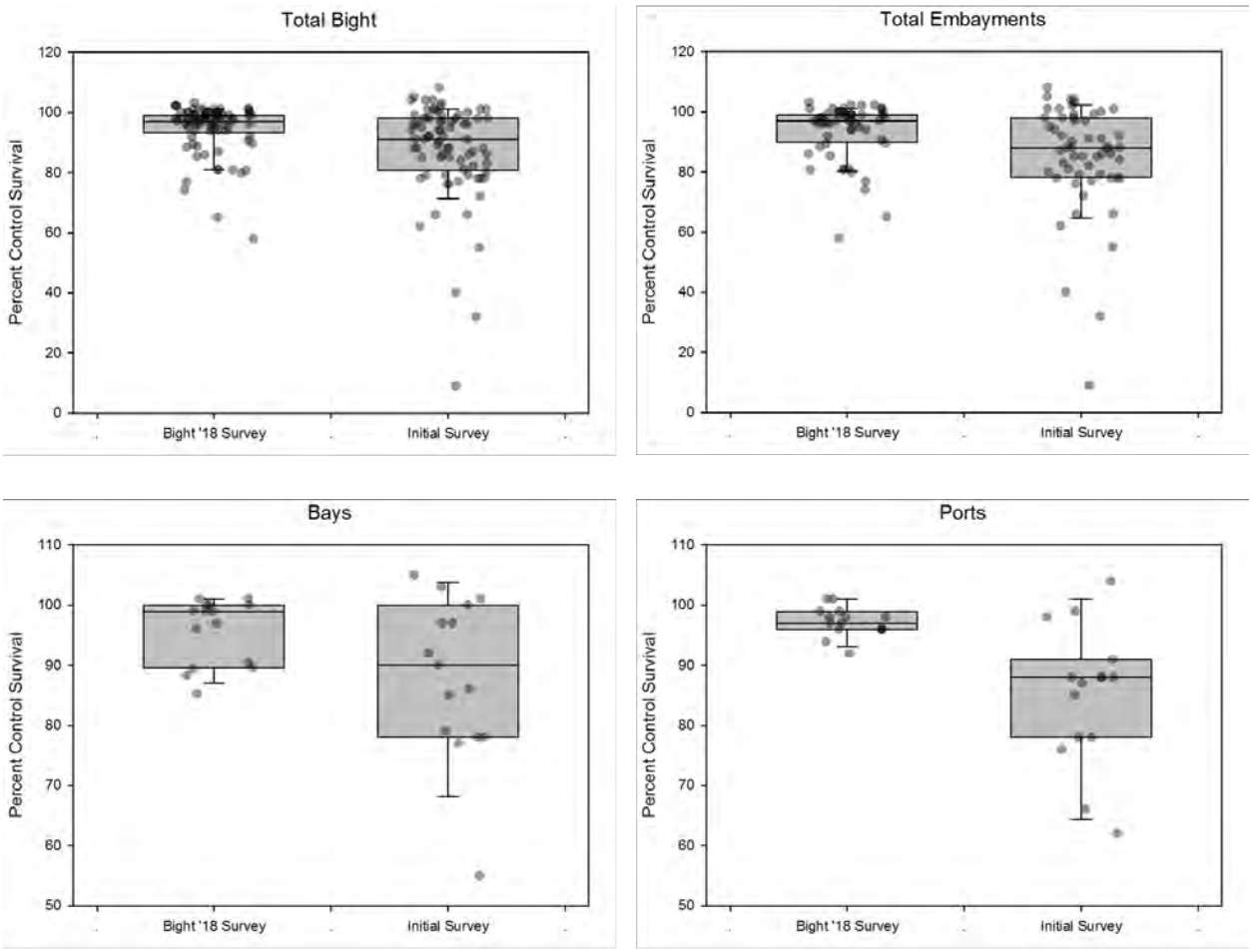

Figure V-13. Wilcoxon Signed Rank tests on the revisit stations indicated significant differences between the amphipod toxicity magnitude in the Bight '18 survey compared to the initial survey. Only Total Bight, Total Embayments, Bays, and Ports were found to be significantly different.

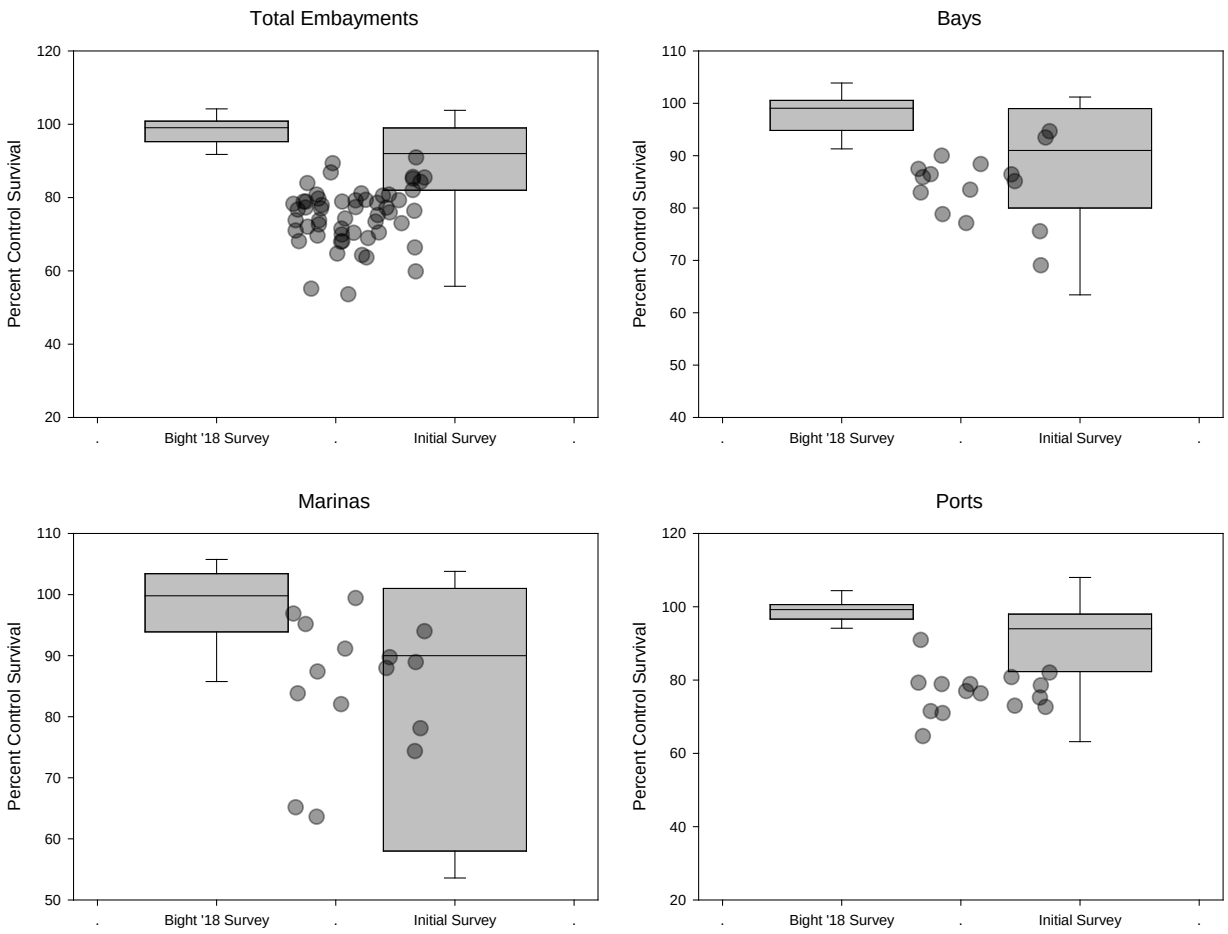

Figure V-14. Wilcoxon Signed Rank tests on the revisit stations indicated significant differences between the mussel embryo toxicity magnitude in the Bight ’18 survey compared to the initial survey. Only Total Embayments, Bays, Marinas, and Ports were found to be significantly different.

VI. DISCUSSION

Overall, the Bight ’18 sediment toxicity survey indicated continued improvement in sediment quality throughout the SCB over the past three surveys. Toxicity remains the greatest in both extent and magnitude in the embayments, specifically the Marinas and Estuaries. The Offshore, Ports, and Bays showed the largest decline in toxicity. In the offshore stratum, only two stations exhibited Low Toxicity, one from the Channel Islands and one from the Outer Shelf. All other offshore stations were Nontoxic.

Embayment strata had the greatest range in amphipod toxicity response during the Bight ’18 survey. As in Bight ’13, the Estuary stratum continued to have some of the highest extent and magnitude of toxicity. For the amphipod, there was a slight increase in the proportion of samples and area considered to be toxic, as well as a higher proportion of samples considered to have Moderate and High Toxicity in the Estuary stratum relative to that observed in Bight ’08 and ’13.

However, results for the mussel test and integrated SQO scores showed a slight decrease in extent and magnitude of toxicity over time (Figures V-8 and V-9). This pattern of lower toxicity in the mussel test relative to the amphipod test is consistent with Bight ’13 and may be indicative of the types of contaminants present as organisms will have variable sensitivity to different compounds. In general, based on the literature, amphipods are more sensitive to organic contaminants and the mussel embryos are more sensitive to metals.

Similar to the Estuary stratum, the Marina stratum also showed an overall increase in the extent and magnitude of toxicity, but in this case, all three measures (amphipod, mussel embryo, and integrated results) followed the same trend on the stratum level (Figures V-7). This agreement between test organisms could be due to a different sediment contaminant exposure in the Marina sediments compared to the Estuary sediments. The chemical analysis for Bight ’18 is ongoing, however, the Bight ’13 Sediment chemistry report (Dodder et al. 2016) found higher concentrations of metals in Marina sediments relative to Estuary sediments which could contribute to higher mussel embryo toxicity. Chemistry results for Bight ’18 will be related to the data presented in this report as part of the integrated sediment quality report.

Brackish Estuaries were sampled and tested as a new stratum during Bight ’18. The sampling frame for this stratum did not provide high sample completeness which resulted in a reduced number of stations for this stratum. Nine of the 21 stations successfully sampled for Brackish Estuaries were reclassified as Estuaries due to the higher salinity at those stations, reducing the total number of stations representing the Brackish Estuaries stratum to 12. Due to the low sample size and the additional two sediment samples excluded due to holding time exceedance (mussel test only), there is a greater level of uncertainty for these results. No additional testing was performed in this stratum to determine the potential cause of toxicity. Based on the frequency and magnitude of toxicity observed in this stratum during this first round of sampling, continued testing with improved sampling frame and additional toxicity characterization is warranted.

Similar to the Bight Regional Marine Monitoring Program, the San Francisco Estuary Institute (SFEI) runs the San Francisco Bay Regional Monitoring Program (RMP), which uses the same sediment toxicity tests for their sediment quality analysis. The last RMP report that summarized yearly monitoring data for sediment toxicity found 70% to 80% of the monitoring stations in San Francisco Bay between 2008 and 2012 to be toxic (SFEI 2013). These results illustrate a much

more impacted area than the Bight ’18 embayments where only 6% of stations were considered toxic based on the integrated toxicity test results.

The previous comparison to the San Francisco Bay RMP made in the Bight ’13 sediment toxicity report (Bay et al. 2015) showed the Bight sediments to be less toxic than San Francisco Bay sediments with only 3% of stations considered toxic. An increase in toxic stations from 3% to 6% between the Bight ’13 and ’18 surveys can be accounted for by the addition of the Brackish Estuary stratum which accounted for half (3%) of the toxic embayment stations in 2018.

Bight ’18 was the third survey to apply the toxicity test methods to embayments as detailed in the California Sediment Quality Objectives Program (SWRCB 2009). As observed in Bight ’13, the use of two toxicity tests resulted in an integrated SQO score that was intermediate between the two individual organisms. This approach provides a more representative evaluation of the sediment quality based on the differing sensitivities of the two test species.

SQO toxicity thresholds were applied to the offshore strata for the single toxicity test using the amphipod. This application is not an intended use for the SQO program; however, this approach provides a standard point of comparison for the amphipod test results over space and time. Although these thresholds are not calibrated to offshore strata, the general evaluation of condition is one of Nontoxic or Low Toxicity response, both of which are considered Not Toxic.

Temporal analysis of the toxicity category for specific revisit stations indicated a consistently not toxic result or a trend from toxic to not toxic. A smaller fraction of locations (0% to 5%) were found to be increasing in toxicity over time, while 15.2% of the amphipod test results and 1.8% of the integrated results identified inconsistent toxic responses over time. In order to further evaluate these observations, additional statistical analyses were performed. The Pearson’s Chi-squared test found no significant relationship between toxicity (toxic versus not toxic) and time (initial versus final survey) for amphipods. This analysis focused on categorical changes in toxicity that may not capture smaller changes which may be more apparent using the toxicity magnitude results. For a more sensitive evaluation of changes over time, a Wilcoxon Signed Rank test was used to determine significant differences in toxicity magnitude over time (initial versus final survey) for both amphipods and mussels. These analyses found significant trends toward lower toxicity for several strata, including Total Embayments for both toxicity test species. Although these trends were statistically significant, the decreases in toxicity magnitude frequently fell within the not toxic category, rather than changing from a toxic to not toxic category. These results align well with the categorical temporal data analysis.

The toxicity test results described here provide only one piece of information on the overall sediment quality. As described in California’s SQO policy, the chemical exposure and benthic community health are also required to provide a more complete assessment of sediment quality. (SWRCB 2009). Sediment toxicity test results provide information about the biological response to natural and anthropogenic characteristics of the sediment; however, the toxic response needs to be paired up with contaminant concentration data to determine if anthropogenic contaminants are a potential stressor. Additionally, sediment toxicity tests are performed under tightly controlled laboratory conditions with specific test organisms and may not represent the true environmental condition of the sediment which may impact the benthic community health. Benthic community condition assessment is necessary to determine if the laboratory toxicity results are ecologically relevant. Once these three measures are evaluated, this sediment quality

triad approach is used to determine an overall SQO score and assessment of the sediment quality. This integrated assessment will be reported in the main sediment quality summary report for Bight ’18.

VII. CONCLUSIONS

The Bight ’18 regional monitoring survey provided a regional assessment of sediment toxicity in the SCB using two standard marine test species. Based on the results of this survey, the Toxicology Technical Committee concluded that:

- **Most of the sediments in the SCB were not toxic.**
  Based on the amphipod toxicity test, which was the only species tested at every site, 96% of SCB sediments were categorized as Nontoxic. Less than 1% was categorized as Moderate or High Toxicity. The remaining sediments were categorized as Low Toxicity. Similarly, low toxicity was observed for the mussel toxicity test, where it was utilized.
- **Bight-wide temporal analysis showed toxicity stayed the same or declined relative to past surveys.**
  Less than 1% of SCB area was considered toxic in 2018, which is as low or lower than previous surveys dating back to 1998 (< 1% in 2008 to 17.5% in 2003). This trend was largely driven by a reduction in toxicity in the Offshore, Port, and Bay strata over the past 20 years.
- **Although Bight-wide trends were minimal between surveys, toxicity in Marinas and Estuaries in 2018 increased compared to 2013.**
  For amphipods, the extent of toxicity increased approximately 20% in the Marina stratum to levels not seen since Bight ’98 (32.5% area). Estuary toxicity followed a similar upward trend. The extent of toxicity increased approximately 18% in the Estuary stratum to levels not seen since Bight ’03. The upwards trend in mussel test results – and hence the integrated SQO scores – were less dramatic for the increases in Marina (integrated SQO score up 1.5%) and Estuary (integrated SQO score up 3.2%) strata.
- **The new Brackish Estuary stratum had the greatest extent and magnitude of toxicity to amphipods compared to all other strata.**
  Brackish Estuaries (estuaries with salinity less than 27 parts-per-thousand) were a new habitat for the Bight Program. Thirty-three percent (33%) of the stations in the Brackish Estuary were categorized as having High Toxicity to amphipods, more than double the frequency of any other stratum, and only 50% of the stations were categorized as Nontoxic. The increased frequency of toxicity in Brackish Estuaries compared to all other strata was also observed for mussel test. The observed toxicity at these stations was not due to salinity because salinity controls survived well. Although high toxicity was observed, however, Brackish Estuaries had a limited sample size because many of the Brackish sites were re-assigned to the Estuaries stratum due to higher than expected salinity.

VIII. RECOMMENDATIONS

A. Bight 2018

Based on the efforts from Bight ’18, the Sediment Quality Planning and Toxicity Technical Committee agree on the following recommendations to follow up on current survey results or to improve the next regional survey implementation.

- **Further investigate increased toxicity in Estuaries and Marinas between 2013 and 2018.**
  Unlike sediment toxicity results from the other strata, particularly for the amphipod test, there was an increase in the percent area of toxicity in both the Estuaries and Marinas strata. These strata should continue to be evaluated in future Bight surveys, including the repeated sampling stations in these strata.
- **Confirm the extent and magnitude of toxicity in Brackish Estuaries.**
  Bight ’18 provided the first evaluation of the Brackish Estuary stratum. The initial results from this survey found a high frequency of toxicity, but the sample size was low relative to what was measured in other strata. Salinity in brackish estuaries is often dynamic, fluctuating with freshwater inputs, which can alter the sampling frame dramatically. There was a drought during the winter of 2017-18, which led to the reduction of freshwater inputs and a reduction in the brackish estuary spatial extent. An improved sampling frame and test methods are recommended to better evaluate this stratum in future surveys. Additionally, sediment toxicity identification evaluations (TIEs) are recommended for stations classified as having Moderate or High Toxicity to provide additional causal assessment.
- **Evaluate the comparability of alternative test species and include in the pre-Bight intercalibration exercise.**
  At the start of the Bight ’18 sampling season, there was concern over the amphipod supply following a population crash observed by the supplier. Additionally, obtaining mussels in good spawning condition is becoming more difficult with warmer ocean conditions during the summer months. Possible alternate test species include *Neanthes* (28-day growth test, SQO species), oysters (SWI embryo development, non-SQO species), or an organism better suited to test sediments from Brackish Estuaries.
- **Investigate the impact of sediment homogenization on the comparability between chemistry and toxicity results.**
  Bight ’18 was the first survey to homogenize all of the sediment grabs at one station prior to distribution to the respective chemistry and toxicity sample containers. The purpose of this change was to improve the potential relationships between the chemistry and toxicity test results. These relationships between chemistry and toxicity should be critically evaluated once the chemistry data becomes available. In addition, the relationships between chemistry and toxicity in Bight ’18 should be compared to the relationships from previous Bight surveys to see if the relationships improved, perhaps as a result of the field method enhancement.

B. Bight 2013

To ensure the Bight Program continues to progress and improve over time, this section addresses the Program's ability to follow through on previous recommendations. Here, we list the recommendations from Bight '13 and hold ourselves accountable for improving the Bight Program's effectiveness and efficiency.

- **Increase emphasis in particular habitats.**
  This recommendation resulted from the greater extent and magnitude of toxicity observed in embayment strata, and the potential for even greater toxicity in unexplored habitats of shallow waters near urban runoff discharges. In Bight '18, we incorporated a new stratum – Brackish Estuaries – which are located between freshwater runoff discharges and our currently monitored Estuary stratum. Being closer to runoff discharges, the Brackish Estuaries stratum (salinity <27 ppt) may have increased exposure compared to the Estuary stratum (>27 ppt).
- **Strengthen basis for toxicity data interpretation.**
  This recommendation resulted from the need for improved toxicity thresholds for offshore strata. Currently, toxicity thresholds based on the Sediment Quality Objectives toxicity line of evidence only apply to embayment strata. No efforts were made to improve or refine the toxicity thresholds used for offshore toxicity data interpretation prior to or during Bight '18. The lack of effort was a result of two factors. First, this would require a large level of resources for an effort that is not currently a priority for state or federal regulatory agencies. Second, there was virtually no toxicity observed in the offshore strata, which minimizes the urgency for action.
- **Investigate use of onboard homogenization of chemistry and toxicity samples.**
  This recommendation resulted from the need to compare chemistry and toxicity results. In previous surveys, the first sediment grab sample at a station was utilized for chemistry. Then, the second grab was used for toxicity. Perhaps as a result, chemistry concentrations rarely correlated with toxicity magnitude. Other regional monitoring programs (i.e., San Francisco Bay Regional Monitoring Program, National Coastal Condition Surveys) typically composite the first and second grabs to enhance this potential correlation. For the first time in the Bight Program, all embayment sediment samples were homogenized in the field prior to subsampling for chemistry and toxicity during Bight '18. One recommendation in this Bight '18 report is to analyze the correlation between chemistry and toxicity from embayment strata to see if this relationship improves compared to previous, uncomposited survey results from previous Bight surveys.
- **Consider use of the *Neanthes* growth test in future surveys.**
  This recommendation resulted from the Bight '13 special study on alternative test species which demonstrated the *Neanthes* 28-day growth test was both feasible for routine use and resulted in different toxicity test outcomes compared to the currently utilized mussel and amphipod test species. The addition of *Neanthes* in future surveys could provide a more complete assessment of the extent, magnitude, or cause of sediment toxicity in the region. However, the Planning Committee opted not to implement the *Neanthes* growth test during Bight '18 based on three reasons: a) multiple laboratories were not sufficiently experienced to run this test, b) larger sediment volumes would require substantially more

sampling effort, and: c) a special study to test a different alternative species (Oyster) was implemented.

- **Improve data entry and upload quality.**
  This recommendation resulted from a multitude of compounding data submission errors in past surveys, which made the process of correcting data and ensuring quality assurance difficult and inefficient. The Bight ’18 data portal included many more automated data checkers, data processing tools, personnel training, and data upload/checkers documentation for upload, which significantly reduced the number of errors in the final dataset. As a result, the amount of time needed for post-submission data processing was reduced by over 50%.

- **Revise split sample testing plan.**
  This recommendation resulted from higher variability in split sample results compared to differences observed in the pre-Bight intercalibration exercise. Any potential interlaboratory variability could not be separated from sampling artifacts as the field samples were not homogenized in the same way as the intercalibration samples. For the Bight ’18 survey, the split samples were homogenized onboard the sampling vessel prior to subsampling for the individual laboratories.

- **Improve training and analysis methods for water quality parameters.**
  This recommendation resulted from several cases of suspect water quality data in Bight ’13, which were not discovered until the data review months after the laboratory testing. These errors should have been caught at the time of sampling when QA deviations could be resolved with sample reanalysis. To address this recommendation, water quality analysis and proper QA/QC procedures were reviewed during the pre-Bight ‘18 intercalibration. Additionally, each laboratory performed quality assurance checks on pH measurements by including a seawater-based pH standard. This allowed each laboratory to determine the accuracy and bias of their pH probes. The new data upload portal included water quality parameter checks during data upload for the pre-Bight intercalibration exercise as well as the main survey. The portal data checker highlighted potential data entry errors or indicated which data should be reviewed and appended with any relevant QA codes or notes.

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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
B18-10000	33.75918	-118.16263	Bays	5	Los Angeles/Long Beach	100	101	Nontoxic
B18-10001	33.7531	-118.15018	Bays	9.1	Los Angeles/Long Beach	102	96	Nontoxic
B18-10002	33.744	-118.16873	Bays	10	Los Angeles/Long Beach	99	102	Nontoxic
B18-10003	33.743833	-118.1401	Bays	7.6	Los Angeles/Long Beach	97	100	Nontoxic
B18-10004	33.74288	-118.1533	Bays	10	Los Angeles/Long Beach	97	94	Nontoxic
B18-10005	33.740683	-118.17495	Bays	13.8	Los Angeles/Long Beach	82	99	Low
B18-10006	33.73963	-118.17153	Bays	12	Los Angeles/Long Beach	101	100	Nontoxic
B18-10007	33.73368	-118.21156	Bays	20	Los Angeles/Long Beach	96	91	Nontoxic
B18-10011	33.72443	-118.2243	Bays	18	Los Angeles/Long Beach	89	99	Low
B18-10012	33.71365	-118.24158	Bays	24	Los Angeles/Long Beach	90	93	Nontoxic
B18-10013	33.712433	-118.2582	Bays	25	Los Angeles/Long Beach	88	89	Low
B18-10014	33.71003	-118.27885	Bays	5	Los Angeles/Long Beach	99	98	Nontoxic
B18-10015	32.787306	-117.20999	Bays	0	Mission Bay	98	103	Nontoxic
B18-10016	32.784541	-117.24059	Bays	0.8	Mission Bay	100	98	Nontoxic
B18-10017	32.784391	-117.21531	Bays	4	Mission Bay	85	98	Low
B18-10019	32.76814	-117.24172	Bays	7	Mission Bay	101	97	Nontoxic
B18-10020	32.75827	-117.24439	Bays	2	Mission Bay	103	93	Nontoxic
B18-10022	32.72408	-117.18307	Bays	5	San Diego Bay	99	104	Nontoxic
B18-10023	32.71750	-117.21556	Bays	0.1	San Diego Bay	99	111	Nontoxic
B18-10024	32.7148	-117.18302	Bays	12	San Diego Bay	99	99	Nontoxic
B18-10026	32.7095	-117.1869	Bays	NA	San Diego Bay- NBC	99	97	Nontoxic
B18-10027	32.7074	-117.185	Bays	NA	San Diego Bay- NBC	97	101	Nontoxic
B18-10028	32.707	-177.1899	Bays	NA	San Diego Bay- NBC	94	98	Nontoxic
B18-10029	32.70189	-117.15893	Bays	1	San Diego Bay	98	90	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B18-10030	32.68784	-117.23027	Bays	1.3	San Diego Bay	100	107	Nontoxic
B18-10031	32.68665	-117.13354	Bays	1	San Diego Bay	99	104	Nontoxic
B18-10032	32.67526	-117.14397	Bays	5	San Diego Bay	96	104	Nontoxic
B18-10034	32.66526	-117.14985	Bays	4	San Diego Bay	100	95	Nontoxic
B18-10035	32.66075	-117.14543	Bays	3.2	San Diego Bay	99	97	Nontoxic
B18-10036	32.65816	-117.14437	Bays	5	San Diego Bay	90	99	Nontoxic
B18-10037	32.64698	-117.11822	Bays	10	San Diego Bay	100	99	Nontoxic
B18-10038	32.64268	-117.12624	Bays	3	San Diego Bay	103	78	Nontoxic
B18-10039	32.64158	-117.13904	Bays	2	San Diego Bay	96	98	Nontoxic
B18-10040	32.64175	-117.11708	Bays	1	San Diego Bay	99	91	Nontoxic
B18-10041	32.62848	-117.1254	Bays	2	San Diego Bay	101	100	Nontoxic
B18-10042	32.62559	-117.11127	Bays	1	San Diego Bay	96	94	Nontoxic
B18-10043	32.61635	-117.1032	Bays	1	San Diego Bay	96	95	Nontoxic
B18-10044	32.61409	-117.09877	Bays	1	San Diego Bay	89	98	Nontoxic
B18-10045	34.25844	-119.2669	Marinas	0.1	Ventura Harbor	77	83	Moderate
B18-10046	34.1712	-119.2235	Marinas	3	Channel Islands Harbor	86	107	Low
B18-10047	33.98298	-118.45072	Marinas	2	Marina del Rey	65	85	Low
B18-10048	33.98025	-118.4509	Marinas	0.1	Marina del Rey	84	89	Low
B18-10049	33.9752	-118.45609	Marinas	0.1	Marina del Rey	81	85	Low
B18-10050	33.97037	-118.44776	Marinas	5	Marina del Rey	94	96	Nontoxic
B18-10051	33.96463	-118.45383	Marinas	5	Marina del Rey	97	105	Nontoxic
B18-10052	33.77737	-118.24176	Marinas	0	Los Angeles/Long Beach	97	99	Nontoxic
B18-10053	33.767233	-118.24965	Marinas	4	Los Angeles/Long Beach	81	100	Low
B18-10054	33.76028	-118.18738	Marinas	0	Long Beach	97	97	Nontoxic
B18-10055	33.75551	-118.12986	Marinas	20	Alamitos Bay	81	92	Low
B18-10056	33.7555	-118.11381	Marinas	0	Alamitos Bay	102	88	Low
B18-10057	33.71925	-118.28132	Marinas	0	Los Angeles/Long Beach	72	99	Low

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B18-10058	33.71305	-118.05379	Marinas	0	Huntington Harbor	69	93	Low
B18-10059	33.61909	-117.92715	Marinas	6	Newport Bay	74	100	Low
B18-10060	33.61505	-117.92509	Marinas	0.1	Newport Bay	93	100	Nontoxic
B18-10061	33.61248	-117.90537	Marinas	0.1	Newport Bay	76	99	Low
B18-10062	33.60879	-117.90477	Marinas	6	Newport Bay	100	102	Nontoxic
B18-10063	33.60649	-117.91125	Marinas	1.5	Newport Bay	48	99	Moderate
B18-10064	33.59653	-117.88027	Marinas	0.1	Newport Bay	99	100	Nontoxic
B18-10065	33.46066	-117.70090	Marinas	1	Dana Point Harbor	92	91	Low
B18-10066	33.460092	-117.69398	Marinas	1	Dana Point Harbor	99	95	Nontoxic
B18-10067	33.45884	-117.69925	Marinas	1	Dana Point Harbor	96	94	Nontoxic
B18-10068	33.45762	-117.69140	Marinas	2	Dana Point Harbor	98	93	Nontoxic
B18-10069	33.21276	-117.39514	Marinas	0	Oceanside Harbor	102	88	Nontoxic
B18-10070	33.20929	-117.39532	Marinas	1	Oceanside Harbor	100	96	Nontoxic
B18-10071	33.20798	-117.39754	Marinas	1	Oceanside Harbor	98	89	Nontoxic
B18-10072	33.20428	-117.39137	Marinas	0	Oceanside Harbor	72	95	Low
B18-10073	32.78060	-117.24926	Marinas	3	Mission Bay	103	86	Low
B18-10074	32.77707	-117.24997	Marinas	1	Mission Bay	103	92	Nontoxic
B18-10075	32.76728	-117.23576	Marinas	4	Mission Bay	101	94	Nontoxic
B18-10076	32.72654	-117.17654	Marinas	1	San Diego Bay	101	106	Nontoxic
B18-10077	32.72496	-117.18335	Marinas	6	San Diego Bay	99	104	Nontoxic
B18-10078	32.72304	-117.22373	Marinas	1	San Diego Bay	99	104	Nontoxic
B18-10079	32.72046	-117.22078	Marinas	1	San Diego Bay	100	110	Nontoxic
B18-10080	32.71882	-117.226	Marinas	6	San Diego Bay	95	103	Nontoxic
B18-10081	32.71823	-117.23040	Marinas	4	San Diego Bay	99	99	Nontoxic
B18-10082	32.71643	-117.22662	Marinas	1	San Diego Bay	98	77	Low
B18-10083	32.71256	-117.23131	Marinas	0.1	San Diego Bay	98	93	Nontoxic
B18-10084	32.71208	-117.23282	Marinas	7	San Diego Bay	98	101	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B18-10085	32.62588	-117.13571	Marinas	0	San Diego Bay	101	82	Nontoxic
B18-10086	32.62355	-117.13362	Marinas	3	San Diego Bay	94	97	Nontoxic
B18-10087	32.62166	-117.10217	Marinas	1	San Diego Bay	91	98	Nontoxic
B18-10088	32.62153	-117.13015	Marinas	0	San Diego Bay	84	99	Low
B18-10089	33.769567	-118.22405	Ports	11	Los Angeles/Long Beach	100	99	Nontoxic
B18-10090	33.76622	-118.27768	Ports	15	Los Angeles/Long Beach	97	99	Nontoxic
B18-10091	33.76245	-118.22043	Ports	0	Los Angeles/Long Beach	99	101	Nontoxic
B18-10092	33.759683	-118.26058	Ports	0	Los Angeles/Long Beach	98	100	Nontoxic
B18-10093	33.753367	-118.18808	Ports	9	Los Angeles/Long Beach	97	99	Nontoxic
B18-10094	33.7527	-118.21808	Ports	18	Los Angeles/Long Beach	96	100	Nontoxic
B18-10095	33.751033	-118.2308	Ports	17	Los Angeles/Long Beach	96	99	Nontoxic
B18-10096	33.74522	-118.21597	Ports	20	Los Angeles/Long Beach	101	101	Nontoxic
B18-10097	33.74495	-118.2072	Ports	0.1	Los Angeles/Long Beach	98	100	Nontoxic
B18-10098	33.742283	-118.27417	Ports	0.1	Los Angeles/Long Beach	87	99	Low
B18-10099	33.74000	-118.27618	Ports	0.1	Los Angeles/Long Beach	98	99	Nontoxic
B18-10100	33.73960	-118.20438	Ports	18.2	Los Angeles/Long Beach	99	100	Nontoxic
B18-10101	33.73892	-118.2104	Ports	27	Los Angeles/Long Beach	92	102	Nontoxic
B18-10102	33.738133	-118.22922	Ports	12.5	Los Angeles/Long Beach	97	96	Nontoxic
B18-10103	33.737383	-118.26608	Ports	1	Los Angeles/Long Beach	98	100	Nontoxic
B18-10104	33.72181	-118.05772	Ports	0.1	Huntington Harbor	83	99	Low
B18-10105	33.73165	-118.18108	Ports	15.4	Los Angeles/Long Beach	100	99	Nontoxic
B18-10106	33.7311	-118.192	Ports	15	Los Angeles/Long Beach	98	98	Nontoxic
B18-10107	33.72897	-118.23373	Ports	11	Los Angeles/Long Beach	101	100	Nontoxic
B18-10108	33.72387	-118.26212	Ports	27	Los Angeles/Long Beach	96	100	Nontoxic
B18-10109	33.71975	-118.23177	Ports	13.3	Los Angeles/Long Beach	94	NA	NA
B18-10110	33.719083	-118.24408	Ports	12.6	Los Angeles/Long Beach	95	101	Nontoxic
B18-10111	33.71763	-118.26845	Ports	13.3	Los Angeles/Long Beach	96	98	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B18-10112	32.71627	-117.17632	Ports	13	San Diego Bay	98	108	Nontoxic
B18-10113	32.71614	-117.17398	Ports	12	San Diego Bay	98	97	Nontoxic
B18-10114	32.7026	-117.1618	Ports	9	San Diego Bay	97	96	Nontoxic
B18-10115	32.69442	-117.15254	Ports	6	San Diego Bay	95	111	Nontoxic
B18-10116	32.6914	-117.15337	Ports	13	San Diego Bay	96	102	Nontoxic
B18-10117	32.69189	-117.23837	Ports	15	San Diego Bay	94	96	Nontoxic
B18-10118	32.6904	-117.2342	Ports	NA	San Diego Bay- NBPL	100	103	Nontoxic
B18-10119	32.69004	-117.1432	Ports	0	San Diego Bay	91	106	Nontoxic
B18-10120	32.6895	-117.238	Ports	NA	San Diego Bay- NBPL	98	101	Nontoxic
B18-10121	32.6878	-117.14076	Ports	5	San Diego Bay	97	104	Nontoxic
B18-10122	32.6872	-117.2339	Ports	NA	San Diego Bay- NBPL	100	101	Nontoxic
B18-10123	32.68549	-117.13635	Ports	5	San Diego Bay	93	109	Nontoxic
B18-10124	32.68433	-117.13126	Ports	0.4	San Diego Bay	91	104	Nontoxic
B18-10125	32.6832	-117.1292	Ports	NA	San Diego Bay- NBSD	95	97	Nontoxic
B18-10126	32.68173	-117.13109	Ports	3	San Diego Bay	96	99	Nontoxic
B18-10127	32.6792	-117.12836	Ports	3	San Diego Bay	100	102	Nontoxic
B18-10128	32.6784	-117.1243	Ports	NA	San Diego Bay- NBSD	96	98	Nontoxic
B18-10129	32.6782	-177.1624	Ports	NA	San Diego Bay- NAB	99	100	Nontoxic
B18-10130	32.6759	-117.1271	Ports	NA	San Diego Bay- NBSD	95	100	Nontoxic
B18-10131	32.6748	-117.1541	Ports	NA	San Diego Bay- NAB	99	105	Nontoxic
B18-10132	32.67427	-117.125	Ports	2	San Diego Bay	97	96	Nontoxic
B18-10133	32.67313	-117.12943	Ports	2.5	San Diego Bay	93	99	Nontoxic
B18-10134	32.6731	-117.1206	Ports	NA	San Diego Bay- NBSD	96	102	Nontoxic
B18-10135	32.6721	-117.1181	Ports	NA	San Diego Bay- NBSD	95	95	Nontoxic
B18-10136	32.67028	-117.1235	Ports	3	San Diego Bay	95	99	Nontoxic
B18-10137	32.66776	-117.12199	Ports	2	San Diego Bay	94	97	Nontoxic
B18-10138	32.666	-117.12	Ports	NA	San Diego Bay- NBSD	95	97	Nontoxic

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B18-10139	32.66359	-117.1227	Ports	4	San Diego Bay	98	95	Nontoxic
B18-10140	32.66056	-117.12296	Ports	10	San Diego Bay	99	97	Nontoxic
B18-10141	32.66045	-117.12539	Ports	4.6	San Diego Bay	100	96	Nontoxic
B18-10142	32.66009	-117.11918	Ports	3	San Diego Bay	96	92	Nontoxic
B18-10143	32.65763	-117.12312	Ports	3	San Diego Bay	98	103	Nontoxic
B18-10144	32.65118	-117.12296	Ports	12	San Diego Bay	99	92	Low
B18-10146	33.96393	-118.45195	Estuaries	1	Ballona Creek	1	NA	NA
B18-10148	33.77783	-118.20531	Estuaries	0	Los Angeles River	10	NA	NA
B18-10149	33.76635	-118.10438	Estuaries	8	Los Alamitos Estuary	81	101	Low
B18-10150	33.76144	-118.2001	Estuaries	3	Los Angeles/Long Beach	87	98	Low
B18-10151	33.75301	-118.10528	Estuaries	4	San Gabriel River	99	NA	NA
B18-10152	33.7278	-118.07249	Estuaries	0	Huntington Harbor	91	97	Nontoxic
B18-10155	33.70334	-118.05317	Estuaries	0	Bolsa Chica Wetlands	88	95	Low
B18-10156	33.69625	-118.04604	Estuaries	0	Bolsa Chica Wetlands	96	98	Nontoxic
B18-10158	33.64677	-117.88462	Estuaries	19	Upper Newport Bay	80	97	Low
B18-10159	33.64582	-117.88868	Estuaries	18	Upper Newport Bay	77	95	Low
B18-10161	33.63159	-117.88644	Estuaries	0.4	Newport Bay	97	92	Nontoxic
B18-10162	33.63735	-117.96364	Estuaries	1	Talbert Marsh	81	96	Low
B18-10163	33.62416	-117.893	Estuaries	0.1	Newport Bay	78	96	Low
B18-10164	33.62135	-117.89479	Estuaries	1	Newport Bay	91	96	Nontoxic
B18-10165	33.61797	-117.9039	Estuaries	1	Newport Bay	88	93	Low
B18-10166	33.23306	-117.41336	Estuaries	2	Santa Margarita estuary	105	94	Nontoxic
B18-10167	33.2314	-117.41233	Estuaries	1	Santa Margarita Estuary	102	91	Nontoxic
B18-10168	33.14021	-117.32424	Estuaries	3	Agua Hedionda Lagoon	58	94	Moderate
B18-10169	33.13982	-117.31879	Estuaries	1	Agua Hedionda Lagoon	81	95	Low
B18-10170	33.13903	-117.33747	Estuaries	6	Agua Hedionda Lagoon	97	95	Nontoxic
B18-10171	33.08992	-117.27848	Estuaries	0	Batiquitos Lagoon	81	88	Moderate

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B18-10172	33.0896	-117.29459	Estuaries	0	Batiquitos Lagoon	96	92	Nontoxic
B18-10173	33.08952	-117.28498	Estuaries	NA	Batiquitos Lagoon	78	89	Moderate
B18-10174	32.97309	-117.24952	Estuaries	3	San Dieguito Lagoon	107	100	Nontoxic
B18-10175	32.93258	-117.25812	Estuaries	NA	Los Penasquitos Lagoon	99	75	Low
B18-10176	32.75778	-117.22729	Estuaries	1	San Diego River	102	104	Nontoxic
B18-10177	32.75703	-117.23524	Estuaries	1	San Diego River	102	101	Nontoxic
B18-10178	32.68753	-117.13087	Estuaries	0	San Diego Bay	85	109	Low
B18-10179	32.64968	-117.10863	Estuaries	0	San Diego Bay	99	92	Nontoxic
B18-10180	32.64777	-117.11644	Estuaries	1	San Diego Bay	97	80	Nontoxic
B18-10181	32.64819	-117.1134	Estuaries	1	San Diego Bay	98	109	Nontoxic
B18-10182	32.55694	-117.12755	Estuaries	1	Tijuana River Estuary	98	94	Nontoxic
B18-10184	34.27683	-119.307	Brackish	2	Ventura River	92	NA	NA
B18-10188	33.97929	-118.425	Brackish	0	Ballona Creek	0	NA	NA
B18-10192	33.38717	-117.593	Brackish	0	San Mateo	86	NA	NA
B18-10193	33.20341	-117.391	Brackish	2	San Luis Rey River	103	106	Nontoxic
B18-10194	32.97625	-117.248	Estuaries	3	San Dieguito	102	100	Nontoxic
B18-10195	32.80451	-117.223	Estuaries	3	Rose	95	85	Low
B18-10196	32.76053	-117.21	Estuaries	0.1	San Diego River	104	103	Nontoxic
B18-10197	32.75887	-117.216	Estuaries	1	San Diego River	103	96	Nontoxic
B18-10198	32.75799	-117.225	Estuaries	0	San Diego River	96	105	Nontoxic
B18-10199	32.65833	-117.083	Brackish	0	Sweetwater River	103	94	Nontoxic
B18-10200	32.61784	-117.098	Brackish	1	San Diego Bay	85	80	Low
B18-10201	32.59892	-117.116	Estuaries	0	Otay	79	95	Low
B18-10203	34.44325	-120.43	Inner Shelf	18	West Santa Barbara Channel	98	NA	NA
B18-10210	34.24356	-119.385	Inner Shelf	25.9	East Santa Barbara Channel	99	NA	NA
B18-10217	34.03331	-118.864	Inner Shelf	3.9	Hueneme to Dume	100	NA	NA
B18-10218	34.02313	-118.594	Inner Shelf	23	Santa Monica Bay	94	NA	NA

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B18-10224	33.69525	-118.296	Inner Shelf	27	Palos Verdes Shelf	95	NA	NA
B18-10226	33.6436	-118.079	Inner Shelf	26	San Pedro Shelf	98	NA	NA
B18-10227	33.62776	-117.988	Inner Shelf	13	San Pedro Shelf	99	NA	NA
B18-10228	33.619	-118.042	Inner Shelf	28.1	San Pedro Shelf	100	NA	NA
B18-10229	33.52083	-117.77	Inner Shelf	16	Orange Shelf	100	NA	NA
B18-10233	33.03999	-117.312	Inner Shelf	24	North San Diego Shelf	99	NA	NA
B18-10237	32.65987	-117.169	Inner Shelf	10.2	South San Diego Shelf	102	NA	NA
B18-10238	32.63929	-117.187	Inner Shelf	19	South San Diego Shelf	97	NA	NA
B18-10239	32.61205	-117.143	Inner Shelf	11	South San Diego Shelf	99	NA	NA
B18-10240	32.53442	-117.169	Inner Shelf	22	South San Diego Shelf	100	NA	NA
B18-10242	34.42388	-120.058	Mid Shelf	71.2	West Santa Barbara Channel	96	NA	NA
B18-10244	34.3591	-119.825	Mid Shelf	80.7	East Santa Barbara Channel	98	NA	NA
B18-10260	33.60199	-118.057	Mid Shelf	38	San Pedro Shelf	96	NA	NA
B18-10262	33.59486	-118.194	Mid Shelf	50.7	San Pedro Shelf	99	NA	NA
B18-10263	33.59231	-117.925	Mid Shelf	28.3	San Pedro Shelf	99	NA	NA
B18-10266	33.26554	-117.533	Mid Shelf	62	North San Diego Shelf	99	NA	NA
B18-10267	33.21753	-117.48	Mid Shelf	57.4	North San Diego Shelf	100	NA	NA
B18-10269	33.08759	-117.351	Mid Shelf	73	North San Diego Shelf	100	NA	NA
B18-10270	32.96762	-117.3	Mid Shelf	48	South San Diego Shelf	99	NA	NA
B18-10271	32.85105	-117.326	Mid Shelf	67	South San Diego Shelf	102	NA	NA
B18-10272	32.75187	-117.323	Mid Shelf	74	South San Diego Shelf	100	NA	NA
B18-10273	32.66447	-117.271	Mid Shelf	43	South San Diego Shelf	102	NA	NA
B18-10274	32.66374	-117.296	Mid Shelf	74.4	South San Diego Shelf	101	NA	NA
B18-10275	32.63241	-117.306	Mid Shelf	103	South San Diego Shelf	99	NA	NA
B18-10276	32.59759	-117.245	Mid Shelf	45	South San Diego Shelf	99	NA	NA
B18-10277	32.58963	-117.264	Mid Shelf	58	South San Diego Shelf	100	NA	NA
B18-10278	32.55155	-117.199	Mid Shelf	35	South San Diego Shelf	101	NA	NA

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B18-10279	34.41865	-120.214	Outer Shelf	163.4	West Santa Barbara Channel	96	NA	NA
B18-10286	34.24398	-119.706	Outer Shelf	173.4	East Santa Barbara Channel	96	NA	NA
B18-10301	34.06645	-119.134	Outer Shelf	186	Hueneme to Dume	89	NA	NA
B18-10308	33.91242	-118.588	Outer Shelf	201	Santa Monica Bay	95	NA	NA
B18-10311	33.76675	-118.46	Outer Shelf	127	Santa Monica Bay	97	NA	NA
B18-10315	33.46405	-117.762	Outer Shelf	155	Orange Shelf	99	NA	NA
B18-10316	33.30321	-117.609	Outer Shelf	129.6	San Diego Slope	100	NA	NA
B18-10317	33.22107	-117.511	Outer Shelf	181	North San Diego Shelf	98	NA	NA
B18-10318	32.70575	-117.347	Outer Shelf	185	South San Diego Shelf	101	NA	NA
B18-10319	32.65916	-117.337	Outer Shelf	155	South San Diego Shelf	103	NA	NA
B18-10320	32.58565	-117.341	Outer Shelf	183	South San Diego Shelf	101	NA	NA
B18-10382	34.11582	-119.937	Channel Is.	100	North Channel Islands	97	NA	NA
B18-10383	34.11348	-120.024	Channel Is.	110	North Channel Islands	100	NA	NA
B18-10384	34.10157	-120.142	Channel Is.	101	North Channel Islands	92	NA	NA
B18-10385	34.07972	-119.511	Channel Is.	124	North Channel Islands	87	NA	NA
B18-10386	34.07885	-119.702	Channel Is.	92	North Channel Islands	98	NA	NA
B18-10387	34.07547	-119.75	Channel Is.	88	North Channel Islands	95	NA	NA
B18-10388	34.06655	-119.589	Channel Is.	88	North Channel Islands	94	NA	NA
B18-10389	34.0587	-119.497	Channel Is.	82	North Channel Islands	95	NA	NA
B18-10390	34.04545	-120.49	Channel Is.	75	North Channel Islands	95	NA	NA
B18-10391	34.035	-119.351	Channel Is.	84	North Channel Islands	99	NA	NA
B18-10392	34.03116	-119.424	Channel Is.	82	North Channel Islands	96	NA	NA
B18-10393	34.01204	-120.475	Channel Is.	95	North Channel Islands	95	NA	NA
B18-10394	33.99487	-120.337	Channel Is.	71	North Channel Islands	95	NA	NA
B18-10395	33.96562	-119.853	Channel Is.	21	North Channel Islands	99	NA	NA
B18-10396	33.91262	-119.948	Channel Is.	72	North Channel Islands	97	NA	NA
B18-10397	33.85589	-118.28	Estuaries	NA	Dominguez Channel	66	82	Moderate

Station	Latitude (north)	Longitude (west)	Stratum	Depth (m)	Region	Amphipod Survival (% control)	Mussel Embryo Percent Normal Alive (% control)	Integrated Toxicity Category
B18-10411	33.73637	-118.21693	Bays	13	Los Angeles/Long Beach	97	101	Nontoxic
B18-10417	33.73108	-118.15737	Bays	15	Los Angeles/Long Beach	99	100	Nontoxic
B18-10438	32.76652	-117.21854	Bays	1	Mission Bay	100	97	Nontoxic
B18-10447	32.7139	-117.1885	Bays	0	San Diego Bay	98	100	Nontoxic
B18-10465	32.6751	-117.1654	Bays	0	San Diego Bay	98	99	Nontoxic
B18-10658	34.23463	-119.25753	Estuaries	0	Santa Clara River	26	NA	NA
B18-10672	33.97211	-118.45938	Estuaries	0	Ballona Lagoon	84	NA	NA
B18-10674	33.79426	-118.22942	Estuaries	0	Dominguez Channel	80	82	Moderate
B18-10677	33.7752	-118.20581	Estuaries	0	Los Angeles River	11	NA	NA
B18-10740	34.42128	-119.66	Brackish	0	Andree Clark Bird Refuge	99	NA	NA
B18-10741	34.42166	-119.662	Brackish	0	Andree Clark Bird Refuge	73	97	Low
B18-10760	33.84	-118.27	Estuaries	0	Dominguez Channel	59	87	Moderate
B18-10772	33.80271	-118.205	Brackish	0	Los Angeles River	92	1	Moderate
B18-10774	33.79818	-118.205	Brackish	0	Los Angeles River	1	97	Moderate
B18-10776	33.79285	-118.205	Brackish	0	Los Angeles River	26	102	Moderate
B18-10778	33.78753	-118.205	Brackish	0	Los Angeles River	0	102	Moderate
B18-10824	32.58854	-117.108	Estuaries	0	Otay	79	101	Low
B18-10875	33.17534	-117.404	Inner Shelf	23	North San Diego Shelf	99	NA	NA
B18-10876	33.00712	-117.298	Inner Shelf	27	North San Diego Shelf	99	NA	NA
B18-10880	32.65306	-117.215	Inner Shelf	10	San Diego Shelf	104	NA	NA
B18-10968	33.92353	-118.568	Outer Shelf	182	Santa Monica Bay	84	NA	NA

NA= Not analyzed.

APPENDIX B: STATION ID CROSS REFERENCE

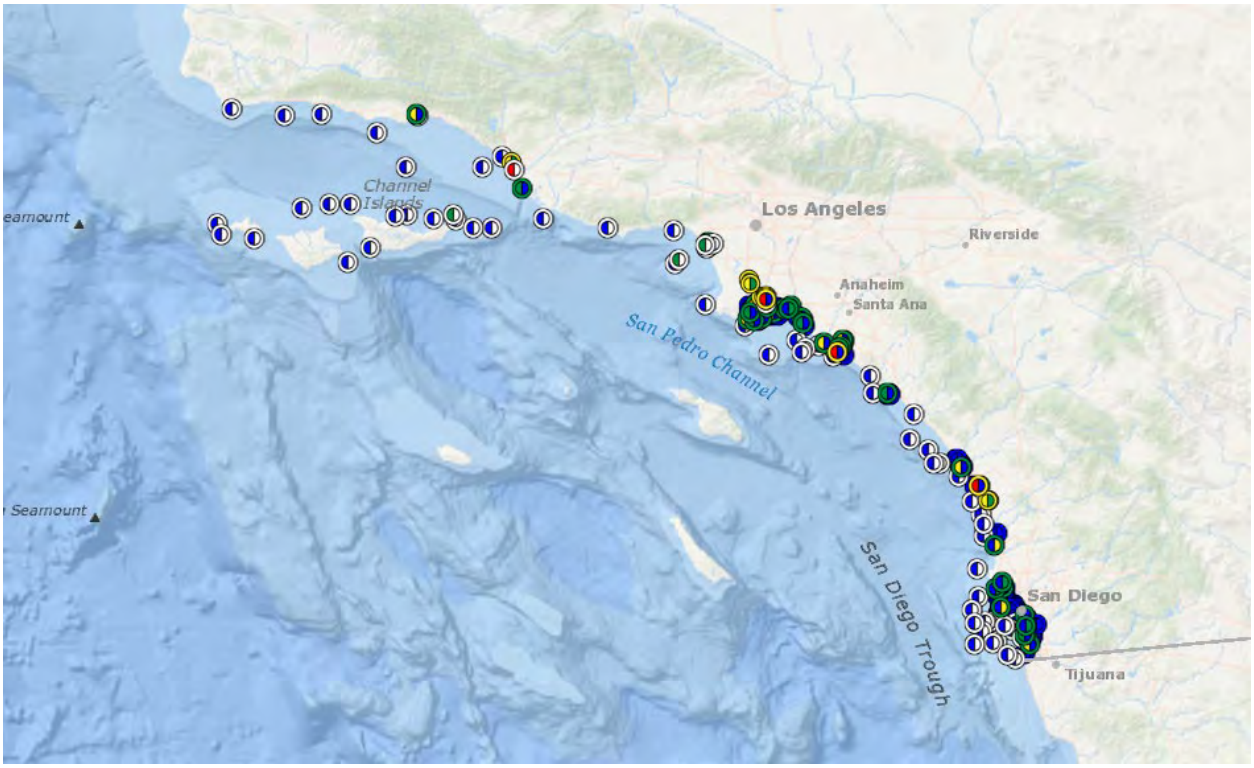
Cross reference of station IDs for revisit stations sampled in Bight ’18 and in at least one previous Bight survey.

Bight ’18	Bight ’13	Bight ’08	Bight ’03	Bight ’98
10000	8388	6478	-	2152
10002	8358	6448	4098	-
10004	8355	6444	-	2157
10006	8350	6437	-	2156
10011	8318	6404	4242	-
10012	8304	6387	-	2162
10013	8302	6386	4178	-
10017	8159	6217	4228	-
10019	8152	6212	4020	-
10022	8122	6172	4092	-
10024	8109	6152	-	2436
10032	8068	6093	4028	-
10034	8060	6080	-	2242
10036	8052	6071	4116	-
10037	8029	6040	4148	-
10046	8425	6549	-	2130
10047	8417	6530	-	2443
10050	8409	6518	-	2448
10051	8407	6513	4085	-
10053	8397	6489	4010	-
10055	8383	6472	4018	-
10059	8280	6350	-	2136
10062	8273	6343	4065	-
10073	8156	6216	-	2423
10075	8151	6211	-	2425
10077	8123	6173	-	2434
10080	8117	6161	-	2222
10081	8116	6159	4076	-
10084	8102	6145	-	2226
10086	8013	6025	4052	-
10090	8396	6487	4266	-
10094	8374	6466	4210	-
10095	8371	6463	-	2432
10096	8360	6450	4146	-
10101	8347	6435	-	2179
10106	8333	6419	4162	-

Bight ’18	Bight ’13	Bight ’08	Bight ’03	Bight ’98
10107	8326	6413	-	2298
10108	8316	6402	-	2182
10112	8112	6155	-	2263
10114	8100	6140	-	2251
10116	8087	6129	-	2252
10117	8085	6128	-	2441
10140	8056	6075	4084	-
10144	8045	6054	-	2262
10149	8394	6485	4118	-
10151	8378	6468	4194	-
10158	8292	6363	4075	-
10159	8290	6362	4017	-
10167	8248	6303	4209	-
10168	8222	6271	4304	-
10169	8219	6270	4087	-
10170	8218	6269	4049	-
10176	8136	6192	4033	-
10177	8129	6181	4264	-
10182	8002	6001	4695	-
10218	9341	7517	-	2382
10224	9229	7321	4042	-
10226	9214	7300	4058	-
10227	9204	7293	-	2325
10229	9171	7231	-	2304
10260	9199	7269	-	2208
10266	9129	7166	4080	-
10269	9105	7122	4048	-
10277	9012	7009	-	2419
10278	9007	7002	4000	-
10301	9359	7542	4093	-
10311	9251	7395	4038	-
10315	9150	7208	4110	-
10317	9125	7158	4144	-
10320	9011	7008	4068	-
10382	-	-	4027	-
10383	-	-	4347	-
10384	-	-	4155	-
10385	-	-	4163	-
10386	-	-	4115	-
10387	-	-	4051	-

Bight ’18	Bight ’13	Bight ’08	Bight ’03	Bight ’98
10388	-	-	-	2516
10389	-	-	-	2522
10390	-	-	-	2490
10392	-	-	-	2520
10393	-	-	-	2491
10394	-	-	4159	-
10395	-	-	-	2467
10396	-	-	-	2492

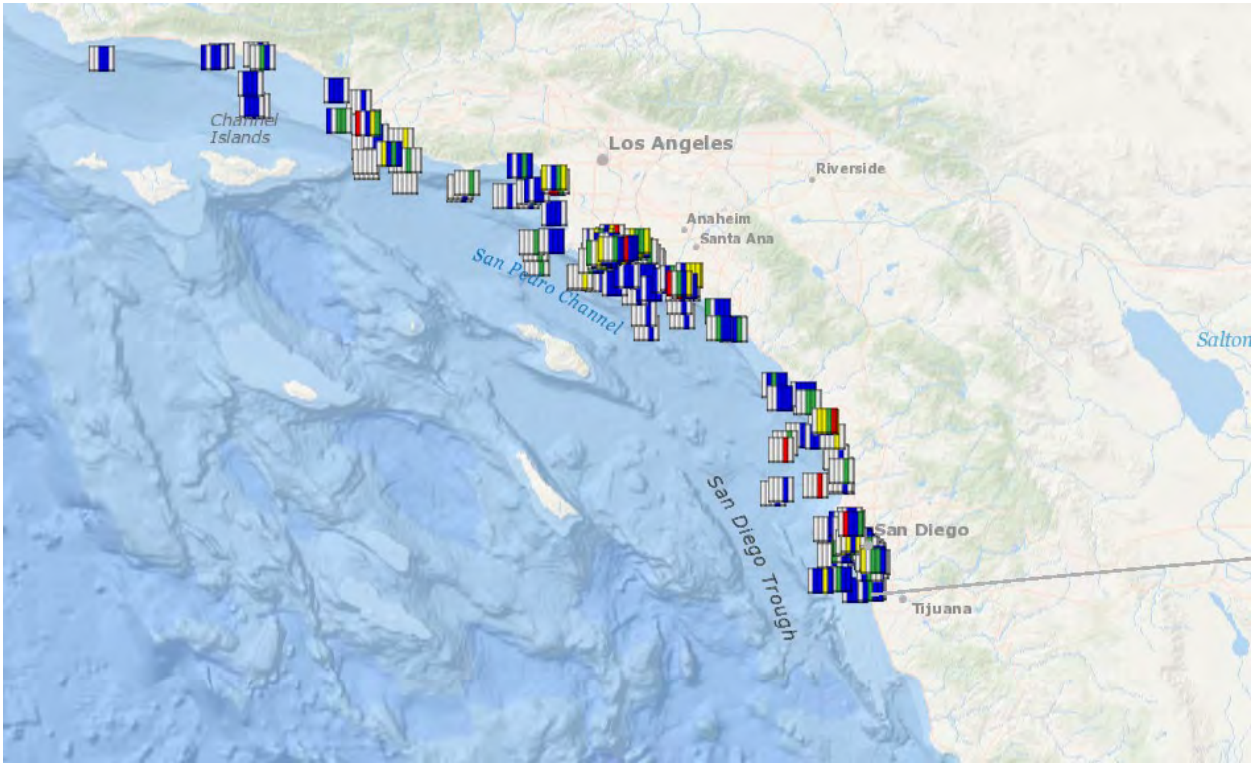
APPENDIX C: INTERACTIVE MAP OF BIGHT '18, BIGHT '13, AND BIGHT '08 TOXICITY RESULTS



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 '08, Bight '13, and Bight '18. 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 only one survey is chosen. 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 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 three surveys. The table also contains the station information from Bight '18 (e.g., latitude and longitude).

Bight'18 Sediment Toxicity Report – Appendix C

APPENDIX D: INTERACTIVE MAP OF AMPHIPOD TOXICITY RESULTS FOR BIGHT '18 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, Bight '13, and Bight '18. 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 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 the surveys represented. The table also contains the station information from Bight '18 (e.g., latitude and longitude).

Bight'18 Sediment Toxicity Report – Appendix D

APPENDIX E: DETAILS OF LABORATORY INTERCALIBRATION AND SPLIT SAMPLE ASSESSMENT METHODS

Comparability of the laboratories for the split samples was based on four factors: the percentage difference from the mean for each sample, a comparison of the toxicity category for each sample, the relative percent difference (RPD) of the duplicate 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 four samples for comparison, the maximum attainable score for this evaluation factor was 12.

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 four samples, the maximum points awarded for this category was 6.

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

For the duplicate sample the following procedure was used:

1. The relative percent difference of the percent mortality of the two duplicate samples was calculated for each laboratory.
2. Assigned points to each laboratory based on their calculated RPD when compared to the thresholds shown in Table 3.

The maximum attainable score for this evaluation factor was 12.

Table 3. Summary of scoring system for duplicate sample results.

Reference Tox. (deviation from grand mean)

Result	Pts.
0 – 10 %	12
>10 – 20 %	9
>20 – 30 %	6
>30 %	0

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 EC50/LC50 data for each species.
3. Pooled intercalibration reference toxicant EC50/LC50 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 4.

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 12.

Table 4. Summary of scoring system for reference toxicant results.

Reference Tox. (deviation from grand mean)	
Result	**Pts.**
Within 1 SD	12
Within 2 SD	9
Within 3 SD	6
> 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 5.

Table 5. Scoring system for sum of all factors

Description	**% of maximum possible score**	**Number of points**
Very High comparability	90	38-42
High comparability	80	34-37.5
Moderate comparability	70	29.5-33.5
Low comparability	< 70	< 29.5

APPENDIX F: EVALUATION OF PACIFIC OYSTERS AS A VIABLE TEST SPECIES OPTION FOR THE SEDIMENT-WATER INTERFACE TEST

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Introduction

The State of California Sediment Quality Objectives (SQO) Policy contains narrative objectives for the protection of aquatic life due to direct effects based on the integration of three primary lines of evidence: 1) sediment chemistry, 2) sediment toxicity, and 3) benthic community. Since 2003, the Bight Program has used two test species in accordance with the SQO Policy to evaluate the toxicity of sediments in bays and estuaries, and some offshore locations; a 10-day acute lethality test using the burrowing amphipod *Eohaustorius estuarius*, and a 48-hr sublethal embryo development using the bivalve mussel *Mytilus galloprovincialis* exposed to the sediment-water interface.

The latest SQO framework (Bay et al. 2014) includes a suite of three amphipod species that may be used to assess acute effects, and only two species that may be used to assess sublethal effects; bivalve embryo development using *Mytilus galloprovincialis*, and a 28-day growth and survival test using the polychaete *Neanthes arenaceodentata*. Tests using the mussel embryos have generally been preferred due to their documented sensitivity to various chemicals of concern, and the short duration of the test enabling a more rapid assessment of results. For these reasons the mussel has been the choice for the sublethal toxicity test in support of the Bight Program for each survey starting in 2003.

A significant drawback to using mussel embryos is that this species is generally in prime gravid state only during the winter and spring months along the west coast. Gravid animals can generally be obtained throughout the remainder of the year, but the frequency of obtaining gravid adults and the quality of the gametes is significantly reduced in the summer months when the Bight Program occurs. Consequently, the laboratories participating in the Bight Program have sometimes struggled to obtain gravid individuals, resulting in purchasing of mussels from multiple suppliers in hopes that one batch might work, and frequent retests or re-spawning of animals from different sources on subsequent days. Each batch of mussels typically costs hundreds of dollars, but quality gametes can be acquired from a single batch of mussels when they are in the prime gravid state. During the summer months, labs participating in the study have purchased up to six batches of mussels to initiate each round of testing, greatly increasing test costs. Additionally, quality gametes can be obtained from prime gravid mussels in less than an hour, however, in the summer months laboratories noted attempting to spawn mussels for up to nine hours before having success. These issues can quickly add up to significant extra costs for the lab, lost time, and questionable gamete quality even when individuals do spawn. The ultimate result is potentially compromised data quality, or data gaps, which fortunately, has not appeared to have been an issue for the Bight Program so far.

A second bivalve, the Pacific oyster *Crassostrea gigas*, is included as an acceptable species within the same EPA and ASTM methods for effluent and receiving water testing that are

referenced for the mussel embryo development test (USEPA 1995 and ASTM 2012). The oyster is also generally listed as an acceptable species in most NPDES permits that require toxicity tests with bivalve embryos. Contrary to the mussel, Pacific oysters are generally in their prime gravid state during the summer months. Laboratories participating in the comparison have also successfully spawned Pacific oysters in less than an hour during the summer and obtained quality gametes using a single batch of organisms. Another benefit associated with oysters is that gametes can be collected by stripping if there are difficulties inducing spawning. Due to their similar sensitivity to various chemicals as mussels, and alternate seasonal spawning cycles between the two species, both mussels and oysters have been considered interchangeable for the purposes of NPDES discharge compliance monitoring.

Oysters were not tested during development of the SQO methods, thus they have not been considered as an acceptable test species under this guidance to assess sediment quality. The Bight ’18 Program provided an exceptional opportunity to test drive the viability of oyster embryos as a test species using the sediment-water interface test. A demonstration was performed by two participating laboratories to evaluate the performance and sensitivity of oyster embryos compared to the mussel *Mytilus galloprovincialis* that is typically used. This document provides the results produced to test oyster embryos as an alternative species in the sediment-water interface test and discusses the use of oysters as a potential option for subsequent Bight monitoring programs under SQO guidance.

Materials & Methods

The evaluation of oysters as an alternative species was performed by two Bight ’18 participating laboratories, Wood Environment and Infrastructure Solutions Inc. (Wood) and Aquatic Bioassay & Consulting Laboratories (ABC). To evaluate the performance of oyster embryos, each laboratory tested two sediment samples (B18-10115 and B18-10178) collected during the Bight ’18 Program and also performed a reference toxicant test with ammonia. A side-by-side comparison of tests using both oysters and mussels was performed by each lab as an additional validation step to compare sensitivity between the two species.

Test methods for both mussels and oysters followed those detailed in the Bight ’18 Toxicology Manual with the one difference being test temperature between the two species (15 ± 1°C for the mussels and 20 ± 1°C for the oysters). For both the mussel and oyster tests, the SQO toxicity category for each sample was determined using thresholds established for the mussel species since threshold levels for oysters have not yet been developed.

Results

A summary of data and statistical results for the mussel and oyster tests is presented in Tables 1 and 2 for ABC and Wood respectively. All sample results were compared to the associated water only lab control for the SWI test.

Tests performed by ABC identified statistically significant effects to mussel embryos exposed to sediments from Sites B18-10115 and B18-10178, although the response was limited at 9.7 and

10.1 percent effect respectively relative to the associated lab control. No statistically significant effects to oyster embryos were observed in either sediment sample tested at ABC. Both sediment samples tested by ABC were considered non-toxic for both the mussel and oyster tests using SQO thresholds.

Tests performed by Wood found no statistically significant effects to either mussel or oyster embryos in both sediment samples tested. Consistent with results at ABC both sediment samples tested at Wood were considered to be non-toxic using both mussel and oyster embryos.

Reference Toxicant Data

Mussels and oyster embryos were exposed to a range of ammonia concentrations in a water-only reference toxicant test, consistent with methods used for the Bight Program to support an evaluation of quality assurance. A summary of median effect (EC50) concentrations for both total and un-ionized ammonia is presented in Table 3 for both labs and both test species.

The reference toxicant tests performed by both Wood and ABC concurrently with the sediment tests met control test acceptability criteria for both mussel and oyster embryos (> 90% mean normal-alive). The median effect concentration for both total and un-ionized ammonia at both labs was very comparable between the two species (less than a factor of 2). Results between the two labs for each species were also comparable and less than a factor of 2.

Additionally, the oyster embryos exhibited similar sensitivity to ammonia as mussels when compared to each laboratory's historical reference toxicant chart for mussel testing. The oyster reference toxicant test performed by ABC resulted in an EC50 of 0.12 mg/L un-ionized ammonia, which was within the laboratory's historical EC50 range for mussels (0.08 – 0.12 mg/L). The un-ionized ammonia EC50 for oyster testing at Wood was 0.24 mg/L, which was slightly higher than the historical range for mussels of 0.09 to 0.23 mg/L.

Available literature also provides support that both mussel embryos and oyster embryos experience similar sensitivity to other toxicants. Several studies evaluated the effects of various metals on the bivalve embryos and reported an EC50 of 7.8 µg/L copper for mussels (Phillips et al. 2003) while the copper EC50 for oysters ranged from 5.3 – 11.5 µg/L (USEPA 1984). Exposure to zinc resulted in an EC50 of 178 µg/L for mussels (Phillips et al. 2003) and the reported EC50 for oyster embryos ranged from 119 – 207 µg/L zinc (Martin et al. 1981 and Burgess and Nacci 1993).

Quality Assurance

Samples were received in good condition and were immediately placed in cold storage until test initiation. All tests met minimum test acceptability criteria (TAC) established in the Bight '18 Toxicology Manual. Poor embryo recovery occurred in one Lab Control replicate and two B18-10178 replicates of the oyster test performed at Wood E&I, therefore, those replicates were excluded from statistical analyses. The replicates were considered statistical outliers using Grubb's test. The reason for the low recovery from these test replicates remains unknown.

Table 1. Bivalve SWI Results - ABC Laboratories

Mediterranean mussel (*Mytilus galloprovincialis*)			
Sample ID	Mean Normal-Alive (%)	Percent Effect[1] (%)	SQO Result
Lab Control	94.1	N/A	Non-toxic
B18-10115	**85.0**	**9.7**	Non-toxic
B18-10178	**84.5**	**10.1**	Non-toxic
Pacific Oyster (*Crassostrea gigas*)			
Sample ID	Mean Normal-Alive (%)	Percent Effect[1] (%)	SQO Result[2]
Lab Control	95.2	N/A	Non-toxic
B18-10115	95.8	-0.6	Non-toxic
B18-10178	92.7	2.7	Non-toxic

[1] A negative value for %Effect indicates the sample outperformed or had a positive effect relative to the Lab Control.

[2] SQO result calculated using thresholds for mussel test since thresholds for oyster test not yet developed.

Bold values a statistically significant effect compared to the Lab Control. N/A – Not applicable

Table 2. Bivalve SWI Results - Wood E&I

Mediterranean mussel (*Mytilus galloprovincialis*)			
Sample ID	Mean Normal-Alive (%)	Percent Effect[1] (%)	SQO Result
Lab Control	88.2	N/A	Non-toxic
B18-10115	83.7	5.1	Non-toxic
B18-10178	84.6	4.1	Non-toxic
Pacific Oyster (*Crassostrea gigas*)			
Sample ID	Mean Normal-Alive (%)	Percent Effect[1] (%)	SQO Result[2]
Lab Control	88.2	N/A	Non-toxic
B18-10115	79.3	10.1	Non-toxic
B18-10178	80.0	9.4	Non-toxic

[1] A negative value for %Effect indicates the sample outperformed or had a positive effect relative to the Lab Control.

[2] SQO result calculated using thresholds for mussel test since thresholds for oyster test not yet developed.

Bold values a statistically significant effect compared to the Lab Control. N/A – Not applicable

Table 3. Summary of Reference Toxicant Test Results

ABC Laboratories			
Test Endpoint	Test Species	Total Ammonia EC_{50} (mg/L)	Un-ionized Ammonia EC_{50} (mg/L)
Combined Normal-Alive	Mediterranean mussel (*Mytilus galloprovincialis*)	5.9	0.10
	Pacific Oyster (*Crassostrea gigas*)	5.6	0.12
Wood E&I			
Test Endpoint	Test Species	Total Ammonia EC_{50} (mg/L)	Un-ionized Ammonia EC_{50} (mg/L)
Combined Normal-Alive	Mediterranean mussel (*Mytilus galloprovincialis*)	11.3	0.18
	Pacific Oyster (*Crassostrea gigas*)	10.7	0.24

Conclusion

Results of this initial intercalibration study provide good confidence that SWI tests using oyster embryos can successfully be performed. Tests conducted during this study with both oyster and mussel embryos met current EPA and Bight protocol test acceptability criteria and provided comparable results between two laboratories in clean laboratory water, natural sediments, and a water only reference toxicant test using ammonia. Results of this initial study, consistent with available literature, suggest further that the sensitivity of both mussel and oyster embryos is comparable. Two key limitations of this study are the desire and need to have more participating laboratories and sediments with a range of toxicity (both sediments in this case being non-toxic). Results from this study however are very encouraging and a suggestion to continue pursuing oyster embryos as a potential alternative SQO-approved test species is viable and warranted.

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