

**Naval Information
Warfare Center**



PACIFIC

TECHNICAL REPORT 3311
JUNE 2023

Final Report Derivation and Demonstration of an Environmentally Relevant Approach for Stormwater Toxicity Testing Compliance Monitoring ESTCP Project #ER-201727

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ADMINISTRATIVE INFORMATION

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EXECUTIVE SUMMARY

ES-1 Introduction

Methods to assess environmental impacts from episodic discharges on receiving water bodies need a more environmentally relevant and scientifically defensible toxicity test design. Many permittees are regularly required to conduct 96-h toxicity tests on discharges associated with events that are generally less than 24 h in duration. Current standardized methods do not adequately reflect these episodic discharge conditions at either the point of compliance or as it mixes with the receiving environment. In order to evaluate more representative biological effects, an alternative toxicity approach is described incorporating pulsed exposures of effluents and subsequent transfer of test organisms to clean water for the remainder of the test.

A more realistic assessment of the toxicological impacts of stormwater runoff, or other episodic discharges (e.g., intermittent discharges of stormwater and relief water from dry dock outfalls at Naval shipyards, chlorinator/dechlorinator cooling water from pier-side ship, etc.) on beneficial uses in the receiving waters is critical to support decisions related to the need and prioritization of appropriate best management practices (BMPs), and meaningful compliance with Clean Water Act goals. Results from pulsed toxicity exposures have been well documented in the peer-reviewed literature as a means of improving the characterization of exposure and potential for toxicity associated with episodic contaminant exposures (Dupuis et al. 2003, Butcher et al. 2006, Diamond et al. 2006, Hoang et al. 2007a, b, c, Angel et al. 2010, AMEC 2015, Rosen et al. 2019), however, the development and application of standardized protocols that are accepted by the regulatory community are currently lacking.

ES-2 Objectives

The objective of this project was to derive, demonstrate and validate a standardized environmentally relevant and scientifically defensible exposure design for laboratory toxicity testing, i.e., whole effluent toxicity (WET) to assess impacts to receiving waters from episodic discharges such as stormwater runoff.

This goal was accomplished through implementation of the following primary six tasks (Figure 1-1):

1. Engaged and established a panel of well-recognized toxicity experts, regulators, and organizations for critical review/debate, and to gain concurrence and support for ‘pulsed’ exposure methodologies;
2. Reviewed existing published pulsed exposure results for relevant contaminants, species and endpoints and conducted a critical review of historical rainfall duration, flow, and receiving water mixing dynamics at Department of Defense (DoD) facilities. This review helped to develop an improved understanding of exposure potential at distinct geographic locations, and subsequently helped guide site-specific exposure regimes;
3. Designed, demonstrated, and implemented a standardized pulsed exposure toxicity test procedure based on modifications of the U.S. Environmental Protection Agency (US EPA) WET methodology to better represent site-specific exposure regimes at end-of-pipe monitoring locations and associated receiving waters during storm events;
4. Conducted concurrent validation exposures using the new methodology along with traditional end-of-pipe collection and testing methods. Concurrent *in situ* testing was also performed in the receiving environment to validate the representativeness and protectiveness of the new laboratory-based methodology in both the receiving water and sediments;

5. Conducted a comprehensive interlaboratory comparison study to assess the ability of a variety of research and contract laboratories across the nation to provide consistent and defensible data using the modified test methodology for pulsed exposure assessment;
6. Presented findings from the interlaboratory comparison study with recommendations to regulators and end-users towards consideration of implementation of a modified WET testing procedure as they pertain to monitored discharges that are episodic in nature.

ES-3 Methodology Description

The pulsed toxicity methodology conducted for this project included modification of exposure duration building on the existing literature using pulsed exposures for stormwater (e.g., Clark et al. 1987, Burton et al. 2000, Reinert et al. 2002, Dupuis et al. 2003, Diamond et al. 2006, Hoang et al. 2007a, b, c); and intermittent discharges from wastewater treatment plants (DOE 2004). In summary, the methods were performed following standard EPA test methods with the exception of exposure duration to the sample of interest. At a given point during the test, organisms were transferred from the exposure chambers with the end-of-pipe effluent sample into test chambers with either clean laboratory water, or the mixed receiving water from the point of compliance. The total exposure period and standard observations continued per EPA protocol requirements (Figure ES-1).

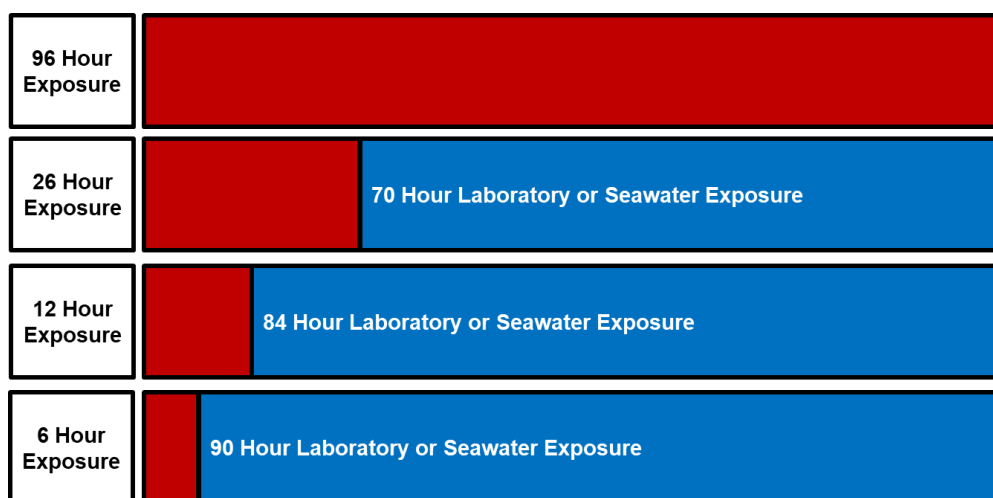


Figure ES-1. Pulsed exposure design for laboratory toxicity testing. Red coloration indicates exposure to spiked solutions or effluent samples (i.e., stormwater) and subsequent transfer into laboratory dilution water or receiving water (blue coloration).

The laboratory study conducted herein was designed to refine the pulsed exposure protocol for two marine species, the purple sea urchin, *Strongylocentrotus purpuratus*, and survival of the mysid shrimp, *Americamysis bahia*, and two freshwater species, the freshwater amphipod, *Hyaella azteca*, and the water flea, *Ceriodaphnia dubia*. These species/methods cover a range of acute and chronic endpoints (US EPA 1995, 2002a, b, c) required under various NPDES Permits were evaluated. This study investigated the effects of various copper (Cu), zinc (Zn) or bifenthrin (a pyrethroid pesticide) concentrations, and exposure durations, on the observed toxicity for all species. Table ES-1 describes the number and type of toxicity tests that were conducted as part of the refinement aspect of this project.

Table ES-1. Total number and type of toxicity test to be conducted for refinement of pulsed exposure testing with spiked stock solutions.

Species	Test Endpoint/Consideration			Total Number of Toxicity Tests
	Standard 96 h Exposure	6, 12, 26 h Pulsed Exposure	Life Stage Sensitivity	
<i>Americamysis bahia</i>	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 9 total	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 27 total	NA	36
<i>Hyalella azteca</i>	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 9 total	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 27 total	NA	36
<i>Ceriodaphnia dubia</i>	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 9 total	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 27 total	NA	36
<i>Strongylocentrotus purpuratus</i>	3 x Cu 3 x Zn <u>3 x Bifenthrin</u> 9 total	3 x Cu 3 x Zn <u>3 x Bifenthrin</u> 27 total	3 x Cu <u>3 x Zn</u> 24 total	60

^a – Latent effects were evaluated for the standard and pulsed exposures by carrying out the test for a longer period. These tests do not contribute to the total number of tests calculation. NA = not applicable.

Stormwater and/or episodic industrial discharge samples from DoD installations were evaluated using both standard and pulsed exposure laboratory methods to ensure representativeness and protectiveness of actual site conditions during dynamic runoff events. Efforts at all demonstration sites was made to collect representative water samples consistent with the Navy industrial NPDES permit to collect a grab sample during the first 4 h of runoff. Table ES-2 describes the number and type of toxicity tests that were conducted as part Task 4 of this project.

Table ES-2. Total number and type of toxicity test conducted for validation of pulsed exposure testing with field collected samples.

Site Name	Stations Sampled	Sample Type	Species/Endpoint	# of Tests	# of Sampling Events	Exposure Durations Tested	Total Toxicity Tests
NBSD	Pier 13, OF39	Stormwater	Mysid acute; Urchin chronic	2	2	Standard (96 h), 6, 12, 26 h Pulsed	32
NBC	OF 9, OF111	Stormwater	Mysid acute; Urchin chronic	2	2	Standard (96 h), 6, 12, 26 h Pulsed	32
NBPL	OF 28	Stormwater	Mysid acute; Urchin chronic	2	2	Standard (96 h), 6, 12, 26 h Pulsed	16
PSNS	OF18ab, OF19, OF21, OF96	Drydock Discharge	Urchin Chronic	2	2	Standard (96 h), 24 h Pulsed	10
NSBNL	OF6, OF15, OF17, OF22A, OF27	Stormwater	<i>Ceriodaphnia</i> acute	1	1	Standard (96 h), 6, 12, 26 h Pulsed	20

OF = Outfall

An Interlaboratory Calibration Study (ICS) was hosted to assess the ability of the pulsed exposure methodology to provide consistent and defensible data for monitoring of episodic discharges and is fully described in Colvin et al. (2021b). The organization, implementation and performance goal objectives of the study was comparable to that performed by EPA's interlaboratory calibration study of standardized freshwater and marine WET methods conducted in 1999-2000 (USEPA 2001). The following study design was followed for conducting this effort:

- Laboratory Procurement;
- Sample Preparation;
- Preliminary Testing;
- Sample Distribution;
- Interlaboratory Calibration Study Testing;
- Data Review and Analysis.

ES-4 Performance Assessment

Table ES-3. Performance objectives.

Quantitative Performance Objectives				
#	Objective	Data Requirements	Success Criteria	Results
1	Task 2: Historical rainfall analysis and literature review	Summary of literature review; rainfall, runoff, and mixing dynamics at a minimum of 5 DoD sites with a diversity of climates.	Summary document providing site-specific exposure duration recommendations for all locations evaluated. Proposed pulsed exposures should fall within the 50 th to 95 th percentile of observed rainfall durations.	✓ Met. Two technical documents generated: 1. Literature Review: “Approaches for Evaluation of Episodic Discharges” (Colvin et al. 2020c) 2. Rainfall analyses of 21 DoD relevant locations: “Long-term Rainfall Analysis at Select DoD-relevant Sites for the Determination of Appropriate Toxicity Exposure Durations” (Colvin et al. 2020a)
2	Task 3: Pulsed protocol development and demonstration	Demonstrate applicability in the lab with minimum of 3 toxicants, 4 species, 4 time points.	Data quality objectives met, and all test acceptability criteria met for all toxicity tests performed. Meaningful EC/LC ₅₀ and confidence intervals for each species/constituent/exposure durations were generated. A standardized, easy to perform method that is representative and protective of diverse receiving water conditions.	✓ Met. A total of 144 toxicity tests were conducted. Test acceptability criteria met for all tests conducted. Point estimate (EC/LC₅₀) values and confidence intervals for each species/constituent/exposure durations were generated. A standard operating procedure was published for each of the species evaluated (Colvin et al. 2020b). Manuscripts published on marine (Colvin et al. 2021a) and freshwater efforts (Karic et al. 2022).

Quantitative Performance Objectives				
#	Objective	Data Requirements	Success Criteria	Results
3	Task 4: Pulsed protocol validation	Concurrent end-of-pipe laboratory and <i>in situ</i> receiving water and sediment exposures (two events).	All data quality objectives met, and all test acceptability criteria met for all toxicity tests performed. Statistically similar results ($p < 0.05$ using students t-test) between pulsed exposure laboratory and <i>in situ</i> exposure methods.	✓ Met, partially. Test acceptability criteria were met for all tests conducted. Stormwater sampling and both laboratory and <i>in situ</i>-based testing efforts were conducted for Navy Region Southwest, encompassing the three metro installations: NBSD, Naval Base Coronado (NBC) and Naval Base Point Loma (NBPL) (2 events, 10 samples, 6, 12, 26 h and static exposures). Statistical analyses for the <i>in situ</i> results were compared against the pulsed exposure closest in duration to the event-specific storm duration.
		Develop acceptability criteria for the interlaboratory study.	Consensus among majority of peer review team, and protocol for laboratories.	✓ Met. Criteria used for the conducting and evaluation of the interlaboratory calibration study was modeled after the USEPA's Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Vol. 1 (USEPA 2001).

Quantitative Performance Objectives				
#	Objective	Data Requirements	Success Criteria	Results
4	Task 5: Interlaboratory calibration study (ICS)	Toxicity and associated water quality data from all participating labs.	Procurement and successful testing by at least 5 qualified laboratories. Specific success criteria: Completion Rate: $\geq 90\%$; False Positive Rate: $\leq 5\%$; Precision Rate: all test acceptability criteria met for each species tested; $\leq 35\%$ CV among laboratories for EC/LC ₅₀ point estimates generated; overlapping 95% confidence intervals among laboratories.	✓ Met, partially. Data from 7, 8 and 7 laboratories were generated during the ICS using the <i>Ceriodaphnia dubia</i>, <i>Americamysis bahia</i> and the <i>Strongylocentrotus purpuratus</i> organisms, respectively. Completion rates were 100% for all methods for both the pulsed exposure and the standard methods. False positive rates in the blank (negative control) sample were 0% for all methods/species tested. Variability observed in the Pulsed ICS averaged 67.2 and 22.6% for LC₅₀ values for the <i>C. dubia</i> and mysid acute tests for all sample types, respectively. Variability averaged 55.5% for EC₅₀ values for the short-term sea urchin embryo-larval development tests for all sample types. Full report published through NIWC publications office (Colvin et al. 2021b).
Qualitative Performance Objectives				
#	Objective	Data Requirements	Success Criteria	Results
5	Task 1: Establish a diverse panel of toxicological experts, regulators, and dischargers.	Multiple meetings/webinars were coordinated. Convene at least one panel discussion at a national scientific conference (e.g., SETAC).	General consensus among panel experts on the importance and need for a suitable pulse-specific protocol, and a final standardized methodology.	✓ Met. A Pulsed Exposure Scientific Advisory Panel (PESAP) was established with roughly 21 participants from diverse backgrounds including toxicological experts, regulators, and end-users. Biannual updates (either in person or through tele-conferences) were conducted each year of the project program. Feedback on work-products were incorporated into final versions prior to publication.

Qualitative Performance Objectives				
#	Objective	Data Requirements	Success Criteria	Results
6	Task 5: Ease of use of new methodology	Feedback from participating labs on usability of methodology.	Positive feedback from participating labs.	✓ Met, partially. ✓ A survey was provided to laboratories requesting their feedback on their participation in the ICS and implementation of the pulsed exposure methods. Four laboratories provided feedback and the primary concern was the increased labor hours that would be required for performing the pulsed exposure method. An additional concern was the one-time cost of constructing the materials needed for performing organism transfers. However, the labs recognized that the cost was an appropriate investment if pulsed exposure methodologies were integrated into future monitoring efforts.
7	Task 6: Engagement of regulatory community	Meetings/presentations to regulatory community on results from interlaboratory calibration study and suggestion implementations.	Positive feedback and interest from regulatory community.	✓ Met. ✓ From the onset of the project with the formation of the PESAP, and throughout the project with numerous presentations and technical sessions of the pulsed exposure work that we have conducted, communication of the efforts of this project have been ongoing and frequent. Biannual updates to the PESAP allowed for feedback opportunities from the participants. There was an overall consensus that there is a need for an alternative approach for the monitoring of episodic discharges. ✓ The regulatory community has also recognized the need discuss and understand the state of science for frequency and duration as it pertains to water quality criteria and thus the EPA hosted a workshop in September 2019 in Washington, D.C. Two project members were invited with numerous other experts to attend this 2-day workshop.

ES-5 Cost Assessment

In order to successfully transition this methodology to end-users and laboratories for use, the costs associated with implementing the pulsed exposure methodology should be comparable to existing standard methods or at a minimum, provide a greater benefit and understanding of episodic discharge impacts per unit of cost.

Operational costs for performing standard toxicity test methods were compared to costs associated with conducting the pulsed exposure methodology. Table ES-4 shows the range and average of costs for each methodology performed.

Table ES-4. Range of costs for performing both standard and pulsed exposure toxicity test methods.

	Species					
	<i>Ceriodaphnia dubia</i> acute		<i>Americamysis bahia</i> acute		<i>Strongylocentrotus purpuratus</i> chronic	
	Standard	Pulsed	Standard	Pulsed	Standard	Pulsed
Min	\$1,160.00	\$1,000.00	\$900.00	\$960.00	\$1,020.00	\$1,200.00
Max	\$1,700.00	\$1,500.00	\$1,120.00	\$2,000.00	\$1,600.00	\$1,400.00
Average	\$1,430.00	\$1,248.16	\$1,010.00	\$1,321.93	\$1,310.00	\$1,343.70

NOTE: This is assuming that a single pulsed exposure test is being conducted.

The main notable difference in costs between standard toxicity test methods and the pulsed exposure methodology is the number of labor hours that are required to conduct the transfer of organisms from the test/effluent solution into receiving or laboratory control water at the appropriate time point.

ES-6 Implementation Issues

The pulsed exposure methodology is a fairly straightforward adaptation of existing toxicity methods (US EPA 1995, 2002a, b, c) that are widely used for the evaluation of effluent samples. The data generated in the laboratory refinement studies were in line with our expectations. The project team had been using the pulsed exposure methods for a few years prior to the ICS and were very familiar with nuances associated with the methods for the different species. It was reassuring that participant laboratories were able to execute the pulsed exposure methods and made for a successful ICS effort.

The primary issue in getting the method accepted as an alternate test method has been due to that there is no specific EPA procedure for getting a WET test method evaluated through the EPA's Alternate Test Procedure (ATP) process. It has also been a learning process determining the optimal way to approach getting the method submitted to the ATP process. It was discovered that the project team would be unable to submit the method for review for a nationally accepted method, but rather the pulsed method would be reviewed for regional and permit specific acceptance. Therefore, since the primary demonstrations for this project took place within Navy Region Southwest, the ATP submission will apply to the future NPDES permit that will cover the three regional bases on San Diego Bay. This will set a precedent for other end-users to request use of the pulsed exposure method for their monitoring requirements. We expect that there will be pushback by permit writers at some stage of the permit negotiation process where it will be perceived that the pulsed exposure methodology is not conservative enough to fully replace standard testing and won't protect the beneficial uses of the receiving environment. We have developed a potential monitoring framework

where toxicity testing is one component of an overall risk evaluation. The approach is fairly site-specific and would not be appropriate for discharges to small ponds, lakes, or other water bodies that have limited assimilative capacity. However, many or most NPDES monitoring programs that require toxicity testing include end-of-pipe monitoring to larger, more dynamic water bodies (i.e., large streams, rivers, bays, harbors, and ocean). These locations will undoubtedly have rapid mixing due to flow, tides, currents, wave action where pulsed exposures approaches may be more appropriate and representative than standard static exposures. A pulsed exposure assessment can also be an added tool for areas identified as having high risk with prior documented toxicity in the outfall samples using standard continuous exposure methods. The idea is not to replace existing methods, but rather to supplement them to provide a more representative and meaningful measure for subsequent management decisions.

ACRONYMS

ASTM	American Society for Testing and Materials
ATP	Alternate Test Procedure
BMP	Best Management Practices
CETIS	Comprehensive Environmental Toxicity Information System
COC	Contaminants of Concern
CORMIX	Cornell Mixing Zone Expert System
Cu	Copper
CuSO ₄	Copper Sulfate
CV	Coefficient of Variation
DO	Dissolved Oxygen
DOC	Dissolve Organic Carbon
DoD	Department of Defense
DMW	Diluted Mineral Water
DoD	Department of Defense
EC ₅₀	Median-Effect Concentration
ELAP	Environmental Laboratory Accreditation Program
ENVVEST	Environmental Investment
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
FSW	Filtered Seawater
HDPE	High Density Polyethylene
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICS	Interlaboratory Calibration Study
JBPHH	Joint Base Pearl Harbor-Hickam
LC ₅₀	Median Lethal-Effect Concentration
LOEC	Lowest Observed Effect Concentration
MSD	Minimum Significant Difference
NAB	Naval Amphibious Base
NBC	Naval Base Coronado
NBPL	Naval Base Point Loma
NBSD	Naval Base San Diego
NESDI	Navy's Environmental Sustainability Development to Integration Program
NIWC Pacific	Naval Information Warfare Center Pacific
NOEC	No Observed Effect Concentration

NPDES	National Pollutant Discharge Elimination System
PMSD	Percent Minimum Significant Difference
PSNS	Puget Sound Naval Shipyard
RPD	Relative Percent Difference
SERDP	Strategic Environmental Research and Development Program
SOP	Standard Operating Procedure
QA	Quality Assurance
QC	Quality Control
TAC	Test Acceptability Criteria
TST	Test of Significant Toxicity
USEPA	United States Environmental Protection Agency
WET	Whole Effluent Toxicity
Zn	Zinc

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1. INTRODUCTION

The purpose of this project was to derive, demonstrate and validate a modified pulsed exposure toxicity test methodology for the evaluation of episodic discharges. This section provides a general overview of the project including background, objectives and regulatory drivers.

1.1 BACKGROUND

The Department of Defense (DoD), like other permittees across the nation, is required to comply with increasingly stringent water quality requirements for stormwater runoff and other episodic (non-continuous) discharges. These requirements generally include end-of-pipe monitoring, enforced by National Pollutant Discharge Elimination System (NPDES) permits, prior to mixing in the receiving water. Regulatory concern with stormwater discharges is associated with the Environmental Protection Agency's (EPA) Clean Water Act's goal to prevent discharge of toxics in toxic amounts (USEPA 1972). As a result, the existing EPA whole effluent toxicity (WET) test methods developed to assess continuous point source discharges (USEPA 1995, 2002 a, b, c) are now being applied to episodic discharges such as stormwater as well. DoD facilities and other industrial and municipal discharges, in fact, are frequently unable to comply with current stormwater NPDES requirements under the current test methodologies, even with the implementation of rigorous Best Management Practices (BMPs). Passing or failing toxicity and chemical concentration limits in any given end-of-pipe stormwater discharge can be a function of the size of the storm, antecedent dry period, when the sample was taken, or type of sample (grab or composite) as shown effectively in pollutograph studies (Kayhanian et al. 2008). These variables increase the complexity with regard to interpreting the results for a given end-of-pipe stormwater sample, necessitating a more realistic and standardized sample collection and toxicity exposure regime. The key element of exposure duration and integration with receiving waters is missing from current methodologies.

Toxicity tests are desirable because: 1) they take into account contaminant bioavailability, which can vary from that in uncontaminated filtered water used to develop water quality criteria (Stephan et al. 1985); and 2) they incorporate the potential for adverse effects associated with exposure to complex mixtures (i.e., multiple contaminants), many of which may not be monitored. However, stormwater is often monitored from end-of-pipe samples during the first flush (first few hours of rainfall) and then evaluated with laboratory toxicity tests using a continuous (i.e., static or static-renewal) exposure of up to 7 days, depending on the species and test endpoint. This methodology does not adequately replicate the dynamic nature of the stormwater exposure at either the point of compliance or as it mixes with the receiving environment (Katz et al. 2006; AMEC 2015), which may have substantially different physical and chemical properties than the runoff itself. The limitations of using current WET methods for stormwater testing at end-of-pipe for compliance has been recognized by regulators, as reflected in a new NPDES Permit for Naval Base San Diego (Permit R9-2013-0064). This permit includes a provision where a special chronic toxicity study may be conducted to propose modifications to the Water Quality Control Plan for the San Diego Region (Basin Plan) to incorporate mixing zone dilution credits, recognizing that end-of-pipe monitoring is not representative of actual conditions in the bay. Use of a more applicable and representative toxicity test method would provide a sound basis for supporting such a change.

A more realistic assessment of the toxicological impacts of stormwater runoff, or other episodic discharges (e.g., intermittent discharges of stormwater and relief water from dry dock outfalls at Naval shipyards, chlorinator/dechlorinator cooling water from pier-side ship, etc.) on beneficial uses in the receiving waters is critical to support decisions related to the need and prioritization of appropriate BMPs, and meaningful compliance with Clean Water Act goals. Results from pulsed

toxicity exposures have been well documented in the peer-reviewed literature as a means of improving the characterization of exposure and potential for toxicity associated with episodic contaminant exposures (Dupuis et al. 2003, Butcher et al. 2006, Diamond et al. 2006, Hoang et al. 2007a, b, c, Angel et al. 2010, AMEC 2015, Rosen et al. 2019), however, the development and application of standardized protocols that are accepted by the regulatory community are currently lacking.

1.2 OBJECTIVES OF THE DEMONSTRATION

The objective of this project was to derive, demonstrate and validate a standardized environmentally relevant and scientifically defensible exposure design for laboratory toxicity testing i.e., (WET) to assess impacts to receiving waters from episodic discharges such as stormwater runoff. A key objective was to gain regulatory approval of this alternative test procedure for episodic discharges. A modified WET test procedure was also employed as an option to determine compliance where end-of-pipe numerical (chemical) concentration limits may be unattainable or potentially overprotective.

This goal was accomplished through implementation of the following primary six tasks (Figure 1-1):

1. Engaged and established a panel of well-recognized toxicity experts, regulators, and organizations for critical review/debate, and to gain concurrence and support for ‘pulsed’ exposure methodologies;
2. Reviewed existing published pulsed exposure results for relevant contaminants, species and endpoints and conducted a critical review of historical rainfall duration, flow, and receiving water mixing dynamics at DoD facilities. This review helped to develop an improved understanding of exposure potential at distinct geographic locations, and subsequently helped guide site-specific exposure regimes;
3. Designed, demonstrated, and implemented a standardized pulsed exposure toxicity test procedure based on modifications of the EPA WET methodology to better represent site-specific exposure regimes at end-of-pipe monitoring locations and associated receiving waters during storm events;
4. Conducted concurrent validation exposures using the new methodology along with traditional end-of-pipe collection and testing methods. Concurrent in situ testing was also performed in the receiving environment to validate the representativeness and protectiveness of the new laboratory-based methodology in both the receiving water and sediments;
5. Conducted a comprehensive interlaboratory comparison study to assess the ability of a variety of research and contract laboratories across the nation to provide consistent and defensible data using the modified test methodology for pulsed exposure assessment;
6. Presented findings from the interlaboratory comparison study with recommendations to regulators and end-users towards consideration of implementation of a modified WET testing procedure as they pertain to monitored discharges that are episodic in nature.

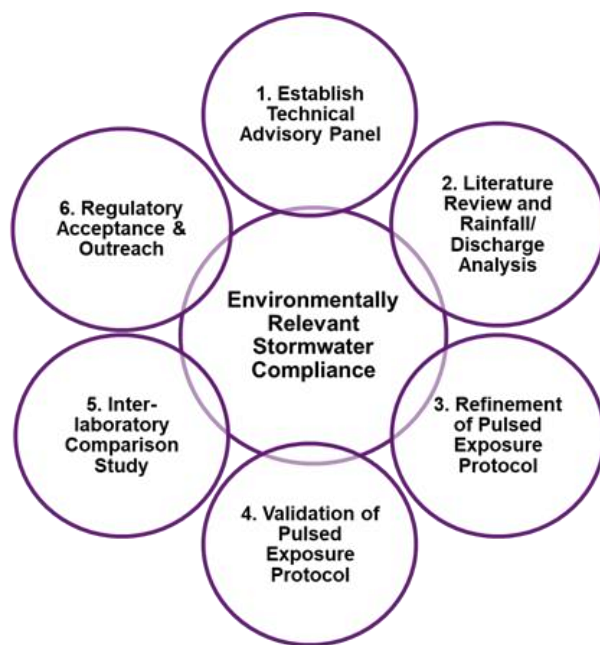


Figure 1-1. Overview of tasking towards improved stormwater compliance.

1.3 REGULATORY DRIVERS

A variety of NDPES permits require EPA WET methods to test for compliance with toxicity guidelines for episodic discharges. There are a variety of episodic discharges regulated by NPDES permits including stormwater, dry dock discharges, discharges from submarine mounted dechlorination units, batch treatment industrial discharges, and some mining operations as prime examples. Standard methods do not account for discharge duration or dilution as these intermittent discharges enter the receiving environment, and the chemical and physical properties of the receiving environment (i.e., dissolved oxygen, hardness, salinity, pH) that may alter the susceptibility of organisms to toxicants present in the stormwater (Burton et al. 2000, Schiff et al. 2003, Diamond et al. 2006, Gordon et al. 2012).

Existing WET test methods as currently applied are not appropriate for episodic discharges as the methods were originally designed to assess continuous point-source discharges (US EPA 1995, 2002a, 2002b, 2002c). For example, samples collected for the assessment of stormwater toxicity from industrial areas are frequently collected within the first few hours of an event (i.e., the “first-flush”) and storm duration or 24-hour composite samples are often collected for watershed locations. Test organisms are then exposed to these samples for the entire duration of the toxicity test, which can range from 48 hours (acute and short-term chronic tests) to 7 days (chronic toxicity tests). This type of exposure might not be a realistic or representative of what is occurring at the point of compliance at the end-of-pipe nor in the receiving environment.

Following is a clear example of how WET testing is currently applied to episodic stormwater discharges in San Diego, CA and the limitations and concerns of current methods. The first NPDES Permit with a stormwater toxicity testing requirement was finalized in 2002 as Order No. R9-2002-0002, NPDES Permit No. CA0109363, for the Naval Base Point Loma (NBPL). Additional separate and similar NPDES Permits quickly followed and are in place for Naval Base San Diego (NBSD) and Naval Base Coronado (NBC). Each of these permits requires the collection and testing of stormwater from a number of locations for analysis of a suite of chemical constituents and acute toxicity. Increasingly, chronic toxicity tests are being incorporated at the end of pipe in metro San

Diego Naval Base permits, which signifies increasing challenges for stormwater compliance for the Navy.

Stormwater monitoring for other locations in California have encountered similar challenges meeting permit requirements at the end-of-pipe using existing methods. The State of California has been a leader with regard to permits that require toxicity testing, but other states are also now starting to incorporate similar requirements in select NPDES Permits (e.g., Washington, Virginia, Hawaii, and Florida). Joint Base Pearl Harbor Hickam (JBPHH) also has numerous industrial discharges from outfalls that are routinely monitored for compliance associated with NPDES Permit No. HI0110230 including stormwater management and water compliance programs. Additionally, Project ENVVEST (Environmental Investment) and the Puget Sound Naval Shipyard and Intermediate Maintenance Facility (PSNS&IMF) NPDES Water Environmental Compliance has been monitoring dry dock discharges as well as the receiving environment since September 2000. To meet requirements under NPDES Draft Permit No. WA0002062, technical data is generated to proactively assess regulatory and stakeholder concerns about industrial and stormwater discharges from the shipyard. Another example is the Norfolk Naval Shipyard which has an NPDES Permit (No. VA0005215) that requires short-term toxicity testing for dry dock discharges to the Elizabeth River that are episodic in nature. Naval Submarine Base New London has monitoring requirements for stormwater discharges with the freshwater organism, *Daphnia pulex*, under Connecticut's Department of Energy and Environmental Protection (DEEP) General Permit #GSI000679.

2. METHODOLOGY

This section provides an overview of the origin of standardized toxicity testing as well as describes the pulsed exposure methodology to provide a better understanding of its purpose as well as advantages and limitations of the modified methodology.

2.1 METHODOLOGY DESCRIPTION

Aquatic toxicity testing for compliance with NPDES permits follows highly standardized procedures, with the earliest standard method being developed in 1945, and subsequently adopted by the American Society for Testing Materials (ASTM; Hoffman et al. 1995). Today, there are dozens of standard laboratory methods commonly used to assess WET for regulatory compliance (e.g., ASTM 2000, USEPA 1995, 2002a, b, c; CEPA 2004; ASTM 2012).

The pulsed toxicity methodology conducted for this project included modification of exposure duration building on the existing literature using pulsed exposures for stormwater (e.g., Clark et al. 1987, Burton et al. 2000, Reinert et al. 2002, Dupuis et al. 2003, Diamond et al. 2006, Hoang et al. 2007a, b, c); and intermittent discharges from wastewater treatment plants (DOE 2004). In summary, the methods were performed following standard EPA test methods with the exception of exposure duration to the sample of interest. At a given point during the test, organisms were transferred from the exposure chambers with the end-of-pipe effluent sample into test chambers with either clean laboratory water, or the mixed receiving water from the point of compliance. The receiving water was collected at the same time as the discharge sample. The total exposure period and standard observations continued per EPA protocol requirements to evaluate potential latent effects from the short-term pulse.

Applicability of a pulsed exposure method for other episodic discharges that require monitoring was considered including, for example, dry dock discharges, as regulated under current NPDES Permits.

Establishing appropriate exposure durations for planned episodic discharges is relatively straightforward, however, deriving an exposure regime for unmanaged events such as stormwater runoff is more challenging. To assist with the development of methods to derive appropriate exposures for stormwater a separate rainfall analysis exercise was performed in support of this project for a variety of locations across the U.S. including Hawaii and Guam (Colvin et al., 2020a). An evaluation of relationships between rainfall and runoff in highly urbanized environments (characterized by impervious surfaces) has found that runoff durations and the volume of runoff can be predicted using rainfall data, which is now widely available (see Figure 2-1 with data from Katz et al. 2006). An analysis presented in Colvin et al. (2020a) using rainfall data from 21 locations across the U.S. over the last 20 years found that, on average, 95% of storms resulted in 28 or fewer hours of discharge over a 96 h period directly after the onset of the storm.

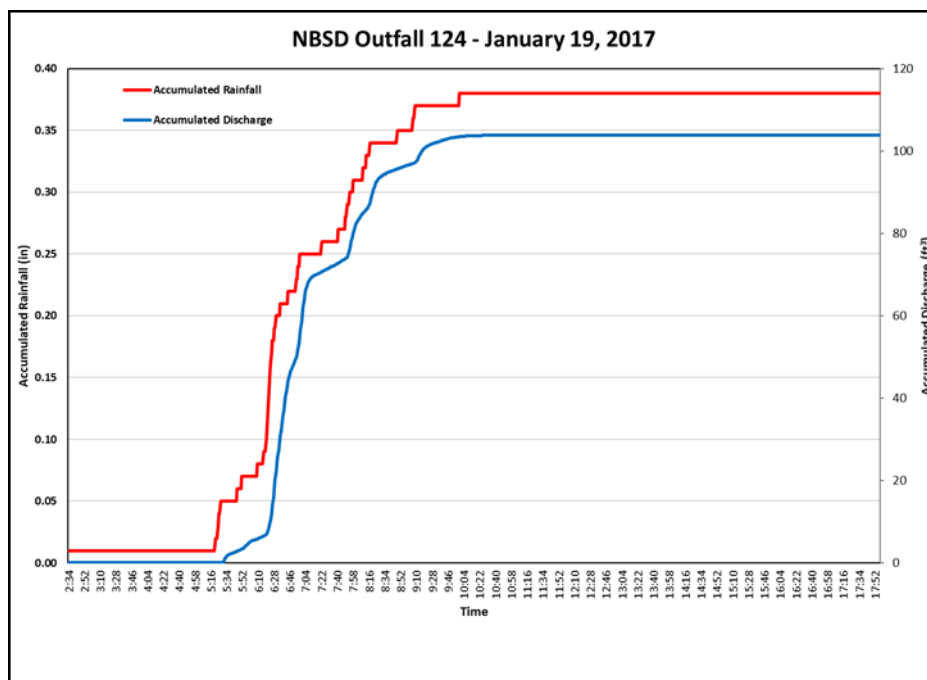


Figure 2-1. Accumulated rainfall and stormwater discharge from Outfall 124 at NBSD on 19 Jan 2017. Relationship between rainfall and discharge volume during one storm and resulting good correlation ($r^2 = 0.9939$) shows the appropriateness of using rainfall duration as a proxy for discharge duration. (From Colvin et al., 2020a).

Documented results from pulsed toxicity exposures show improved characterization of exposure and potential for toxicity associated with episodic contaminant exposures (e.g., Reinert et al. 2002, Dupuis et al. 2003, Butcher et al. 2006, Diamond et al. 2006, Hoang et al. 2007a, b, c, Angel et al. 2010, AMEC 2015, Rosen et al. 2016) in comparison to standard continuous exposures (e.g., USEPA 1995, 2002a, b, c). These studies generally show that adverse effects increase as a function of exposure duration (e.g., Figure 2-2). Some pulsed exposure studies, however, have shown latent effects associated with uptake and accumulation kinetics, requiring an adequate post-exposure observation period in the laboratory to ensure protectiveness (Burton et al. 2000) which were also conducted with our methodology. Recent investigations involving modification of the standard methods based on realistic stormwater exposure duration to multiple test species and endpoints were supported by the City of San Diego (Stransky et al. 2015) and Naval Base San Diego (Rosen et al. 2019), both of which helped form a basis for the new methodology. Development of a modified test method, validated through both state of the art *in situ* monitoring during test method development, and a rigorous interlaboratory-calibration effort based on previous USEPA interlaboratory validation approaches, were key components of this project.

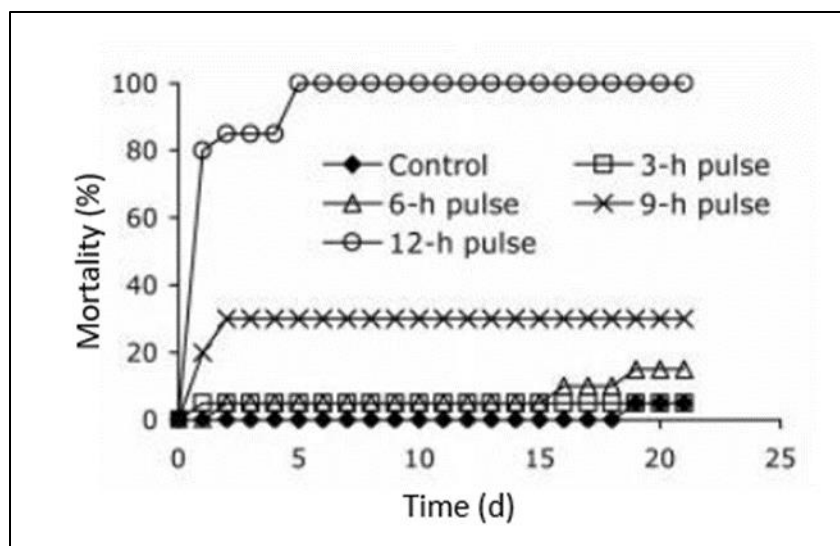


Figure 2-2. Daily cumulative mortality of *Daphnia magna* exposed to a single pulsed exposure of 6 mg/L arsenic for 3 to 12 h over a 21-d period (Hoang et al. 2007a).

It is important to point out that both duration and magnitude of exposure are important with regard to manifestation of toxicological effects. As physical properties and chemical concentrations often vary dramatically during stormwater runoff events (e.g., Kayhanian et al. 2008) and other episodic discharges, proper collection of a representative sample was an important consideration that was addressed during this demonstration. An additional suggestion has been proposed to add a concurrent test treatment that represents the concentration of stormwater or other discharge type immediately upon entering the associated receiving water. This sample would better represent the overall magnitude of exposure at a conservative location near the point of discharge and would be tested following a traditional continuous exposure to the same material.

2.2 METHODOLOGY DEVELOPMENT

Numerous studies have been conducted throughout the world to modify toxicity exposure durations, frequencies and other test parameters to better reflect the nature of episodic discharges with laboratory-based evaluations. A review was performed as part of this current project effort (Colvin et al. 2020c) and provided a thorough background of the state-of-the-science and reference to various considerations related to the design of pulsed exposure studies.

Modifications to standard toxicity test methods were initially conducted and described in Rosen et al. (2019). In brief, preliminary research was conducted to modify WET testing protocols designed for continuous flow discharges for application to episodic and/or ephemeral discharges such as those associated with storm water runoff. The effort was undertaken in response to a Naval Base San Diego industrial stormwater NPDES permit (R9-2013-0064) condition that allows the Navy to assess and propose alternative testing parameters. The technical approach taken was to modify the WET testing method to simulate a range of exposure conditions found at the end-of-pipe. The test conditions matrix included: acute and chronic endpoints with commonly used test organisms; copper, zinc, and a combination of the two toxicants at various concentrations found to cause toxicity under standard WET testing.

2.3 ADVANTAGES AND LIMITATIONS OF THE METHODOLOGY

A primary weakness of current, regulatory-approved WET test methods is that they do not adequately replicate exposure conditions at either the point of compliance (i.e., where samples are collected) or as the discharge mixes with the receiving environment. Instead, exposure durations of standard methods are based on established test protocols, which focus on continuous exposures during specific portions of a test species early life history. The pulsed exposure method is a modification to standard exposure methods that allows for increased environmental realism.

For example, an exposure of organisms to end-of-pipe stormwater samples for an entire 96 h testing period is not appropriate for most storm events as it is not a relevant contaminant exposure regime; as noted previously, stormwater discharges across the U.S. generally occur for less than 28 hours. Furthermore, testing only end-of-pipe samples, whether a continuous or pulsed exposure, fails to account for changes in the sample properties as it mixes with receiving water environments which may have very different physical and chemical properties. Results for end-of-pipe tests may over or underestimate toxicity in the mixing zone depending on effluent and receiving water characteristics. Another potentially significant limitation with current continuous exposure methods relates to changes in water quality characteristics that may occur in holding after the sample is collected, particularly for volatiles, surfactants, and dissolved metals as described in the EPA WET methods. Since the sample is episodic, the ability to go out and collect a new representative sample for longer duration tests is limited or may not be possible depending on the situation.

The slight modification of the exposure duration of organisms to the effluent sample and subsequent transfer to receiving or laboratory control water also allows for a latent effect observation period that is not standard practice in the standard exposure methods.

An inherent advantage to both standard and pulsed exposure methodologies is that they take into account contaminant bioavailability and can incorporate the potential for adverse effects associated with exposure to complex mixtures (i.e., multiple contaminants).

Dilution/Mixing Zones

Incorporating an appropriate dilution that may be tested based on site-specific mixing zone characteristics is a primary alternative approach identified for testing samples that are derived from episodic events. Extensive mixing zone studies in the receiving environment are required to come up with an appropriate dilution credit that can be applied to end-of-pipe measurements/results from NPDES monitored discharges. Determination of appropriate mixing zones can be conducted through a number of different means. Two such methods are the USEPA-approved steady-state model Cornell Mixing Zone Expert System (CORMIX) or dye-studies. These methods can be used either alone or in conjunction depending on site-specific requirements to develop representative conservative dilution factors within zones of initial dilution that may be incorporated into permit calculations (Earley et al. 2007).

However, mixing zones or dilution credits may not always be authorized by water quality regulators as is the current case for Naval Base San Diego. Currently, the San Diego Regional Water Quality Control Board does not authorize mixing zones or dilution credits in water bodies that have current water quality impairment listings as is the case for San Diego Bay, which has a number of different water quality listings that vary depending on the location. Many states have different mixing zone regulations including some that disallow mixing zones completely where certain fauna may be present (e.g., salmon in Alaska or threatened and endangered mussel species in some southeastern states).

While certain locations may allow for dilution credits, modeling exposure with very conservative assumptions, or potential costly field studies, must still be conducted on a site-specific basis to determine appropriate allowable mixing zones. Furthermore, applying a dilution to a test sample addresses magnitude of exposure, but fails to account for time of exposure; both of which are important factors with regard to toxicological effects and protection of aquatic life.

Toxicity Modeling

Toxicological modeling techniques have also been applied in some circumstances to predict toxicity due to intermittent discharges to organisms in the receiving environment. These models, while improving, still have too much uncertainty and are too context dependent to realistically be available for regulatory use (Diamond et al. 2006a, Hoang et al. 2007b, Gordon et al. 2012, Gosset et al. 2016). This will require additional inputs from quality ecotoxicological studies to hopefully minimize this uncertainty, which led Gordon et al. (2012) to create a database of pulsed exposure studies. Once again, a detailed review of these models is beyond the scope of this document, but they have been thoroughly reviewed by Diamond et al. (2006a), Reinert et al. (2002), and Gosset et al. (2016). In addition, the EPA also has recommended modeling approaches to determine appropriate criteria for intermittent exposures (USEPA 1991) and these approaches are thoroughly discussed and analyzed by Butcher and Diamond (2003).

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3. PERFORMANCE OBJECTIVES

The Performance Objectives for this study are divided into quantitative (objectives that were measured against a standard or set criteria to demonstrate success), and qualitative objectives (objectives that required a particular quality during use of the technology or in the end result). Table 3-1 outlines the performance objectives that provided the basis for evaluating the success of the pulsed exposure methodology. Meeting these performance objectives was critical for successful demonstration and validation of the pulsed exposure methodology.

Table 3-1. Performance Objectives.

Quantitative Performance Objectives				
#	Objective	Data Requirements	Success Criteria	Results
1	Task 2: Historical rainfall analysis and literature review	Summary of literature review; rainfall, runoff, and mixing dynamics at a minimum of 5 DoD sites with a diversity of climates.	Summary document providing site-specific exposure duration recommendations for all locations evaluated. Proposed pulsed exposures should fall within the 50 th to 95 th percentile of observed rainfall durations.	✓ Met. Two technical documents generated: 1. Literature Review: “Approaches for Evaluation of Episodic Discharges” (Colvin et al. 2020c) 2. Rainfall analyses of 21 DoD relevant locations: “Long-term Rainfall Analysis at Select DoD-relevant Sites for the Determination of Appropriate Toxicity Exposure Durations” (Colvin et al. 2020a)
2	Task 3: Pulsed protocol development and demonstration	Demonstrate applicability in the lab with minimum of 3 toxicants, 4 species, 4 time points.	Data quality objectives met, and all test acceptability criteria met for all toxicity tests performed. Meaningful EC/LC ₅₀ and confidence intervals for each species/constituent/exposure durations were generated. A standardized, easy to perform method that is representative and protective of diverse receiving water conditions.	✓ Met. A total of 144 toxicity tests were conducted. Test acceptability criteria met for all tests conducted. Point estimate (EC/LC₅₀) values and confidence intervals for each species/constituent/exposure durations were generated. Testing was conducted for Cu, Zn and bifenthrin using the marine species, <i>Americamysis bahia</i> and <i>Strongylocentrotus purpuratus</i>, and the freshwater species, <i>Ceriodaphnia dubia</i> and <i>Hyalella azteca</i>. All tests were compared against standard static exposures and factors of difference determined for each species/metal/duration combination; latent effects associated with the <i>A. bahia</i> exposures were evaluated; life stage sensitivity of the purple urchin was evaluated. A standard operating procedure was published for each of the species evaluated (Colvin et al. 2020b). Manuscripts published on marine (Colvin et al. 2021a) and freshwater efforts (Karic et al. 2022).

Quantitative Performance Objectives																												
#	Objective	Data Requirements	Success Criteria	Results																								
3	Task 4: Pulsed protocol validation	Concurrent end-of-pipe laboratory and <i>in situ</i> receiving water and sediment exposures (two events).	All data quality objectives met, and all test acceptability criteria met for all toxicity tests performed. Statistically similar results (p < 0.05 using students t-test) between pulsed exposure laboratory and <i>in situ</i> exposure methods.	✓ Met, partially. Test acceptability criteria were met for all tests conducted. Stormwater sampling and both laboratory and <i>in situ</i>-based testing efforts were conducted for Navy Region Southwest, encompassing the three metro installations: NBSD, Naval Base Coronado (NBC) and Naval Base Point Loma (NBPL) (2 events, 10 samples, 6, 12, 26 and static exposures). Statistical analyses for the <i>in situ</i> results were compared against the pulsed exposure closest in duration to the event-specific storm duration. P-values shown in bold below indicate statistical differences if present. Observed differences are discussed in 3.3. <table><tr><th colspan="2" rowspan="2">Deployment Date/Species</th><th colspan="2">Deployment Site</th></tr><tr><th>NBSD Pier 13</th><th>NAB OF09</th></tr><tr><td rowspan="2">Jan-2019</td><td>Sea Urchin</td><td><0.05</td><td>0.347</td></tr><tr><td>Mysid</td><td><0.05</td><td>0.007</td></tr><tr><td></td><td></td><td>NBSD Pier 13</td><td></td></tr><tr><td rowspan="2">May-2019</td><td>Sea Urchin</td><td>0.914</td><td></td></tr><tr><td>Mysid</td><td>0.374</td><td></td></tr></table>	Deployment Date/Species		Deployment Site		NBSD Pier 13	NAB OF09	Jan-2019	Sea Urchin	< 0.05	0.347	Mysid	< 0.05	0.007			NBSD Pier 13		May-2019	Sea Urchin	0.914		Mysid	0.374	
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		Develop acceptability criteria for the interlaboratory study.	Consensus among majority of peer review team, and protocol for laboratories.	✓ Met. Criteria used for the conducting and evaluation of the interlaboratory calibration study was modeled after the USEPA’s Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Vol. 1 (USEPA 2001).																								

Quantitative Performance Objectives				
#	Objective	Data Requirements	Success Criteria	Results
4	Task 5: Interlaboratory calibration study (ICS)	Toxicity and associated water quality data from all participating labs.	Procurement and successful testing by at least 5 qualified laboratories. Specific success criteria: Completion Rate: $\geq 90\%$; False Positive Rate: $\leq 5\%$; Precision Rate: all test acceptability criteria met for each species tested; $\leq 35\%$ CV among laboratories for EC/LC₅₀ point estimates generated; overlapping 95% confidence intervals among laboratories.	✓ Met, partially. Data from 7, 8 and 7 laboratories were generated during the ICS using the <i>Ceriodaphnia dubia</i>, <i>Americamysis bahia</i> and the <i>Strongylocentrotus purpuratus</i> organisms, respectively. Completion rates were 100% for all methods for both the pulsed exposure and the standard methods. False positive rates in the blank (negative control) sample were 0% for all methods/species tested. The precision of test methods evaluated in the Pulsed Exposure Interlaboratory Calibration Study was estimated by calculating a CV for point estimates (i.e., LC₅₀s and EC₅₀s) for samples that elicited a toxic response. Additionally, to compare the pulsed exposure method to standard continuous exposure methods, the CVs of the 6.25 and 100% concentrations were calculated for each sample. Variability observed in the Pulsed ICS averaged 67.2 and 22.6% for LC₅₀ values for the <i>C. dubia</i> and mysid acute tests for all sample types, respectively. Variability averaged 55.5% for EC₅₀ values for the short-term sea urchin embryo-larval development tests for all sample types. For comparison, USEPA (2001) reported multi-laboratory precision CVs for acute WET test methods that ranged from 20.0 to 38.5% and averaged 28.4%. Previous CVs reported from USEPA (1988) were 22-167% (with a weighted mean of 50%) for acute WET methods testing reference toxicants. Full report published through NIWC publications office (Colvin et al. 2021b).

Qualitative Performance Objectives				
#	Objective	Data Requirements	Success Criteria	Results
5	Task 1: Establish a diverse panel of toxicological experts, regulators, and dischargers.	Multiple meetings/webinars were coordinated. Convene at least one panel discussion at a national scientific conference (e.g., SETAC).	General consensus among panel experts on the importance and need for a suitable pulse-specific protocol, and a final standardized methodology.	✓ Met. A Pulsed Exposure Scientific Advisory Panel (PESAP) was established with roughly 21 participants from diverse backgrounds including toxicological experts, regulators, and end-users. Biannual updates (either in person or through tele-conferences) were conducted each year of the project program. Feedback on work-products were incorporated into final versions prior to publication. A specific session entitled “Assessment and monitoring of risks to aquatic environments from short-term (episodic) contaminant exposures” was included at the SETAC North America 39th Annual Meeting (2018). A side meeting was also conducted for this project specifically at the SETAC North America 40th Annual Meeting (2019).
6	Task 5: Ease of use of new methodology	Feedback from participating labs on usability of methodology.	Positive feedback from participating labs.	✓ Met, partially. A survey was provided to laboratories requesting their feedback on their participation in the ICS and implementation of the pulsed exposure methods. Four laboratories provided feedback and the primary concern was the increased labor hours that would be required for performing the pulsed exposure method. An additional concern was the one-time cost of constructing the materials needed for performing organism transfers. However, the labs recognized that the cost was an appropriate investment if pulsed exposure methodologies were integrated into future monitoring efforts.

Qualitative Performance Objectives				
#	Objective	Data Requirements	Success Criteria	Results
7	Task 6: Engagement of regulatory community	Meetings/presentations to regulatory community on results from interlaboratory calibration study and suggestion implementations.	Positive feedback and interest from regulatory community.	✓ Met. From the onset of the project with the formation of the PESAP, and throughout the project with numerous presentations and technical sessions of the pulsed exposure work that we have conducted, communication of the efforts of this project have been ongoing and frequent. Biannual updates to the PESAP allowed for feedback opportunities from the participants. There was an overall consensus that there is a need for an alternative approach for the monitoring of episodic discharges. Considerations or concerns that were discussed during the development of the demonstration plan were addressed and communicated to the ESTCP program office in White Paper dated 11 April 2019. The regulatory community has also recognized the need discuss and understand the state of science for frequency and duration as it pertains to water quality criteria and thus the EPA hosted a workshop in September 2019 in Washington, D.C. Two project members were invited with numerous other experts to attend this 2-day workshop.

3.1 PERFORMANCE OBJECTIVE #1: HISTORICAL RAINFALL ANALYSIS AND LITERATURE REVIEW

Description

Acquisition, review and analysis of historical rainfall, runoff and mixing zone dynamics at a variety of DoD facilities were conducted. Target sites included those representing diverse climates and land use around the country (and territories), including but not limited to NBSD, San Diego, CA; Naval Base Kitsap, Puget Sound, WA; Pearl Harbor Naval Shipyard, HI; Marine Corps Base Quantico, VA; Norfolk Naval Shipyard, VA; Naval Station Pensacola, Pensacola, FL; and Naval Base Guam. An assessment of the rainfall duration at each of the sites evaluated was important to provide a realistic testing regime for a given region.

An extensive literature review and summary of published pulsed exposure toxicity studies were also conducted, with a focus on those relevant to stormwater and other episodic industrial discharges representative of those that occur at various DoD facilities, such as dry dock discharges, ballast water exchange, boom cleaning operations, and chlorinator/dechlorinator discharges. This review expanded the awareness of the state of the science associated with episodic discharges, in addition to stormwater, potentially resulting in broader appeal and potential application of the testing technology.

Data Requirements

A review of rainfall, runoff and mixing dynamics at a minimum of five DoD sites with a diversity of climates was conducted. A review of published literature was conducted, summarized and published as a technical document.

Extent Success Criteria Were Met

Two technical documents were published through Naval Information Warfare Center (NIWC) Pacific's Publications Office and made publicly available through Defense Technical Information Center (DTIC). The first was a summary document that details analyses conducted on historical rainfall data at numerous locations across the United States in order to develop site-specific recommendations for exposure durations for laboratory toxicity testing specifically for assessment of stormwater discharges (Colvin et al 2020a). This document was leveraged with a project through the Navy Environmental Sustainability Development to Integration (NESDI) program (Project #547, entitled *Demonstration of Improved Toxicity Methodology to Link Stormwater Discharges to Receiving Water Impacts at Navy Sites*; PI: M. Colvin). Leveraging allowed for more DoD locations to be included in this analysis. In brief, 21 locations were selected based on their proximity to DoD locations and for each location, two analyses were performed on each of the data sets obtained. Total rainfall duration was determined for both any given rolling 24 h period and 96 h continuous basis at the onset of a qualifying rain event. The mean, standard deviation and the 50th, 75th and 95th percentile of rainfall duration was calculated for each location and summarized within the technical document:

The second document developed was a literature review (also leveraged with NESDI Project #547) that discusses the advantages of pulsed toxicity methodologies, and various considerations for designing pulsed exposure testing protocols (Colvin et al. 2020c).

Some of the considerations that are discussed within the review document include the following:

- Pulse concentration, duration and magnitude;
- Pulse exposure frequency;

- Latent effects;
- Organism age at initiation;
- Mixture of toxicants and complex samples; and
- Selection of toxicity testing endpoints.

Alternative and supplementary approaches to evaluate episodic discharges were also evaluated in this review document, such as:

- Dilution and mixing zones;
- Toxicity modeling;
- Time-averaged concentrations (TAC); and
- Media-effect time criteria.

In addition, there is a brief discussion of potential options to incorporate pulsed exposure testing methods into regulatory frameworks and the review concludes with implications for the pulsed exposure work at NIWC Pacific under this ESTCP project and the NESDI project.

3.2 PERFORMANCE OBJECTIVE #2: PULSED PROTOCOL DEVELOPMENT AND DEMONSTRATION

Description

The approach for laboratory pulsed exposures was refined with relevant contaminants of concern (COCs) spiked in clean water along with the testing of actual effluent and receiving water samples for comparison purposes. This included COCs that have been used previously in the literature. The laboratory testing was conducted with multiple permit-relevant marine and freshwater species that cover a range of acute and chronic endpoints (USEPA 1995, 2002a, b, c). Pulsed exposure testing included simple modifications to standard methods by transfer of test organisms from either the spiked solutions or effluent samples (e. g. stormwater) into laboratory dilution water or receiving water. These studies built on the preliminary work conducted by NIWC Pacific (Rosen et al. 2019), which resulted in recommendations to verify repeatability and reproducibility through multiple tests for each species and test regime, expand species and contaminant lists, consider the timing of onset of pulses with respect to species life stage, identify the most sensitive life stage for pulsed exposures, and examine potential for latent effects. This aspect of the project was also leveraged with NESDI Project #547 (PI: Colvin) where additional considerations were assessed as a result from the literature review and included evaluations of latent effects (with the opossum shrimp, *Americamysis bahia*) and life stage sensitivity/delayed onset of pulsed exposure (with the purple sea urchin, *Strongylocentrotus purpuratus*).

Data Requirements

This project demonstrated applicability of the pulsed exposure methodology in lab-based exposures with a minimum of 3 toxicants (e.g., copper, zinc, and a pesticide), 4 species (Figure 3-1), and 4 time points (e.g., standard exposure, and 3 pulsed exposure durations). Four different laboratories participated in this effort (NIWC Pacific Bioassay Lab, University of California Davis Granite Canyon, Loyola University, and the Wood Toxicology Lab).



Figure 3-1. Toxicity test species from left to right: the purple sea urchin *Strongylocentrotus purpuratus* (with larval development endpoint shown), the mysid shrimp *Americamysis bahia*, the freshwater amphipod *Hyalella azteca*, and the water flea *Ceriodaphnia dubia*.

Extent Success Criteria Were Met

All data generated from lab-based exposures had individual test acceptability criteria and objectives met for all toxicity tests performed. Test acceptability criteria for each test endpoint were:

- *Americamysis bahia* acute survival tests: minimum 90% survival in controls;
- *Strongylocentrotus purpuratus* chronic embryo-larval development tests: minimum 80% survival in controls;
- *Hyalella azteca* acute survival tests: minimum 90% survival in controls;
- *Ceriodaphnia dubia* acute survival tests: minimum 90% survival in controls.

All species were tested in triplicate for each exposure duration (standard, 6 h, 12 h and 96 h) using either Copper (Cu), Zinc (Zn) or Bifenthrin-spiked test solutions. This resulted in at least 96 toxicity tests.

All water quality parameters (dissolved oxygen, pH, salinity/conductivity, and temperature) were maintained within recommended ranges for the duration of all toxicity tests performed (USEPA 1995, 2002a, b, c). A thorough review of the data and test procedures for all tests did not identify any likely impacts on data because of these deviations; therefore, all presented data were deemed acceptable for reporting purposes.

A standardized operating procedure (SOP) was generated for all species included in this performance objective and published as a technical document through the NIWC Pacific's Publications Office and made publicly available through DTIC (Colvin et al. 2020b).

3.3 PERFORMANCE OBJECTIVE #3: PULSED EXPOSURE PROTOCOL VALIDATION

Description

The pulsed exposure methodology was validated to ensure that it is indeed representative and protective of actual site conditions during dynamic runoff events. This was performed through end-of-pipe monitoring of stormwater/episodic discharge samples along with concurrent *in situ* toxicity testing at select locations within the San Diego Region. SEA Rings served as the platform for exposing the same species selected for the laboratory exposures and housed passive sampling devices and water quality loggers (e.g., salinity, temperature, dissolved oxygen) for characterization of the stormwater runoff and assessment of potential confounding factors.

Data Requirements

Laboratory evaluations of stormwater and/or episodic industrial discharges from a minimum of three DoD facilities were tested for toxicity using the methods developed and demonstrated in the previous performance objective including standard exposure methods for appropriate comparison. A

minimum of 10 effluent samples from at least two distinct events were tested resulting in at least 60 toxicity data points for each relevant test species. Concurrent *in situ* toxicity testing with marine species in the adjacent receiving waters was conducted at two marine DoD facilities in San Diego using techniques demonstrated previously (e.g., SEA Ring under ESTCP #ER-201130; Rosen et al. 2012, Rosen et al. 2017). *In situ* validation was performed at NBSD and NBC during a minimum of two storm events. *In situ* testing during each event was to be performed at multiple locations covering a gradient away from the primary end-of-pipe source. One representative location was positioned outside the influence of the discharge as a receiving water “reference” location for comparison purposes. End-of-pipe sampling locations included the sites at NBSD and NBC in addition to sites at Puget Sound Naval Shipyard (PSNS) and Naval Submarine Base New London (NSBNL).

Extent Success Criteria Were Met

Data generated from laboratory-based and *in situ*-based exposures was met for all toxicity tests performed. Test acceptability criteria for each test endpoint were:

- *Americamysis bahia* acute survival tests: minimum 90% survival in controls;
- *Strongylocentrotus purpuratus* embryo-larval development tests: minimum 80% survival in controls;
- *Ceriodaphnia dubia* acute survival tests: minimum 90% survival in controls.

In situ testing at NBSD and NBC was conducted concurrently with laboratory-based tested. Several other Navy installations were also evaluated in laboratory-based exposures using the pulsed exposure methodology.

- Navy Region Southwest (NBSD, NBC and NBPL) –
 - Jan 2018: 2 samples
 - Laboratory-based effort using purple sea urchin and mysid shrimp
 - Jan 2019: 15 samples
 - *In situ* and laboratory-based effort using purple sea urchin and mysid shrimp
 - May 2019: 9 samples
 - *In situ* and laboratory-based effort using purple sea urchin and mysid shrimp
- Puget Sound Naval Shipyard –
 - Laboratory-based effort using purple sea urchin
 - Feb 2018: 2 samples
 - Jun 2018: 3 samples
 - Jan 2021: 1 sample
 - Apr 2021: 1 sample
 - Dec 2021: 1 sample
- Navy Submarine Base New London –
 - Jan 2019: 5 samples
 - Laboratory-based effort using *Ceriodaphnia dubia*

All water quality parameters (dissolved oxygen, pH, salinity/conductivity, and temperature) were maintained within recommended ranges for the duration of all laboratory-based toxicity tests performed (USEPA 1995, 2002a, b, c).

A goal for the project was to show statistically similar results between the laboratory pulsed exposure methods and *in situ* exposure methods indicating an appropriate realistic exposure regime in the lab (i.e., using a student's t-test with unequal variances, $p \geq 0.05$). Provided no confounding influences *in situ* (e.g., low salinity), effects in the laboratory should be no less than that observed in the field to ensure protectiveness of the lab-based methods.

For the sea urchin embryo-larvae development test, all laboratory-based exposures exhibited toxicity throughout the concentration series tested. However, the receiving water samples that were collected prior to the storm event and during the storm event, both resulted in no toxicity. The *in situ* exposures in the SEA Rings also exhibited no toxicity.

For the mysid survival test, only the 26 h and static exposures for the sample collected at NBSD-P13 exhibited toxicity. All other stormwater samples and receiving water samples exhibited no toxicity. The *in situ* exposures did show some toxicity for the SEA Ring deployed at NBSD-P13, but only for the SEA Ring deployed at the sediment surface, possibly indicating deposition of materials that led to the toxicity observed. The SEA Ring deployed at NAB-OF09 also exhibited toxicity to the mysids for all SEA Ring deployment depths. The decreased survival that was observed is likely due to the depressed salinity that was observed during the storm events, as even the 100% concentration stormwater sample (salted for laboratory exposures) for the 96 h static exposure did not elicit a toxic response. A study by Weston (2011) found that a reduced salinity to 10 ppt or less for a period of 6 hours affected survival of *A. bahia* with few to no survivors following exposure to 6 ppt for the same timeframe. Salinity tolerance studies conducted for the City of San Diego have found that the marine kelp shrimp, *Holmesimysis costata*, is very sensitive to brief reductions in salinity, resulting in 0% acute survival following exposure to a salinity of 20 ppt for only 30 minutes (AMEC 2015). The AMEC study (2015) found that sea urchin embryos were less sensitive to reduced salinities, which is also supported by the lack of toxic response observed in this current study for *in situ* exposures.

General trends for both species show greater frequency of sea urchin embryo abnormality and juvenile mysid mortality with increasing effluent exposure with both duration and concentration. However, the *in situ* exposures provide basis for comparison and show that standard end-of-pipe exposures over-estimated toxicity in the receiving environment for the highly sensitive chronic sea urchin test and in several instances; also, for the less sensitive acute mysid exposure.

3.4 PERFORMANCE OBJECTIVE #4: INTERLABORATORY CALIBRATION STUDY

Description

In order to assess the ability of the modified methodology to provide consistent and defensible data for the assessment of episodic discharges, a comprehensive inter-laboratory calibration study (ICS) was performed.

Data Requirements

To meet requirements of this objective, a goal of a minimum of 10 laboratories would be solicited for participation and a minimum of five of the 10 laboratories would participate in the inter-laboratory calibration study. Toxicity and associated water quality data generated from all participating labs would be documented and provided to the pulsed project team for final analysis and evaluation.

Extent Success Criteria Were Met

The ESTCP project team modeled the Pulsed Exposure Toxicity ICS after the USEPA's Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity

Test Methods, Vol. 1 (USEPA 2001). A total of 14 laboratories in the United States and one laboratory in Canada were contacted via phone calls and email. Of those contacted a total of 10 of the laboratories in the United States and the one laboratory in Canada expressed interest in participation. After confirming those laboratories that were interested in the study, a request for qualifications (RFQ) and costs package was sent out to each laboratory. A third-party contractor, Edmond Scientific Company, managed the RFQ coordination and cost negotiations with each interested laboratory. A list of selected participant laboratories and species tested by each is shown in Table 3-2.

Table 3-2. Selected laboratories for the pulsed exposure interlaboratory study and species tested.

Laboratory	Location	Test Species		
		<i>Ceriodaphnia</i>	Mysid	Purple Sea Urchin
Aquatic Bioassay Consulting (ABC)	Ventura, CA	X	X	X
EA Engineering	Hunt Valley, MD	X	--	X
EcoAnalysts	Port Gamble, WA	X	X	X
Enthalpy	San Diego, CA	X	X	X
GEI Consultants	Denver, CO	X	--	--
Great Lakes Environmental Co (GLEC)	Columbus, OH	X	--	--
McCampbell Analytical Inc.	Pittsburg, CA	--	X	X
Nautilus Environmental Company	Burnaby, BC	X	X	X
Pacific EcoRisk	Martinez, CA	X	X	X
Tetra Tech	Owings Mills, MD	X	--	--
Naval Information Warfare Center (NIWC) ^A	San Diego, CA	--	X	X
Wood Environment & Infrastructure Solutions Inc. (Wood) ^A	San Diego, CA	X	X	X

^A – referee laboratory

For each test method, two referee laboratories were involved in the Calibration Study. The referee laboratories were responsible for preparing bulk test samples, conducting preliminary testing of the samples and distribution of the “blind” samples to the participating laboratories. The following five samples were prepared for each test method: 1) a blank control water; 2) a non-toxic stormwater sample; 3) a toxic stormwater sample; and 4) a toxic metal-spiked laboratory control sample, and 5) a blind duplicate of the metal-spiked laboratory control.

All laboratories that participated in the study successfully completed both pulsed and standard test methods and met standard EPA laboratory test acceptability criteria including control performance.

Two contracted laboratories that planned to initiate the daphnia tests were unable to do so properly due to a lack of suitable in-house neonates to initiate all tests at once. In addition, one participating laboratory provided questionable supporting water quality results and had insufficient documentation to verify that required test methods were followed and thus their data was excluded from the study.

In addition to successful completion of the tests, two additional key metrics for this evaluation included the frequency of false positives (declaring a clean sample toxic), and interlaboratory precision measured as the percent coefficient of variation (% CV) among labs. False positive rates for the negative control (Sample 1) were 0% for all tests conducted. Overall false positive rate, including the non-toxic stormwater sample (Sample 2), was 2.3%. Precision for the test methods was determined by comparing % CVs based on both laboratory control performance and dose response relationships measured using calculated median lethal or median effect (LC₅₀ or EC₅₀) concentration values. Confidence intervals surrounding the point estimates were also evaluated when a dose response was observed. Interlaboratory CVs of LC₅₀s ranged from 25.6 to 70.1% for acute test methods using *Ceriodaphnia* and mysid shrimp. Interlaboratory CVs of EC₅₀s for the short-term chronic tests using the purple sea urchin ranged from 50.4 to 59.2%. Furthermore, the pooled mean LC₅₀/EC₅₀ values for the pulsed exposure tests for the two duplicate samples were not statistically different from each other for all three test species indicating good repeatability for intra-laboratory performance.

3.5 PERFORMANCE OBJECTIVE #5: ESTABLISH ADVISORY PANEL

Description

The establishment of a panel of well-recognized toxicological testing experts, key regulators, discharger representatives and organizations are essential for the success of this project. The collaboration and consensus of a modified testing methodology among the assembled panel helped guide decisions on experimental approach, validation methodologies, and overall implementation within a proposed framework, to ensure that the new methods for episodic discharges are scientifically defensible, more environmentally representative, and protective of beneficial uses in the associated receiving waters.

Data Requirements

Dissemination of information and progress of the project for review and commentary was completed through multiple meetings/webinars. Such meetings/webinars occurred on a biannual basis and at least one panel discussion at a national scientific conference (e.g., Society of Environmental Toxicology and Chemistry) was convened.

Extent Success Criteria Were Met

At the onset of the project, a letter of request was sent in July 2017 to invite prospective members to participate in the Pulsed Exposure Scientific Advisory Panel (PESAP, or the Panel). The request was well received and over a dozen positive responses were received from toxicological experts, regulators, and end-users/dischargers. While a few participants were asked to be removed from the Panel due to retirement, individuals from the same institution or company were included on the Panel as a replacement (Table 3-3).

Table 3-3. Participants of the Pulsed Exposure Advisory Panel.

Organization	Name
Department of Ecology, WA	Chris Dudenhoeffer
Shell Health - Americas	Sarah A. Hughes
Naval Facilities Engineering Command Mid-Atlantic (NAVFAC Mid-Atlantic)	David Cotnoir (retired)
Naval Facilities Engineering Command Mid-Atlantic (NAVFAC Mid-Atlantic)	Taylor Jones
Pearl Harbor Naval Shipyard (PHNS)	Devon Takara
Scripps Institution of Oceanography University of California, San Diego	Dimitri Deheyn
California Stormwater Quality Association (CASQA)	Geoff Brosseau
U.S. EPA, Office of Compliance	Greg Savitske
Naval Facilities Engineering Command Southwest (NAVFAC SW)	Jessica Palmer
Naval Facilities Engineering Command Southwest (NAVFAC SW)	Melanie Kito
U.S. EPA, Office of Research and Development	Jim Lazorchak
U.S. EPA, Office of Research and Development	Kay T. Ho
San Diego Regional Water Quality Control Board (SDRWQCB)	Keith Yaeger
Port of San Diego	Kelly Tait
Southern California Coastal Water Research Project (SCCWRP)	Steve Bay (retired)
Southern California Coastal Water Research Project (SCCWRP)	Ken Schiff
University of California, San Diego	Kimberly O'Connell
City of San Diego, CA	Leslie Nanninga
Commonwealth Scientific and Industrial Research Organization (CSIRO)	Stu Simpson
Orange County Sanitation District	Violet Renick
Puget Sound Naval Shipyard and Intermediate Maintenance Facility (PSNS&IMF)	Trevor Richardson

Throughout this project, it has been clear that there is a consensus among Panel members and others in the scientific and regulatory community that there is justification for an alternative method for the assessment of episodic discharges.

3.6 PERFORMANCE OBJECTIVE #6: EASE OF USE OF NEW METHODOLOGY

Description

While we were primarily concerned with the development and demonstration of a scientifically defensible and more environmentally representative methodology for the evaluation of episodic discharges, it was not our intention to create an onerous methodology that is fiscally irresponsible with regard to labor and material expenses to do such.

Data Requirements

To gauge the feasibility of use of the new methodology in a commercial setting, feedback from laboratories participating in the inter-laboratory calibration study was collected and evaluated.

Extent Success Criteria Were Met

A survey was provided to laboratories requesting their feedback in their participation of the inter-laboratory calibration study and implementation of the pulsed exposure methods. Four laboratories provided feedback and the primary concern was the increased labor hours that would be required for performing the pulsed exposure method. An additional concern was the one-time cost of constructing the materials needed for performing organism transfers (i.e., screen tubes). However, the labs recognized that the cost was an appropriate investment if pulsed exposure methodologies were integrated into future monitoring efforts.

3.7 PERFORMANCE OBJECTIVE #7: ENGAGE REGULATORY COMMUNITY

Description

Involvement of the regulatory community at the onset of and throughout the project was important to gain concurrence on the pulsed exposure methodology for the ultimate transition of the method into compliance monitoring programs. Additionally, the project team has been working with the EPA's Alternative Test Procedures (ATP) program; <https://www.epa.gov/cwa-methods/alternate-test-procedures>. This is a mechanism in which alternative methods are submitted and reviewed for potential nationwide or limited use as an alternative to approved 40 CFR Part 136 methods.

Data Requirements

As part of Task 1, the establishment of an Advisory Panel, individuals from critical regulatory communities have had regular opportunities to help guide the process. Meetings and presentations to the regulatory community on the results from inter-laboratory calibration study have been delivered allowing for suggestions to the implementation of the methodology.

Extent Success Criteria Were Met

As mentioned in objective 3.5, regulators from several jurisdictions (i.e., San Diego Regional Water Quality Control Board; Department of Ecology, WA; US EPA) were invited and participated in the advisory panel that was established early in the project. Their awareness and participation in the Panel helped refine the Demonstration Plan that guided this project.

Additionally, by integrating pulsed exposure methodologies into current regulatory monitoring with the Navy Region Southwest, the SDRWQCB is being made aware how the pulsed exposure methodology can be incorporated into a monitoring framework and how the data that is generated compliment ongoing efforts at a given site.

The project team has been working with CA Region 9's ATP regional coordinator, Roseanne Sakamoto, with the process that needs to be followed for the submission of the pulse exposure methodology as an alternate toxicity testing method. As this type of submission has not been received before, the process is being developed as we progress. The submission of the pulsed exposure toxicity method will be reviewed for regional application (i.e., for Navy Region Southwest) and not at the national level. Due to the extensive data requirements and review associated with these WET test methods, national applications are not accepted.

4. SITE DESCRIPTIONS

A Site Selection Memorandum and a short addendum were prepared as part of this project and are attached in Appendix C.

Briefly, in order to meet the objectives of the project, specifically Task 4 (concurrent validation of a proposed end-of-pipe laboratory testing and *in situ* receiving water evaluation), selected sites had specific characteristics that demonstrated the validity of the pulsed exposure methodology with regard to representativeness and protectiveness of actual site conditions during episodic runoff events. A series of site selection criteria as shown in Table 4-1 were developed on the basis of the demonstration objectives, an understanding of the sites and conditions where the methodology was targeted for application, and practical logistics for the demonstrations.

Stormwater monitoring and *in situ* assessment

The demonstration sites for this project focused on sites where existing NPDES permits require the capture and testing of stormwater runoff for toxicity. Outfalls or discharges that have been monitored provided a high level of characterization and the ability to leverage with other projects and end user support. Outfalls or discharges of interest have demonstrated “end-of-pipe” toxicity and elevated chemistry (relative to NPDES benchmarks) to allow for the most accurate interpretation of results of the alternative toxicity testing methodology.

Selected outfalls or discharges had safe and secure access to the site during storm events. Adjacent to the outfall or discharge, an appropriate receiving environment was present where *in situ* deployment of monitoring tools (e.g., SEA Ring) and other water quality sensing equipment occurred. Placing test organisms *in situ* ensured they are exposed to the full dynamics of a runoff event or other episodic discharge (changing chemical concentrations, mixture ratios, physical parameters and exposure duration). This method provided a more realistic assessment of potential effects from an episodic discharge. *In situ* results were used to validate results derived from laboratory-based pulsed exposure methodologies. These measures provided an integrated and environmentally realistic assessment of the potential bioavailability and toxicity of contaminant exposure associated with time-varying potentially-contaminated discharge. Table 4-1 provides a site selection criteria evaluation for four sites that were chosen including Navy Region Southwest in San Diego, CA (including NBSD, NBC and NBPL), Puget Sound Naval Shipyard (PSNS), and Naval Submarine Base New London (NSBNL), CT.

Table 4-1. Site Selection Criteria for Demonstration of the Pulsed Exposure Methodology.

Parameter	Preferred Value(s)	Relative Importance ¹	NRSW			PSNS ²	NSBNL ²
			NBSD ²	NBC ²	NBPL ²		
Discharge Location	Discharge to ambient environment in area of interest, sufficient number of outfalls	1	Yes	Yes	Yes	Yes	Yes
Known biological impacts in receiving waters/ history of toxicity	Demonstrated and ongoing toxicological effects related to stormwater	1	Yes	Yes	Yes	No ³	Yes ⁴
Access to location	Safe access to location during storm events	2	Yes	Yes	Yes	NA	NA
<i>In Situ</i> Testing Conducted	Safe access and toxic end-of-pipe discharge in the vicinity	2	Yes	Yes	No	No	No
Potential for leveraging/cost sharing	Concurrent Navy grab sampling of outfalls of interest; other stormwater monitoring efforts at outfalls of interest	2	Yes	Yes	Yes	Yes	Yes
End user support	Interest and support from stormwater compliance manager	3	Yes	Yes	Yes	Yes	Yes
Historical toxicity data	5+ years	4	Yes	Yes	Yes	Yes	Yes
Detectable Contaminants of Concern in Discharges	Metals and/or organics (e.g., copper, zinc, surfactants, PAHs) at concentration levels that may exceed permit limits	2	Yes	Yes	Yes	Yes	Yes

NA – Not applicable;

¹ – Range from 1 to 5, with 1 being highest;

² – NBSD – Naval Base San Diego, CA; PSNS – Puget Sound Naval Shipyard, Bremerton, WA; Norfolk - Norfolk Naval Shipyard, Norfolk, VA; New London – Naval Submarine Base New London, New London, CT;

³ – Current dry dock discharges that are monitored for toxicity do not have a history of toxicity; however, stormwater outfalls that are monitored for chemistry have elevated concentrations of metals that may cause toxicity and are of interest for this study;

⁴ – Toxicity was observed in several outfalls during the monitoring period of 2007 to 2015.

4.1 PUGET SOUND NAVAL SHIPYARD

4.1.1 Site Location and History

Puget Sound Naval Shipyard and Intermediate Maintenance Facility (PSNS & IMF) was selected as a site for the demonstration and validation of the pulsed exposure methodology. This site fit most of the site selection criteria described in Table 4-1. Numerous effluent discharges are routinely monitored for compliance associated with NPDES Draft Permit No. WA0002062.

Under the NPDES program, PSNS is required to implement BMPs to reduce, treat and control discharges from the shipyard (Taylor et al. 2011). Additionally, the PSNS NPDES Water Environmental Compliance and Project ENVVEST (Environmental Investment) generates technical data to meet NPDES requirements and assess regulatory and stakeholder concerns about industrial and stormwater discharges from the shipyard.

Over the past 10 years, discharges at PSNS have had a history of exceeding numerical action limits for copper. Toxicity testing has also been conducted for numerous years evaluating both effluent discharges from dry docks as well as the adjacent receiving environment. These existing monitoring requirements allowed for leveraging opportunities for the pulsed exposure methodology for sample collection, analytical chemistry analysis and concurrent toxicity evaluations using standard methods.

PSNS & IMF is part of the Bremerton Naval Complex (BNC) which is located in the city of Bremerton (Figure 4-1), about 15 miles west of Seattle, Washington. PSNS was established in 1891 as a naval station and then designated Navy Yard Puget Sound in 1901. The Navy Yard constructed a number of ships, submarines, tugs and small boats during World War I and during World War II was primarily involved in efforts to repair battle damage to ships of the U.S. fleet and its allies. Following the second World War, the Navy Yard was designated Puget Sound Naval Shipyard where in subsequent years the shipyard modernized aircraft carriers, activated ships and built new ships when needed. The BNC covers approximately 350 acres of land and an additional 340 acres of tidelands along 11,000 feet of shoreline. There are over 300 buildings and structures, including a steam plant, six deep water piers, six dry docks and numerous moorings. The predominant land cover within the Shipyard is rooftops, paved areas (roads, parking areas, sidewalks, and concrete working areas), and piers.

The stormwater system draining the BNC includes over 150 storm drainage systems with more than 1,000 catch basins and track drains leading to the Sinclair Inlet (Brandenberger et al. 2012). As reported by Brandenberger et al. (2012), industrial and municipal stormwater runoff contains a broad variety of pollutants whose concentrations can vary widely depending on storm event size and other local and regional factors. Stormwater runoff from non-dry dock locations within the BNC all drain directly into the adjacent receiving environment and many of these drainage locations are also tidally influenced. Discharge of stormwater from Shipyard operations is permitted by the USEPA Region 10 under the CWA (NPDES permit WA002062, 1994). Under the NPDES program, the Shipyard is required to implement BMPs designed to reduce, treat, and control discharges of contaminants from operations to the adjacent receiving environment.

Additionally, technical work under PSNS Waterfront Environmental Compliance and Project ENVVEST, starting in 2000, has been and continues to focus efforts on several tasks:

- 1) Establish a baseline for assessing continuous process improvement of Shipyard operations and other sources of contamination into Sinclair and Dyes Inlets.

- 2) Provide data for validation of proposed mixing zones and model verification. Ambient data are needed to inform the development of discharge limits and verify and validate discharge models and assess total loading of all contaminants into the Inlets.
- 3) Obtain data and information on toxicity for NPDES permit requirements for the Shipyard. Specific toxicity tests on effluents and ambient waters are needed to inform the permit process.
- 4) Develop procedures needed to meet ambient monitoring requirements for water, sediment, and biota in support of adaptive management actions.

Several other studies have taken place in or around PSNS evaluating industrial and stormwater runoff, ambient toxicity and copper bioavailability, sediment contamination, effectiveness of reactive amendments in the remediation of contaminated sediments, bacterial monitoring, propeller wash studies, and many more.

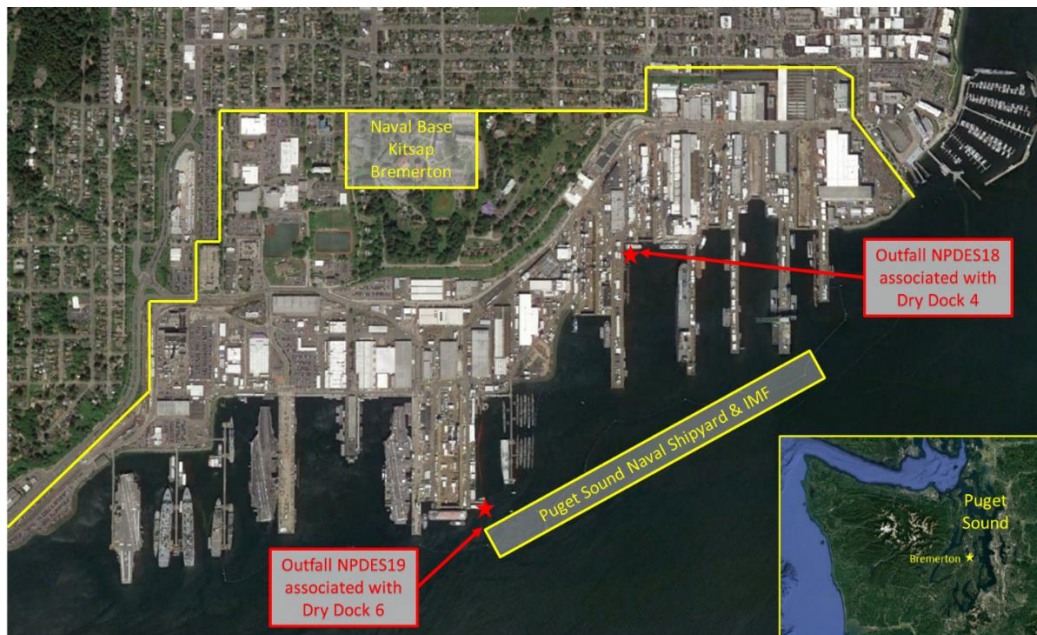


Figure 4-1. Location of the Puget Sound Naval Shipyard and Intermediate Maintenance Facility (PSNS & IMF) and Bremerton Naval Complex on the Sinclair Inlet, Washington (from ER-201131 final report). Outfalls NPDES 18 and 19 as potential sampling sites.

4.1.2 Site Geology/Hydrogeology

The most prominent fluctuations in sea level and currents in Sinclair Inlet are caused by tides. The tidal range in sea level, up to 4 m, is about one-quarter of the water depth. The tidal currents associated with these sea-level oscillations typically have an 8 cm/s amplitude that is uniform with depth; hence, large volumes of water and material suspended across the entire water column move in and out of the inlet once or twice a day. The excursion length for this exchange is about 1.7 km, based on a typical 8 cm/s tidal current and a duration of 6 hours (from slack ebb to slack flood). The net exchange caused by these large fluctuations depends, in part, on (1) the mixing within the inlet caused by the large tides, and (2) by the strength of the currents that flow past the mouth of the inlet, either from Dyes Inlet or from the Port Orchard channel (Noble et al., 2013).

4.1.3 Contaminant Distribution

To support PSNS & IMF's NPDES permit and other waterfront compliance, Project ENVVEST was established to assess the status of environmental quality in Sinclair and Dyes Inlets by conducting ambient monitoring and toxicity testing of marine and nearshore waters collected from the Inlets and from effluents from PSNS&IMF industrial outfalls. Effluents from Dry Docks 4 and 6 discharge to the Sinclair Inlet from Outfalls 18 and 19, respectively. Effluent samples and nearby ambient samples have been sampled on a near-quarterly basis for almost two decades through this monitoring program. Physical, analytical and toxicological characterization of the samples were conducted for an integrated assessment of the watershed draining into Sinclair and Dyes Inlet to develop Total Maximum Daily Loads (TMDL).

Over the past 15 years, 148 exceedances of NPDES discharge limits occurred. Two of the 148 exceedances were for temperature, while the remaining exceedances were for total recoverable copper where the average, daily max, maximum and/or monthly averages were in violation (EPA DMR Tool).

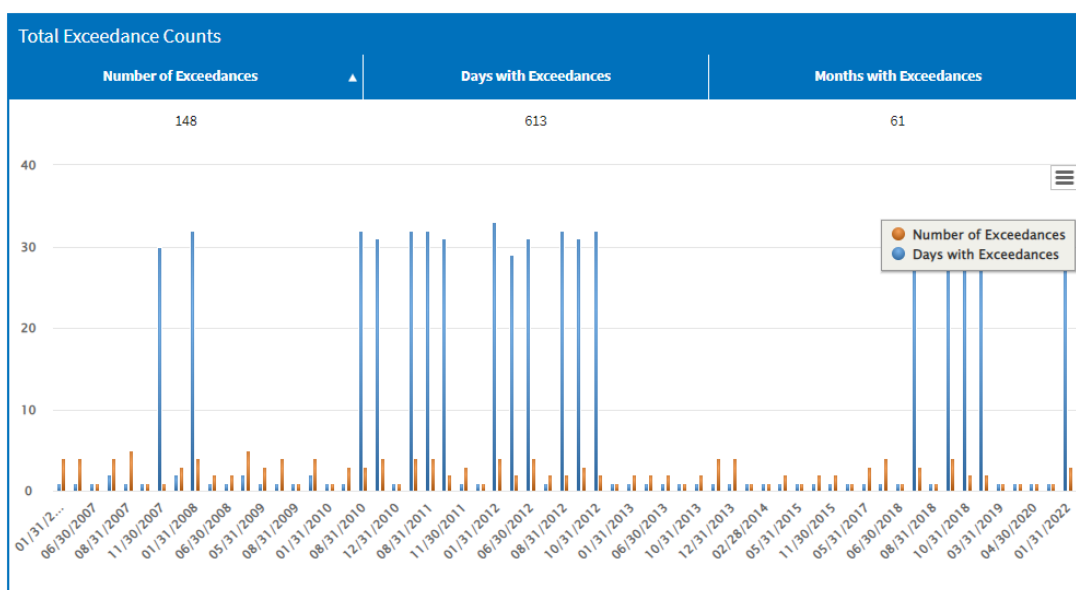


Figure 4-2. Exceedance counts for PSNS&IMF outfalls 18 and 19 (from EPA DMR Tool; accessed 3/15/2022).

Toxicity testing through Project ENVVEST has been conducted by the NIWC Pacific Bioassay lab in support of 35 separate monitoring efforts. Typically, 2-3 monitoring events occur per year at three dry dock discharges (OF18, OF19; occasionally OF96 and OF21) and 9-10 ambient seawater samples are assessed for chronic and acute toxicity (Figure 4-3). Chronic toxicity tests are conducted with purple sea urchin (*Strongylocentrotus purpuratus*) and Mediterranean mussel (*Mytilus galloprovincialis*) embryos using standardized protocols (USEPA 1995). Acute toxicity tests were conducted using the bioluminescent dinoflagellate (*Pyrocystis lunula*) and the opossum shrimp (*Americamysis bahia*) using standardized protocols (ASTM 2000; USEPA 2002, respectively).



Figure 4-3. Sampling locations at PSNS & IMF and throughout Sinclair Inlet as part of Project ENVVEST Ambient Monitoring Program.

Occasional toxicity has been observed in the dry dock discharge associated with OF 18, particularly for the chronic sublethal endpoints including embryo-larval development (e.g., sea urchin embryos) and bioluminescent dinoflagellate light reduction (QwikLite).

For the 27 monitoring events summarized to-date, rarely is there statistically/biologically meaningful adverse responses in the receiving water samples in Sinclair Inlet (Figure 4-5). The occasional toxicity observed has been associated with summer-occurring harmful algal blooms.

	Ambient Monitoring Event																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
	NPDES18																											
MysidSurv	0	5	5	0	0	0	0	0	0	0	5		0	0	0	5	0	0	5	0	16	3	0	0	0		0	
KelpGerm	0	0	6	0	3	0	0	0	0	0																		
KelpTube	1	0	0	1	0	12	8	0	0	1																		
SeaUrchin	9	1	0	0	2	0	0	4	0	0	0		0	0	0	0	9	67	94	86	87	5	4	76	6		2	
QwikLite	0	13	2	0	2	6	3	0	44	0				0	0	0	11	20	27	41	11	11	0	0			0	
MusDev		0		0	0	1	0		1	1				0							1	3		2	2		0	
MusSurv		0		0	0	4	4		3	0				0							1	4		12	6		11	
	NPDES19																											
MysidSurv	0	0			0	0	0	0	0	0	5	5	0	0	0	0	0	0	0	0	0	3	3	7	0	4	-7	
KelpGerm	0	0			0	0	0	0	0	0																		
KelpTube	0	0			0	0	0	0	0	0																		
SeaUrchin	11	0			0	14	0	15	2	0	0	58	0	1	1	0	0	1	15	0	1	0	0	0	1	4	1	
QwikLite	0	0			0	0	0	0	0	0				0	0	0	37	0	0	0	16	25	0	25		0	0	
MusDev		1			0	74	1		0	0		1		0			8			0	1		0	0	0		2	
MusSurv		6			4	71	0		3	0		0		2			8			6	0		10	0		6		
	NPDES21																											
MysidSurv	0		0	5	0	0	0	15	0	5	0	0	0	0	0	5	0	10	10	0	11	7	3	7	0	0	0	
KelpGerm	1	0	4	7	0	3	0	0	0	0							0	0	0	0	0	0	0					
KelpTube	0	4	0	5	0	0	0	0	0	0							0	0	0	0	0	0	0					
SeaUrchin	12	2	0	0	1	2	0	0	0	0	1	0	0	0	0	0	0	2	0	0	1	0	0	0	1	0	0	
QwikLite	0	16	0	6	0	0	0	0	4	0	0			0	0	11	26	4	2	1	11	0	1	0		18	0	
MusDev		13			0	0	98			0	1		0		0			1			0	1		1	0		4	
MusSurv		19			0	3	98	0		0	9		0		5			3			0	2		12	5		0	

Figure 4-4. Percent difference from the control (tested concurrently) for effluent samples OF18, OF19 and OF21 at PSNS & IMF.

		Ambient Monitoring Event																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
		PS01																										
MysidSurv	11	0	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	28	0	0	0	0	0	0	0
KelpGerm	25	2	2	0	6	0	0	0	0	0	2																	
KelpTube	47	13	3	0	20	0	0	0	10	4	3																	
SeaUrchin	54	0	0	0	0	0	0	0	0	0	0	2	0	0	1	30	0	0	0	0	0	0	1	0	0	0	0	100
QwikLite	25	0	2	8	0	0	3	15	0	11					0	6	0	0	0	0	0	4	0	9	0		0	0
MusDev		0	0		0	1	0		3	1				0	0				0			0	3	0	0	4	0	0
MusSurv		0			6	9	3	0		1	0			0	1				10			7	0		10	0		8
		PS03																										
MysidSurv	0	0	0	0	0	0	9	0	0	0	0	10	0	0	0	5	0	0	5	0	6	3	0	0	0	0	0	-3
KelpGerm	0	9	2	1	3	0	1	0	0	0																		
KelpTube	12	15	0	0	13	5	0	8	0	0																		
SeaUrchin	20	0	1	0	0	0	0	0	1	0	0	0	0	0	3	0	0	0	0	0	2	1	0	0	0	0	0	97
QwikLite	0	0	14	0	0	14	19	13	18	7					12	2	15	0	0	1	0	4	48	12	0		8	0
MusDev		0	0	0	0	2	0		0	0				0	0				0		0	1		0	0	0	0	0
MusSurv		0		1	9	2	0		0	5				0	2				6		6	0		6	0	0	0	8
		PS08																										
MysidSurv	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	-3
KelpGerm	0	0	0	1	3	1	14	0	0	0																		
KelpTube	1	8	8	0	5	2	0	12	0	2																		
SeaUrchin	1	1	0	0	0	0	0	0	0	0	5	0	0	0	7	0	0	0	4	2	1	1	0	0	0	0	0	0
QwikLite	0	0	8	0	0	0	0	8	0	0				5	9	9	0	0	0	4	0	4	16	0	0		21	0
MusDev		0	0	0	0	1	0		3	0				0	0				0			0	1		0	2	0	0
MusSurv		0		0	0	3	0		0	5				0	0				7			0	2		4	0		7
		Ambient Monitoring Event																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
		PS09																										
MysidSurv	0	11	0	0	0	0		5	0	0	0			0	0	0	10	0	0	5	0	0	0	0	0	0	0	0
KelpGerm	0	22	0	1	4	0		0	0	2																		
KelpTube	11	15	0	0	13	7		3	0	1																		
SeaUrchin	3	0	0	0	1	0		0	0	0	0		0	1	6	0	0	1	1	1	3	2	0	0	0	0	0	0
QwikLite	0	0	33	10	7	5		13	0					18	0	0	0	0	0	14	27	15	0	0	0	0	0	0
MusDev		0		0	2	2		0	0					0				0			0	2		0	2	0	0	0
MusSurv		1		0	6	5		0	6					0				7			10	0		3	0		5	
		PS14																										
MysidSurv	0	5	0	0	0	0	11	0	0	0	0	5	0	0	0	10	0	5	0	0	50		0	0	0			
KelpGerm	0	0	0	0	0	0	15	0	0	3																		
KelpTube	4	0	3	1	5	9	0	7	0	1																		
SeaUrchin	26	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	8		0	0	0			
QwikLite	0	0	8	14	0	4	18	0	18	0				7	0	0	0	0	0	18	27		0	0				
MusDev		0	0	0	17	0		4	1				0		0			0						0	2			
MusSurv		0		0	0	1	1		1	0			0		0			1			3			0	6			
		PS15																										
MysidSurv	0	5	5	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	5	5	0	6		0	4	0	0	0
KelpGerm	0	0	0	0	1	0	0	0	0	0																		
KelpTube	32	7	25	0	8	0	0	11	0	3																		
SeaUrchin	38	0	0	2	0	0	0	0	0	0	0	3	0	0	4	1	0	2	1	0	1		0	0	2	0	0	0
QwikLite	15	0	0	8	0	0	20	0	12	0				9	0	0	11	0	0	0	27		0	0		26	0	0
MusDev		0	4	0	0	0		6	1				0		0			0	0					0	2		4	
MusSurv		0		0	7	0	0		19	5		10		0				0	0		0			0	10		12	
		Ambient Monitoring Event																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
		M3.1																										
MysidSurv	0	0	5	5	0	0	0	0	5	0	0	0	0	0	5	0	0	0	10	5	0	0	0	0	0	10	0	0
KelpGerm	0	9	1	0	1	0	0	2	2	0																		
KelpTube	24	15	0	0	11	3	8	10	0	1																		
SeaUrchin	4	1	4	2	0	0	0	0	0	0	0	1	1	0	2	0	0	9	1	0	0	1	0	0	1	0	0	0
QwikLite	0	0	39	27	0	0	13	0	22	10				0	0	0	0	0	0	19	0	0	0	0	0	0	0	0
MusDev		0	3	0	0	0		2	2				0	1				0						0	0	0	0	0
MusSurv		0		3	7	0	11		0	9		11		0				0			11	2		0	8		5	
		M4																										
MysidSurv		0	0	5	0	0	0	0	5	0	0	0	0	0	0	0	0	5	0	0	28	0		0	0	4	79	
KelpGerm		2	4	1	8	0	0	0	1	0																		
KelpTube		0	29	29	13	0	4	14	2	0																		
SeaUrchin		0	0	2	0	0	0	0	0	0	0	0	2	0	3	0	0	0	1	0	1	1		100	1	0	100	
QwikLite			30	8	10	4	19	7	11	15				13	0	0	0	0	0	22	0	0		0	0		0	0
MusDev		0		2	0	1	0		7	1			1	1				0				0	0		47	1	100	100
MusSurv		5		2	9	1	3		0	7		10		0				0			17	0		38	10		100	100
		BJEST																										
MysidSurv		0	0	5	0	0	0	0	5	5	0	0	5	10	0	0	0	0	0	0	0	39	7		4	5	0	14
KelpGerm		0	0	0	2	0	0	4	0	0																		
KelpTube		0	8	0	12	0	1	8	0	8																		
SeaUrchin		0	0	0	0	0	0	2	0	0	0	1	0	0	6	0	0	0	1	0		6	0		2	0	0	0
QwikLite		0	10	8	4	0	25	4	27	3				0	0	0	0	0	8	18	0	0		0	0		9	0
MusDev		0		1	0	0	0		6	1			0	1								4	0		0	0		0
MusSurv		0		0	7	1	6		6	8		4	0	0				0				13	0		7	12		14

4.2 NAVY REGION SOUTHWEST

Three Navy installations located in the Metro Region of San Diego: Naval Base San Diego (NBSD), Naval Base Point Loma (NBPL) and Naval Base Coronado (NBC), were selected as the sites to demonstrate and validate the pulsed exposure methodology to ensure that it is indeed representative and protective of actual site conditions during dynamic runoff events. Toxicity and chemistry of wet weather runoff and industrial discharges from outfalls and the receiving environment have been routinely measured for compliance associated with NPDES permits (NBSD: Permit No. CA0109169; Order No. No. R9-2013-0064; NBPL: Permit No. CA0109363; Order No. No. R9-2015-0037; NBC: Permit No. CA0109185; Order No. No. R9-2015-0117).

These locations fit all the site selection criteria as described in Table 4-1. Numerous discharges at these installations have a history of stormwater toxicity and numerical criteria exceedances (Katz et al. 2006) allowing for the pulsed exposure methodology to demonstrate the differences in toxicity effects relative to standard exposure methods. The location of the San Diego Metro installations and associated discharges allowed for ease of access in a timely fashion to capture stormwater related samples as well as for *in situ* deployments (SEA Rings). Concurrent stormwater sampling efforts conducted under the NPDES permits allowed for leveraging and cost sharing for both toxicity and analytical chemistry characterization.

4.2.1 Site Location and History

NBSD and adjacent Naval Installations (Figure 4-6) are the principal homeport to the Pacific Fleet, consisting of Naval, Coast Guard, Military Sealift Command logistical support platforms and several research and auxiliary vessels. NBSD is comprised of over 1,000 acres of land, about 90% of which is made up of impervious surfaces. The area west of Harbor Drive (or wet side) of NBSD consists of the Bay front area west of Harbor Drive. The area east of Harbor Drive (or the dry side) consists of the community facilities complex. The shoreline along the west side of NBSD is intensively developed and supports waterfront operations, ship berthing and maintenance, station maintenance, training, administration, and logistics functions. Operational facilities include piers, quay walls, small craft berthing facilities, fueling facilities, armories, and waterfront operations buildings. NBSD contains 13 berthing piers, a mole pier, two channels, and various quay walls that have a total shoreline measurement of approximately 5.6 miles.

Numerous studies have been performed in the sediments and waters around NBSD over the years including stormwater assessments and impacts in the receiving environment of San Diego Bay (i.e., SERDP/ESTCP projects ER-201130, ER-201432, ER-2428; Katz et al. 2006). Additionally, the Regional Water Quality Control Board (RWQCB) requires the Navy to monitor numerous outfalls for toxicity and/or analytical chemistry from each facility during storm events. Therefore, as part of the validation and demonstration of the pulsed exposure methodology, concurrent laboratory-based toxicity testing using both standard and pulsed exposures as well as *in situ* testing was conducted at several of the stormwater discharges already under a monitoring schedule.

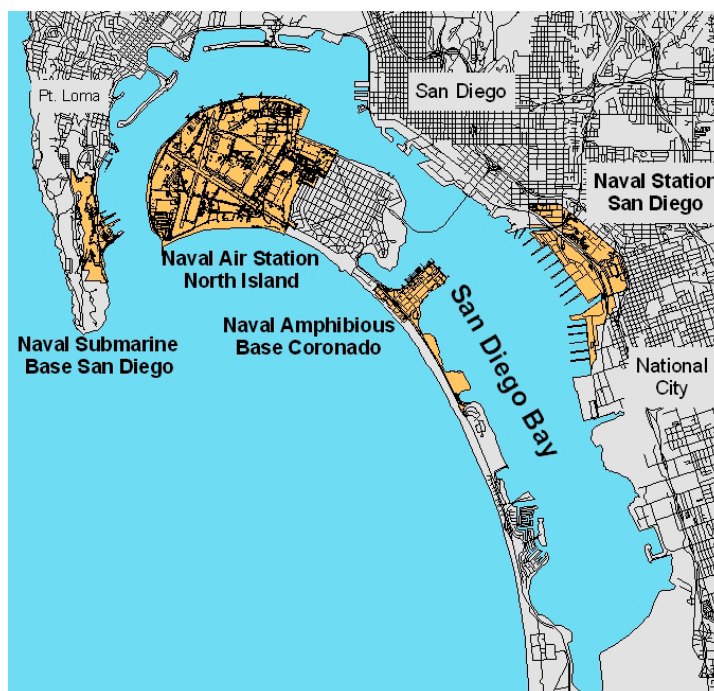


Figure 4-6. Navy Bases bordering San Diego Bay including Naval Station/Base San Diego, Naval Amphibious Base Coronado, Naval Air Station North Island and Naval Submarine Base San Diego/Point Loma (From Katz et al. 2006).

4.2.2 Site Geology/Hydrogeology

San Diego Bay is approximately 15 miles long and varies from 0.2 to 3.6 miles in width. At MLLW, the Bay is approximately 11,000 acres in area (Wang et al. 1998). The Bay has depths ranging from less than 1m to about 18m. Average depths are 6.5m measured from mean sea level (Wang et al. 1998).

Currents within the bay are dominated by mixed diurnal-semi-diurnal tidal exchange (Peeling 1975) with a tidal range averaging approximately 1.7m with occasional extreme tidal amplitudes close to 3m. The twice daily tidal exchange results in a constant replenishment of the bay with ocean water with a tidal prism volume of ~14.5 billion gallons. Tidal exchange and mixing in the bay lead to residence times of between 0 days at the mouth to ~40 days at the innermost region of the bay (Chadwick and Largier 1999a).

Freshwater contributions to the Bay are primarily from the Otay and Sweetwater Rivers, and Chollas Creek, Switzer Creek, and Paleta Creek. The drainage watershed feeding these inputs to the Bay is nearly 283,000 acres. Additional freshwater inputs originate from surface runoff from urban areas through the storm drainage system. The average annual mass load of freshwater entering the Bay is estimated to be between 1 and 4 billion gallons over the typical wet weather season, from October through March (Tetra Tech 2008). However, for about 9 months of the year, the Bay receives no significant amount of fresh water. Annual rainfall averages about 10.5". The lack of rainfall during summer months can result in hypersaline conditions in the shallower south parts of the Bay (Chadwick and Largier 1999b). The summer conditions can lead to vertical and longitudinal gradients in temperature and salinity toward the shallower south Bay. Gradients typically break down in winter months and result in relatively well-mixed bay waters. Mixing, fate and transport in the Bay

have been reasonably well-characterized and modeled for San Diego Bay (Chadwick et al. 2008a, 2008b; Chadwick and Largier 1999 a,b; Largier et al. 1995; Wang et al. 1998, 2007, 2008, 2012, 2016).

4.2.3 Contaminant Distribution

Many areas of San Diego Bay have been listed as impaired water bodies under the Clean Water Act (CWA) 303(d) List by the State of California Water Resources Control Board (SWRCB). The 2014/2016 integrated report identified causal pollutants to be organic compounds (i.e., PCBs, etc.), bacteria, heavy metals, and pesticides. Sources of pollutants potentially include surface runoff from urban watersheds, industrial facilities, vessel activities from Navy operations, recreational and commercial ports, aerial deposition, storm drains, hazardous material and sewage spills, among others. With the long history of industrial, marina, and military use of the Bay, "legacy" pollutants continue to remain from past practices despite curtailment of new discharges (U.S. Navy and Port of San Diego, 2011). Urban runoff is now considered to be the largest source of pollutants in the region.

Historical measurements show that storm water entering the Bay from Navy outfalls impact a relatively small area within the piers, primarily in the top few meters of the water column. These discharges are mixed relatively quickly, returning to pre-storm conditions within 12 to 24 hours of the discharge (Katz et al. 2006). The limited spatial and temporal extent of these discharges led the Navy to conclude that mixing and low exposure conditions in bay waters was the reason that there was virtually no measured toxicity in ambient waters during storm flows.

Water quality standards (WQS) are generally met throughout the Bay though there are no long-term bay-wide datasets to evaluate. There are some limited exceptions to this including observations of elevated bacteria along three shoreline locations and elevated copper in multiple marinas throughout the bay. These impairments were identified under the State's 2006 total maximum daily load (TMDL) program. Bay waters monitored for storm impacts during and after storm events adjacent to Navy facilities showed limited exceedances of copper and zinc water quality criteria though no exceedances of other commonly measured metal and organic contaminants (Katz et al. 2006). Using Toxicity Identification Evaluation (TIE) procedures, copper and zinc were found to cause acute toxicity to the mysid shrimp *Americamysis bahia* in end-of-pipe stormwater samples (Katz et al. 2006).

The most recent bay-wide analysis of sediment quality was generated by the Bight Regional Monitoring Program in 2018 (Port of San Diego, 2021). The focus of the program for San Diego Bay was to characterize sediment quality using the California Sediment Quality Objectives (SQO) approach. Trends observed from these monitoring efforts every five years are showing that the San Diego Bay sediments are relatively stable and in good condition. For the 2018 assessment, the study found that port and marina sites were improving their condition with 60 and 46% in good condition (unimpacted or likely unimpacted), respectively. This was an increase from the 2013 assessment where 48 and 42% of port and marina sites were unimpacted or likely unimpacted. For freshwater-influenced sites, 75% were in good condition in 2013 and 72% were in good condition in 2018. In 2013, 95% of the open bay deep sites were in good condition and in 2018, 100% of the sites were considered in good condition.

Concentration distribution plots for copper in San Diego Bay as measured by the San Diego Regional Monitoring Program as part of Bight '18 are shown below in Figure 4-7 and Figure 4-8. Final sediment quality objective scores for San Diego Bay that integrate results for chemistry, toxicity, and benthic community for the 2013 and 2018 monitoring years are shown in Figure 4-9 and Figure 4-10, respectively.



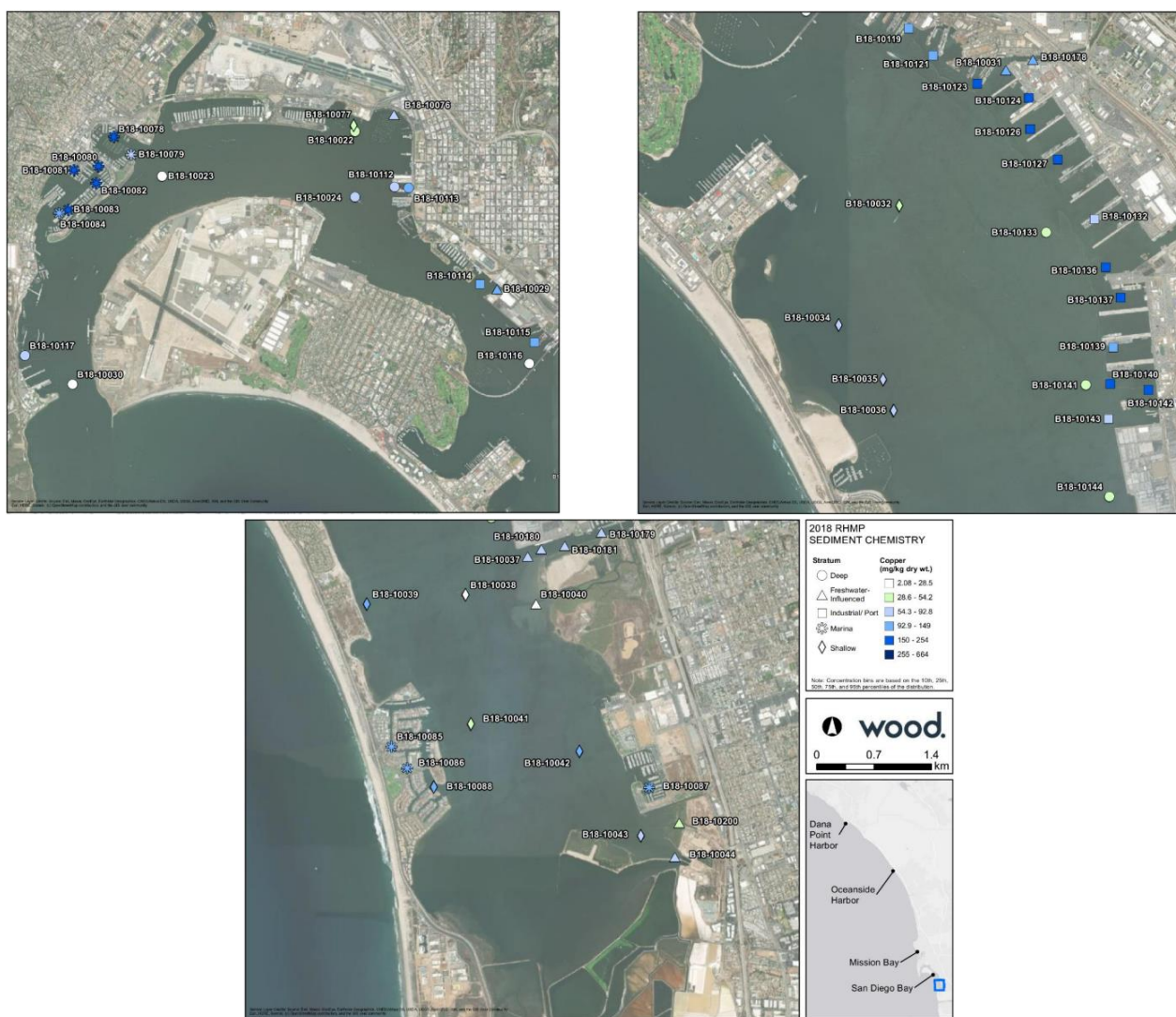


Figure 4-8. Distribution of copper in sediments in North San Diego Bay (upper left), Central San Diego Bay (upper right) and South San Diego Bay (bottom) during the 2018 Regional Harbor Monitoring Program (Port of San Diego, 2021).



Figure 4-9. Integrated SQO scores derived for San Diego Bay during the 2013 Regional Harbor Monitoring Program (Port of San Diego, 2021).



Figure 4-10. Integrated SGO scores derived for San Diego Bay during the 2018 Regional Harbor Monitoring Program (Port of San Diego, 2021).

4.3 NAVAL SUBMARINE BASE NEW LONDON

Naval Submarine Base New London (NSBNL) was selected as an additional East Coast site for the demonstration of the pulsed exposure methodology. This location fit all the site selection criteria as described in Table 4-1. As an additional benefit, this is a freshwater discharge site, whereas the other demonstration sites for this project are marine. Currently, NSBNL holds a MS4 General Permit (GSM 000117) as well additional permit coverage for stormwater discharges associated with industrial activities (No. GSI 000679), and several outfall discharges are monitored during storm events for compliance purposes. Several of these outfalls have exceeded numerical benchmarks over the recent years for chemistry and toxicity endpoints. The pulsed toxicity exposure methodology would help confirm the applicability of site-specific objectives while also providing a more realistic exposure assessment to demonstrate differences in potential adverse effects.

4.3.1 Site Location and History

NSBNL is a base command for submarine activities in the Atlantic Ocean. The base also accommodates training facilities, housing, offices, medical facilities and facilities for submarine servicing. The submarine base is approximately 687 acres of property located on the eastern bank of the Thames River in the Towns of Groton and Ledyard, Connecticut, approximately six miles north of Long Island sound (Figure 4-11).

NSBNL is divided by the Providence and Worcester Railroad tracks into “Upper Base” and “Lower Base”, with the Lower Base consisting of the area west of the tracks down to the Thames River waterfront (U.S. Navy, 2017). As reported in the Stormwater Management Plan (U.S. Navy, 2017), the Upper and Lower Base activities consist of the following:

Upper Base:

- | | | |
|--|--|--|
| • Facilities providing personnel support and administrative services | • Bulky Waste Transfer Facility (BWTF) | • Recreational activities |
| • Maintenance and repair activities | • Defense Logistics Agency (DLA) Disposition Services facility | • Various parking, loading and storage areas associated with activities within proximate buildings |
| • Submarine Learning Center | • Hazardous Waste HW Treatment, Storage and Disposal Facility (TSDF) | • Medical research facilities |
| • Base maintenance activities | • Naval Branch Health Clinic | |
| • Weapons storage facilities | | |

Lower Base:

- | | | |
|--|--|--|
| • Facilities dedicated to submarine support, maintenance, and repair | • SUBASENLON Fire Department (SBFD) | • Oily waste treatment facility |
| | • Steam and power plant (auxiliary power only) | • Morale, Welfare, and Recreation (MWR) Marina |
| | | • Berthing piers |

- Medium Auxiliary Repair Dry Dock (ARDM-4)
- Maintenance and repair activities and small craft dockage
- Various parking, loading, and storage areas associated with activities within proximate buildings

Stormwater runoff from NSBNL drains to the west towards the river via steams and the stormwater drainage system (U.S. Navy, 2017). The specific outfalls at NSBNL that are targeted for sample collections during the 2018-2019 wet season are shown in Figure 4-12 .

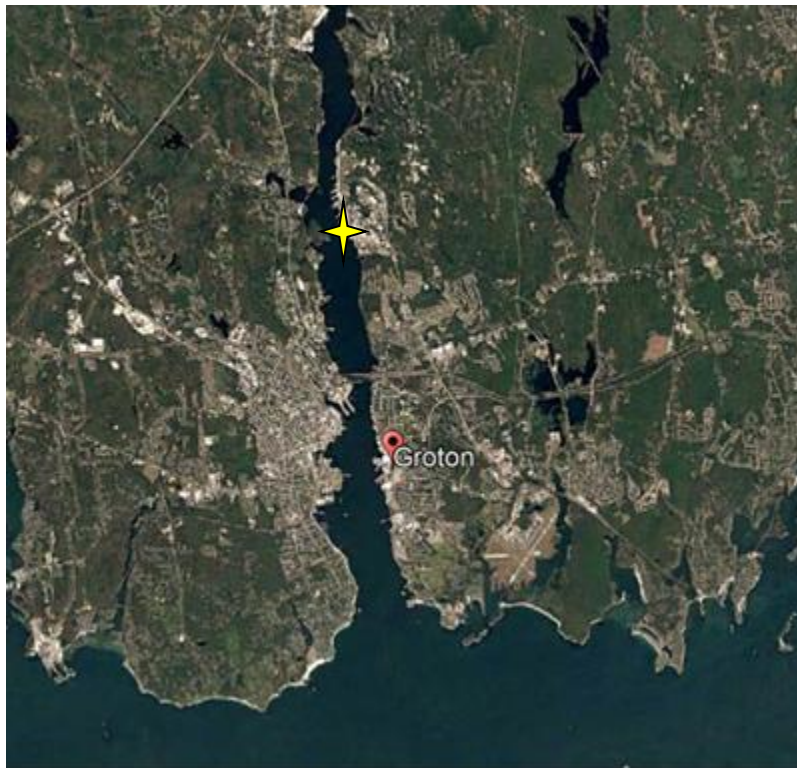


Figure 4-11. Groton, CT with star indicating location of Naval Submarine Base New London (NSBNL; Google Earth).

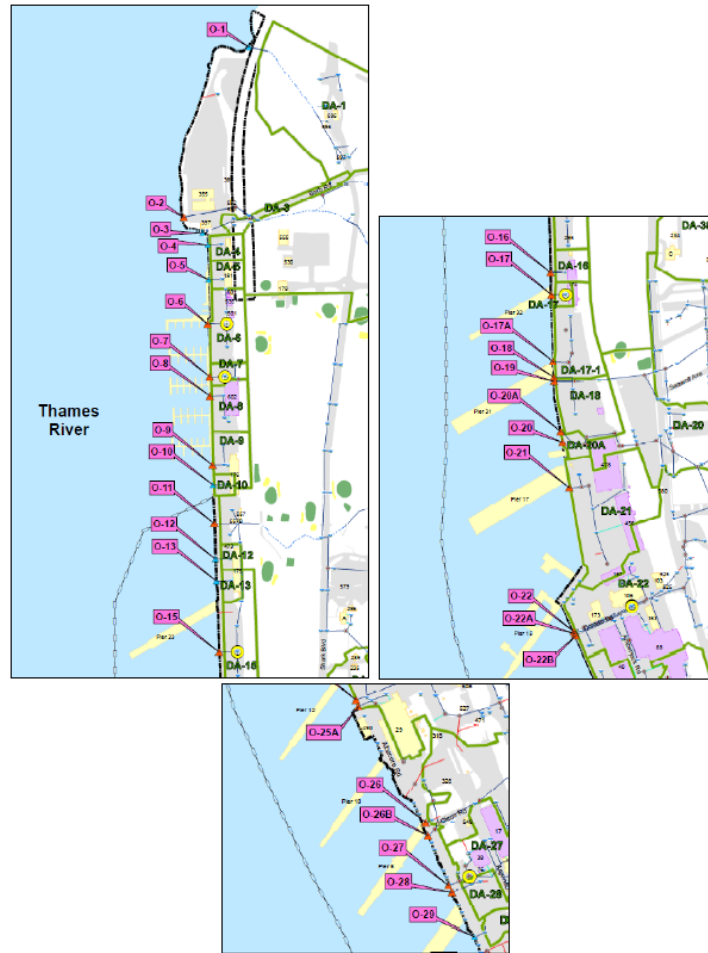


Figure 4-12. Stormwater Sampling Locations at NSBNL.

4.3.2 Site Geology/Hydrogeology

NSBNL is located in the eastern bank of the Thames River. The Thames River watershed drains approximately 1,500 square miles of eastern Connecticut, western Rhode Island and south-central Massachusetts. The Thames River adjacent to the NSBNL has a dredge channel to approximately 40 feet below mean sea level between the base and Long Island Sound. The channel of the river is approximately 600 to 900 feet wide and narrows upstream and becomes shallower upstream from the Base. Outside of the dredged channel, depths vary from two to 10 feet. The Thames River is a fresh/salt water stratified estuary that does not receive wind-induced mixing due to steep banks and narrow channel, leading to a well-defined freshwater outflow at the surface of the river as it empties to the Sound approximately six miles downstream (U.S. Navy, 2016).

Surface water flow on Base mainly consists of perennial and intermittent streams, ponds, a couple lakes, a small wetland as well as the stormwater drainage system. Groundwater at the site is influenced by the tidal exchange observed in the Thames River with increased groundwater flow to the River during low tides that create a greater elevation difference. During high tides, there can be localized reversed flow into the groundwater from the river due to the change in the relative elevation of the River (U.S. Navy, 2016). The Thames River is designated as impaired for bacteria in the vicinity of the Base.

4.3.3 Contaminant Distribution

The Thames River is the receiving water body for numerous outfalls on the NSBNL. The river is currently classified as an impaired body of water for bacteria and as a result, wet weather screening of stormwater outfalls on the Base must be conducted (U.S. Navy, 2017). Analyses for fecal coliform and Enterococci must be conducted during wet weather for each outfall throughout the Base. In addition, per the Industrial Stormwater General Permit, semi-annual stormwater monitoring must be conducted on a semi-annual basis with the analyses of the following parameters with associated benchmarks shown:

- Chemical Oxygen Demand (mg/l) - 75
- Total Oil and Grease (mg/l) - 5
- pH (S.U.) – 5 to 9
- Total Suspended Solids (mg/l) – 90
- Total Phosphorus (mg/l) – 0.40

- Total Kjeldahl Nitrogen (mg/l) – 2.30
- Nitrate as Nitrogen (mg/l) – 1.10
- Total Copper (mg/l) – 0.059
- Total Lead (mg/l) – 0.076
- Total Zinc (mg/l) – 0.160

Benchmarks shown above, except for metals, are based on the 80th percentile of the cumulative relative frequency graphs that were developed from stormwater results reported under the General Permit for the Discharge of Stormwater Associated with Industrial Activity for the sampling years 2003 to 2007. Benchmarks for metals are based on state Water Quality Standards. Following the collection of four semiannual stormwater samples at a given outfall, if the average value for a given parameter is below the benchmark value, monitoring requirements for that outfall have been fulfilled for the permit term and further monitoring is no longer required. For data exceeding benchmarks, the Base must review control measures (i.e., BMPs) and continue monitoring as required by the permit.

Additionally, toxicity testing using neonate water fleas (*Daphnia pulex*) must be conducted during the first two years of the permit cycle.

For the outfalls that were targeted by this Pulsed Exposure Study, during the previous permit cycle 44% of the sampling events showed dissolved copper concentrations exceeding the benchmark concentration of 59µg/L (Figure 4-13). For dissolved zinc, 51% of the samples exceeded the benchmark concentration of 160µg/L for the monitoring events conducted (Figure 4-14).

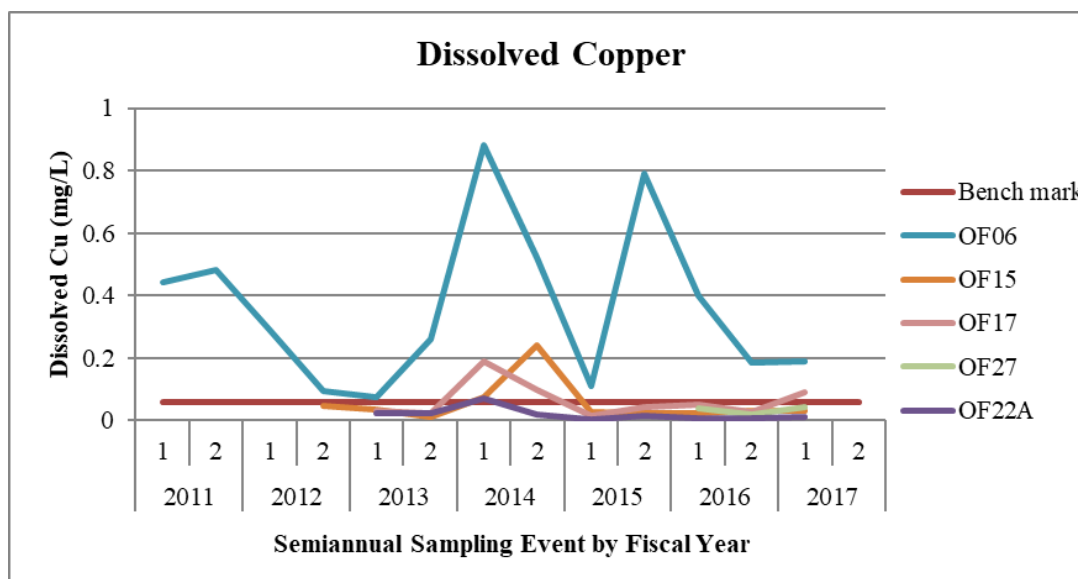


Figure 4-13. Dissolved copper concentrations at selected stormwater outfalls at NSBNL over the previous General Permit Cycle (2012-2017).

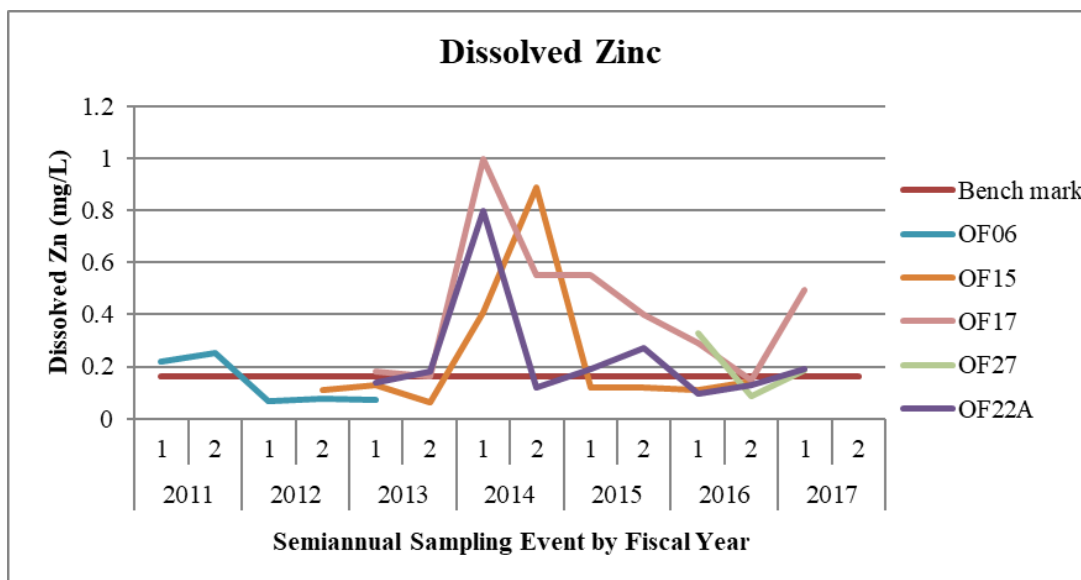


Figure 4-14. Dissolved zinc concentrations at selected stormwater outfalls at NSBNL over the previous General Permit Cycle (2012-2017).

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5. TEST DESIGN

This section provides the detailed description of the experimental design, sampling, and analytical methods to be used to evaluate the utility of the Pulsed Exposure methodology.

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

5.1.1 Refinement of Pulsed Exposure Methodology

Laboratory based toxicity exposures with relevant contaminants of concern (COCs) spiked in clean water were evaluated during this demonstration. Multiple marine and freshwater species that cover a range of acute and chronic endpoints (US EPA 1995, 2002a, b, c) required under various NPDES Permits were evaluated. Concurrently, pulsed exposure testing included modifications to the standard continuous methods by transfer of test organisms from the spiked solutions into either laboratory dilution water or receiving water. An example of the testing regime is shown in Figure 5-1. This approach involves multiple pulse time points in comparison with concurrently tested static or static renewal status quo exposures.

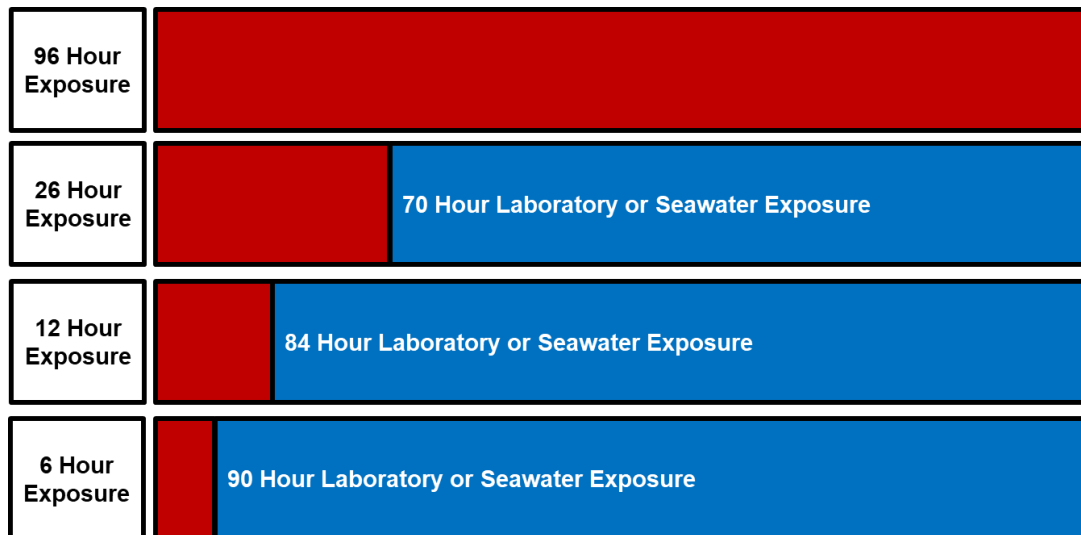


Figure 5-1. Pulsed exposure design for laboratory toxicity testing. Red coloration indicates exposure to spiked solutions or effluent samples (i.e., stormwater) and subsequent transfer into laboratory dilution water or receiving water (blue coloration).

5.1.2 Validation of Pulsed Exposure Methodology

A variety of samples were collected from multiple Naval Installations for the validation of the pulsed exposure method using both end-of-pipe monitoring as well as concurrent *in situ* toxicity testing. Sites included: Puget Sound Naval Shipyard (end-of-pipe only), Naval Submarine Base New London (NSBNL) and San Diego Region Naval Bases – San Diego, Point Loma and Coronado (end-of-pipe, receiving water, and *in situ*).

Stormwater and/or episodic industrial discharge samples from DoD installations were evaluated using both standard and pulsed exposure laboratory methods to ensure representativeness and protectiveness of actual site conditions during dynamic runoff events. Additionally, *in situ* toxicity testing in adjacent receiving waters using techniques demonstrated previously (e.g., SEA Ring under ESTCP #ER-201130; Rosen et al. 2012, Rosen et al. 2017) were conducted concurrently with

laboratory studies to link the toxicity measured at the end-of-pipe with toxicity potential in the receiving environment. The *in situ* testing occurred at multiple Navy Installations locations in San Diego. SEA Rings housed the same species selected for the laboratory exposures and also were fitted with Diffusive Gradients in Thin-Film passive sampling devices (DGTs, Figure 5-2) and water quality loggers (e.g., salinity, temperature, dissolved oxygen) to characterize labile metal and water quality conditions in the receiving water, respectively, to assess potential confounding factors. DGTs were mounted inside as well as outside organism chambers on board the SEA Rings for deployment as they have been shown to be useful in providing an integrated measure of labile metal concentration over the course of full storm events and correlated well with stormwater-relevant dissolved metal concentrations derived from much more labor-intensive composite sampling (Warnken et al. 2005; Rosen et al. 2017). DGTs were deployed for the entire deployment of 96 h and then underwent an acid-extraction of the resin layer followed by metal analysis of the extract via ICP-MS.

Water quality sondes and HOBO loggers were attached to SEA Rings to measure continuous real-time water quality conditions that the organisms were exposed to such as salinity, DO and temperature. These sondes can verify that parameters within test chambers remain within organism tolerance ranges and can characterize the ambient conditions observed during the storm event. Additional parameters that were measured with water quality meters include depth, conductivity, pH and turbidity.

SEA Rings were placed in front of selected outfalls within the top 1 m of the water column to capture the most conservative mixing conditions relative to outfall discharges. Additional SEA Rings were placed at 3m depth and at the sediment surface beneath the outfall to provide a spatial/depth gradient to assess any trends that may or may not be related to the discharge should effects be observed. A distant “reference” location was selected at the NIWC Pacific Pier 169 in San Diego Bay away from point source discharges for comparison purposes.



Figure 5-2. Example of a DGT (Diffusive Gradients in Thin Films) device used to measure labile metals *in situ*.

5.2 BASELINE CHARACTERIZATION ACTIVITIES

For all species and reference toxicants evaluated, standard exposure duration dose-response data was generated. These tests were conducted concurrently with all pulsed exposure tests to allow for direct comparison of results. This allowed for the evaluation of the median effective concentration (EC₅₀) value or median lethal concentration (LC₅₀) within two standard deviations of the internal historical mean, indicating sensitivity to the reference toxicant was consistent with that historically observed for the organism and for methods used in the laboratory.

5.2.1 Selection of Pulsed Exposure Duration

Current data on discharge duration from outfalls across the U.S. is limited, though there are some examples available as shown in the following presentation for Naval Base San Diego (NBSD). It was determined that rainfall duration can be used as a proxy for discharge duration in areas where most of the drainage basin feeding the discharge consists of impervious surfaces, a common characteristic at most industrial sites. As shown in Figure 5-3, accumulated discharge from three outfalls were measured from NBSD during a given storm event over a 14 h period. The three discharges all show different accumulation profiles. Figure 5-4 through Figure 5-6 show each discharge plotted along with the accumulated rainfall monitored during the same period. All three discharges exhibit high correlations (standard linear regression) between the accumulated rainfall and the accumulated discharge (OF72: $r^2 = 0.9917$; OF73: $r^2 = 0.9943$; OF124: $r^2 = 0.9939$). This assessment produces an example indicating the appropriateness of using rainfall duration as a proxy for discharge duration. Figure 5-3 shows how runoff patterns for three different outfalls at NBSD are consistent despite varying volumes due to catch basin. Figure 5-4 through Figure 5-6 show how accumulated rainfall closely matches runoff volume for these same industrial locations.

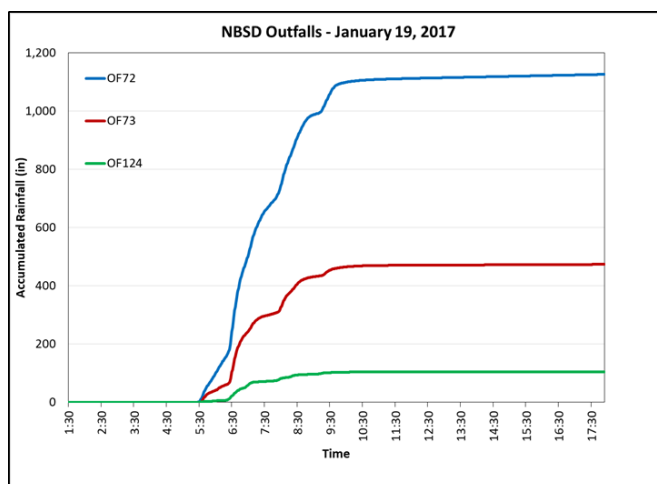


Figure 5-3. Accumulated stormwater discharges as measured from three outfalls from NBSD on 19 Jan 2017. OF = Outfall.

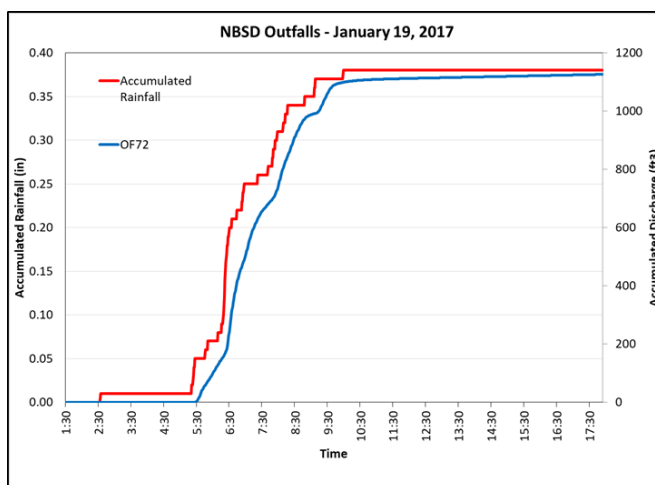


Figure 5-4. Accumulated rainfall and stormwater discharge from Outfall 72 at NBSDs on 19 Jan 2017. OF = Outfall.

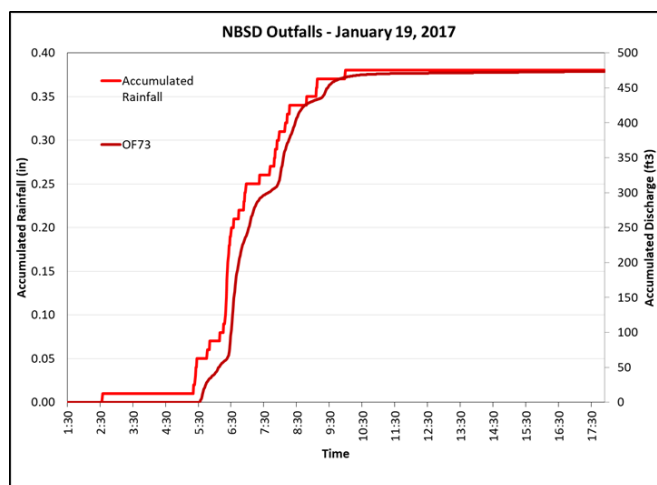


Figure 5-5. Accumulated rainfall and stormwater discharge from Outfall 73 at NBSDs on 19 Jan 2017. OF = Outfall.

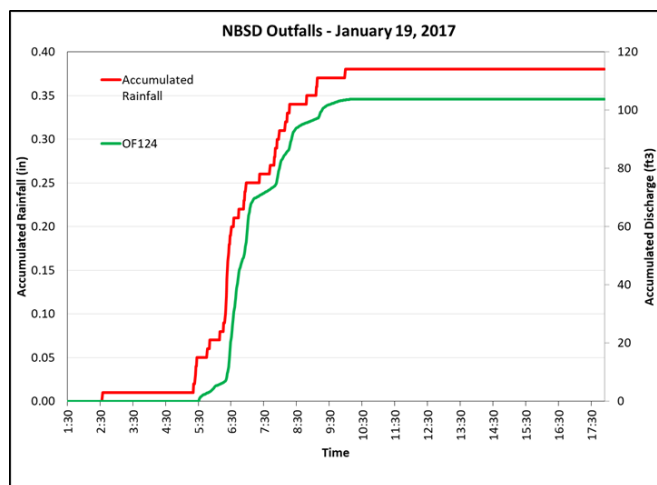


Figure 5-6. Accumulated rainfall and stormwater discharge from Outfall 124 at NBSDs on 19 Jan 2017. OF = Outfall.

Digital data sets covering up to 20 years were downloaded from the National Climatic Data Center (NCDC) hosted by NOAA (NOAA 2017) for the locations shown in Table 5-1 and graphically in Figure 5-7. Locations were categorized by climatic region as defined by NOAA (Karl and Koss 1984).

Table 5-1. Locations and respective regions selected for rainfall duration analysis.

Region	City, State
Northeast	Boston, MA
Northwest	Seattle, WA
Southeast	Charleston, SC
	Jacksonville, FL
	Norfolk, VA
	Pensacola, FL*
Southwest	Tucson, AZ
West	San Diego, CA
	San Francisco, CA
	Paso Robles, CA*
Central	Chicago, IL
	Louisville, KY
South	Corpus Christi, TX
	New Orleans, LA
Pacific	Honolulu, HI
	Piti, Guam*
East North Central	Detroit, MI
	St. Paul, MN
West North Central	Rapid City, SD
	Great Falls, MT
Other	Anchorage, AK

- *All locations had 20yr of data, exceptions: Paso Robles, CA – 11yr; Pensacola, FL – 15yr; Piti, Guam – 2yr.

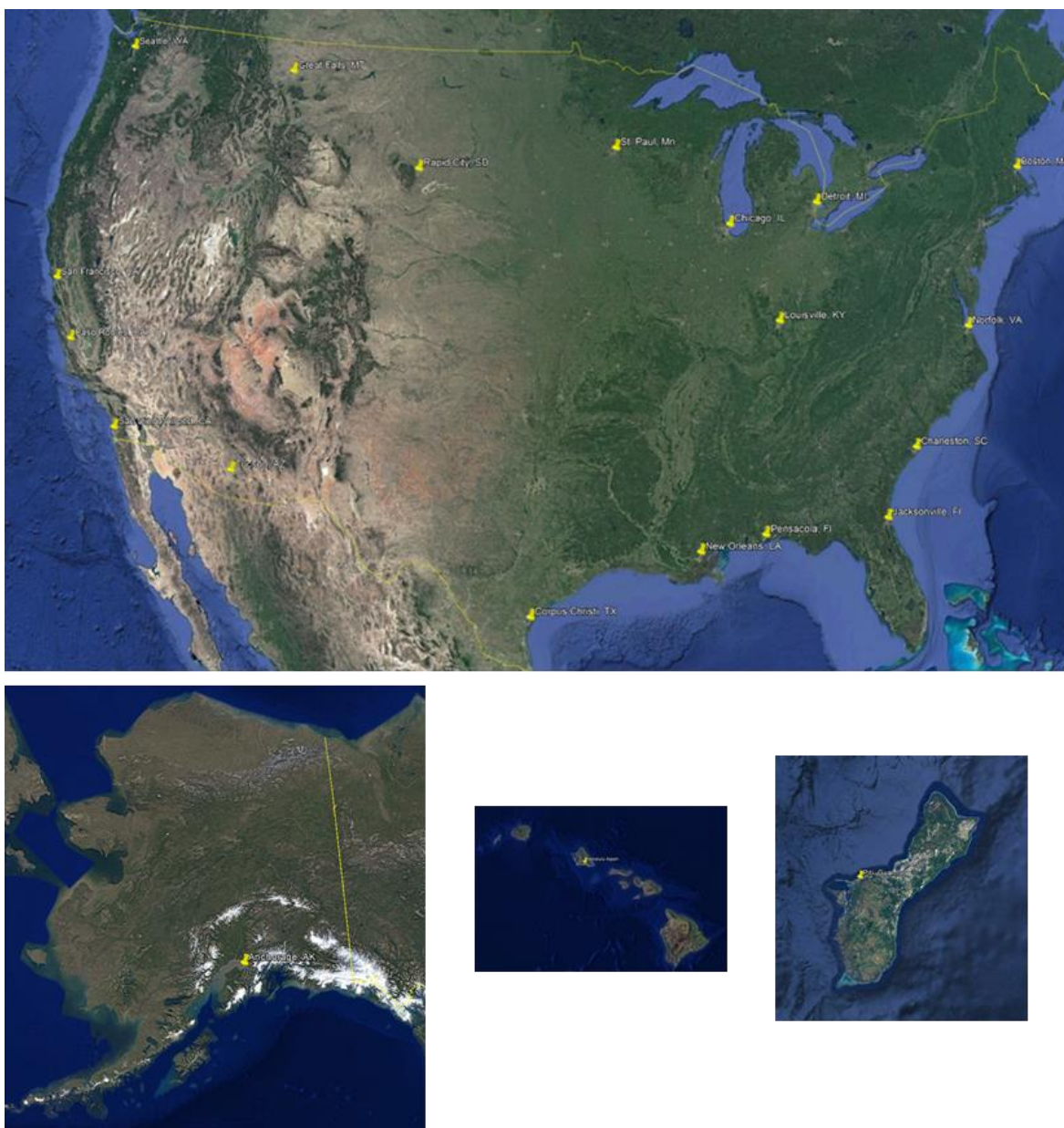


Figure 5-7. Locations Selected for Rainfall Duration Analysis. Top – North America; Bottom left – Anchorage, Ak; Bottom middle – Honolulu, HI; Bottom right – Piti, Guam.

Total rainfall duration was determined for a 96 h continuous basis following the onset of a qualifying rain event for each location. For the purposes of this analysis, a qualifying rain event had to meet two conditions. First, an antecedent dry period of at least 12 h was required prior to the summing of rainfall duration during the subsequent 96 h period. Second, a total of 0.05” cumulative rainfall must be observed prior to the summing of rainfall duration to account for surface saturation to allow for runoff to occur. Following the onset of a qualifying rain event, the total duration of rainfall that was observed over the subsequent 96 h period was calculated and the mean, standard deviation, and the 50th, 75th and 95th percentile of rainfall duration was derived. The 96 h period was

evaluated to be reflective of typical acute (lethal) and certain chronic (sublethal) toxicity testing durations (USEPA 1995, 2002a, b, c).

For the purposes of the refinement, validation and interlaboratory calibration studies to be performed, results of the historical rainfall study from San Diego, CA, were utilized in order to provide a basis for reproducibility and repeatability of test results to be analyzed. The results of the analysis are shown in Figure 5-8. The 50th, 75th, and 95th percentile rainfall durations of 6, 12 and 26 h, respectively, were chosen to span the range of reasonable testing conditions.

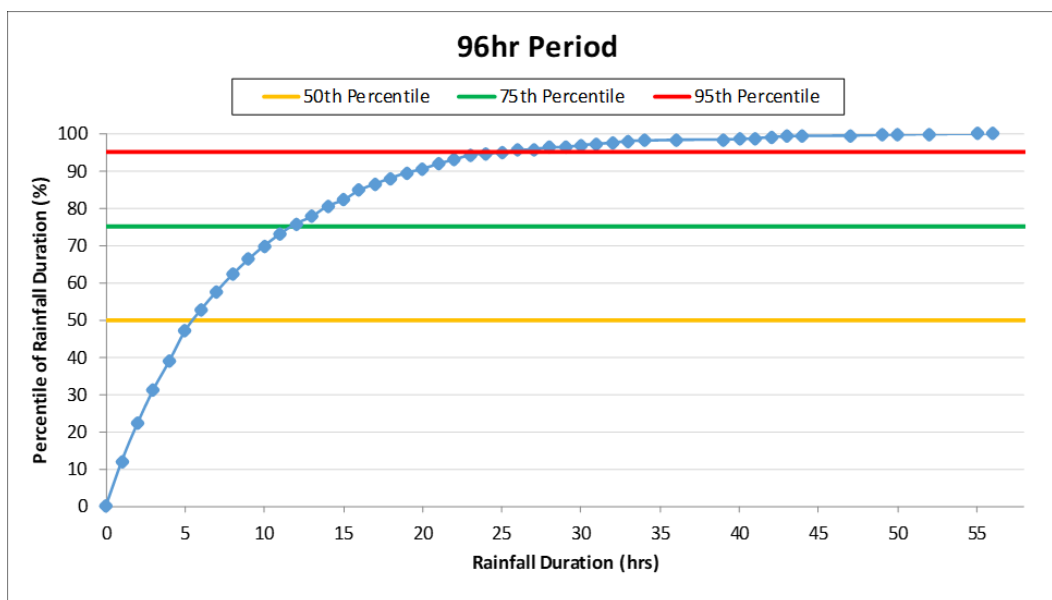


Figure 5-8. Cumulative frequency of rainfall duration over 96 h for San Diego, CA, when rain >0.05" was recorded. The 50th, 75th and 95th percentiles were derived as a basis for pulsed exposure regimes for this study.

5.2.2 Chronic Toxicity Test Methods

Similar to the acute testing, chronic toxicity testing was conducted using modifications to standard USEPA methods (Table 5-2) to conservatively estimate stormwater scenarios that are likely to be observed at the end of pipe. Purple sea urchin embryo-larval development tests were conducted in 25- μ m Nitex screen polycarbonate tubes placed in 400 mL high density polyethylene (HPDE) tri-corner beakers at 15 ± 1 °C (Figure 5-9). For spiked tests with organics, glass beakers were used to minimize the potential for adsorption to the test vessels. Following the pulsed exposure duration, screen tubes were removed from chemical spike or stormwater containing solutions, gently rinsed with FSW, and placed into clean tri-corner beakers containing FSW for the remainder of the 96-h test period (Figure 5-10).

As a quality control measure to ensure that the transfer methods do not negatively impact the embryos, a set of lab controls underwent transfers as well. At the end of the exposure period, the contents of the screen tubes were gently rinsed with FSW into 30 mL scintillation vials and preserved with 1 mL of 10% buffered formalin in seawater. The tests were evaluated for normal larval development on an inverted microscope at 100x magnification. Standard water quality measurements (DO, temperature, salinity and pH) was monitored daily. Concurrent standard reference toxicant tests were conducted as a quality control measure to assess the health of the organisms and technical performance of methods performed. Test specifications are described in Table 5-2.



Figure 5-9. Test set-up for sea urchin embryo-larval development pulsed exposure toxicity test.

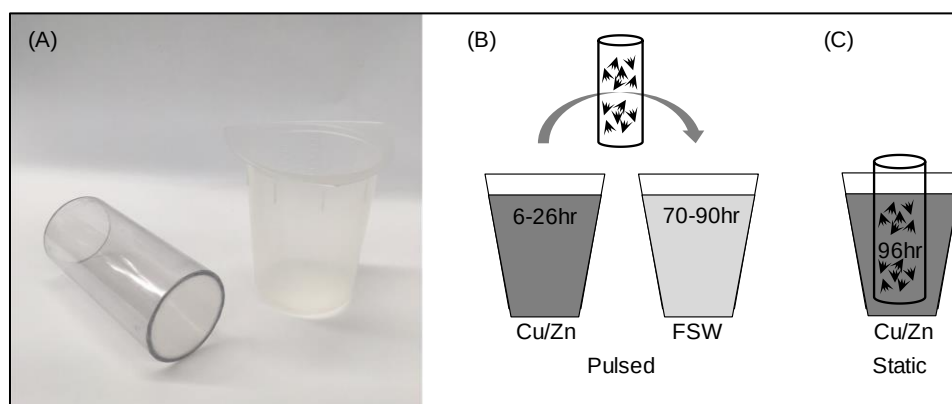


Figure 5-10. (A) Screen tube container (left) and tri-corner beaker (right) (B) Pulsed exposure treatment transfer method (C) Concurrent static exposure.

Delayed Onset/Life Stage Sensitivity

The current EPA and ASTM standard methods recognize the impacts that different ages may have on test organism sensitivity and have, therefore, incorporated restrictions on the ages and/or sizes of test organisms used for all WET testing procedures. Pulsed exposure toxicity test methods to evaluate episodic discharges conform to the age requirements in existing WET protocols because those ages are assumed (or demonstrated to be) most sensitive. However, it is of interest to evaluate the potential for age or life-stage related variance with regard to pulsed exposures to better mimic or validate specific conditions. For example, when conducting special study *in situ* exposures, there may be some age-related variances from standard protocols based on when a pulse occurs in the environment. In addition, prior studies have shown there may be a protective effect against metals early in embryo development, which disappears 10-12 h post-fertilization (Timourian 1968, Buznikov et al. 2007, Stransky et al. 2015).

For sea urchin evaluations, the onset of the exposure of copper in solution was varied to evaluate the effect on the development of the larvae. Following fertilization of eggs, exposure to Cu or Zn-spiked solutions was varied at either 6, 12, 18 or 24 h (Figure 5-11). Exposures were limited to 6 h

followed by transfer to clean, uncontaminated laboratory seawater for the remainder of the 96 h period upon which larvae were transferred to scintillation vials and preserved for evaluation of normal development. Concurrent standard exposures (USEPA 1995) were conducted as a quality control measure to assess the health of the organisms and technical performance of methods performed.

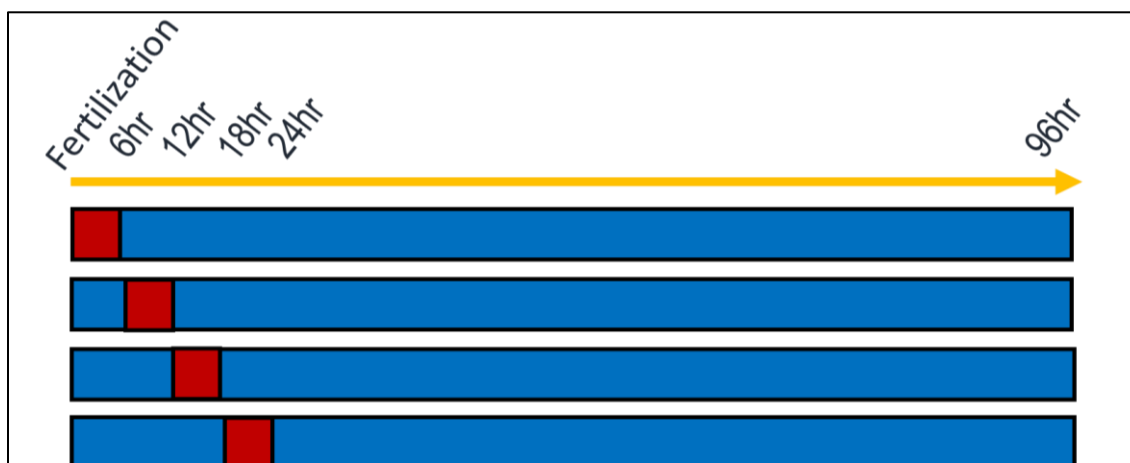


Figure 5-11. Exposure design for life stage sensitivity determination with *S. purpuratus*. Red coloration indicates exposure to spiked solutions into laboratory dilution water or receiving water (blue).

Table 5-2. Purple Sea Urchin embryo-larval development toxicity – laboratory test specifications.

Test organism	<i>Strongylocentrotus purpuratus</i> (purple sea urchin)
Test organism source	Field collected off of Point Loma, San Diego, CA or Monterey, CA
Test endpoints	96 h Embryo-Larval Development Success (Proportion Normal)
Test solution renewal	None
Feeding	None
Test Chamber size/type	Pulsed Exposures: 400 mL polyethylene (HDPE) tri-corner containers with polycarbonate screen tubes with 25 µm mesh. Glass beakers were used for spiked tests with an organic compound. Static Exposures: 30 mL scintillation vial
Test solution volume	Pulsed Exposures: 250 mL Static Exposures: 10 mL
Test temperature	15 ± 1 °C
Test salinity	34 ± 2 ppt
Light quality	Ambient laboratory illumination
Light intensity	10-20 µE/m ² /s (Ambient laboratory levels)
Photoperiod	16 h light/ 8 h dark
Aeration	None.
No. of organisms per chamber	250 eggs, appropriate sperm density to provide > 90% fertilization success (determined in a pre-test trial).
No. of replicates	5
Dilution water	Filtered (0.45 µm) natural seawater
Test duration	Exposure for 6, 12, or 26 h followed by exposure to clean 0.45 µm filtered seawater for the remainder of the 96-h test period
Test acceptability criteria	≥ 80% normal development in surviving controls; < 25% Minimum Significant Difference (MSD)
Reference toxicant	Copper sulfate or copper chloride (for lab control charts and test QA) Spiked pulsed exposure tests to include Copper, Zinc, and Bifenthrin
Test protocol	EPA 600/R-95/136

5.2.3 Acute Toxicity Test Methods

Acute toxicity testing was conducted using modified standard USEPA methods (USEPA 2002a; Table 5-3, Table 5-4, Table 5-5) to conservatively estimate stormwater scenarios that are likely to be observed at the end-of-pipe based on the historical rainfall data (Figure 5-8). Exposures were conducted in species/test specific chambers and following the appropriate pulsed exposure duration (e.g., 6, 12, or 26 h), organisms were carefully transferred to new clean exposure chambers with site receiving water or laboratory control water, as appropriate, for the remainder of the 96 h. To transfer mysid shrimp (*Americamysis bahia*) or amphipods (*Hyalella azteca*), organisms were gently poured over an 80 μm Nitex screen and rinsed into the new clean exposure chambers with receiving water or laboratory control water as appropriate. An alternative approach used consisted of a secondary acrylic chamber with screened bottom placed inside the replicate beaker with the discharge water. The animals were then simply transferred by removing the screened tube insert with test animals, and gently placing it into a new beaker with the receiving water or lab control water. These chambers are commonly used for sediment-interface water tests with a Nitex screen size of 25 to 30 μm , diameter of 4 cm, and height of 8 cm, and 1 cm lip on the bottom holding the screen in place (See Figure 5-12). For the water flea (*Ceriodaphnia dubia*), transfers of the organisms were accomplished with disposable plastic transfer pipettes as typically performed for standard methods.

As a quality control measure to ensure that the transfer methods do not negatively impact organisms, a set of lab controls underwent transfers as well. Daily survival counts in each replicate were conducted and standard water quality measurements (DO, temperature, salinity/conductivity and pH) was monitored daily. Concurrent standard reference toxicant tests were conducted as a quality control measure to assess the health of the organisms and technical performance of methods performed. Test specifications for the acute tests are in Table 5-3, Table 5-4 and Table 5-5.

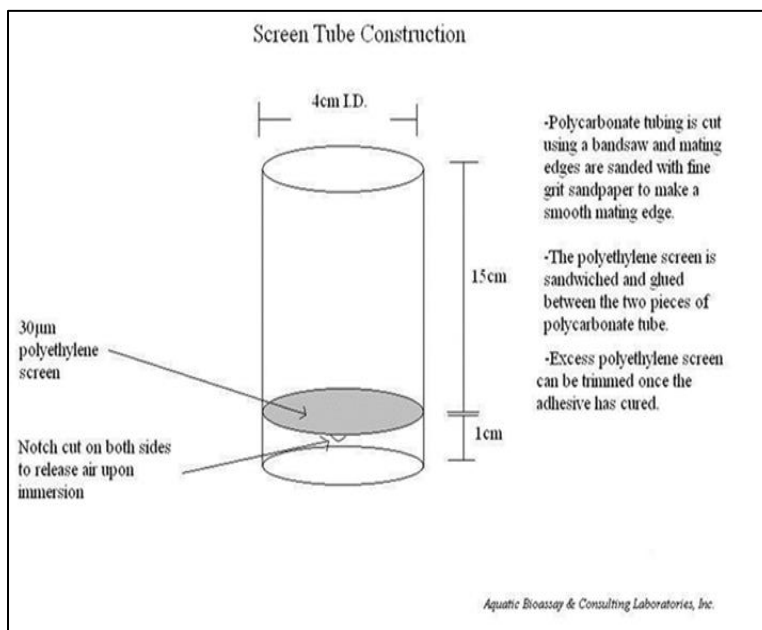


Figure 5-12. Screen tube construction for pulsed studies exposures.

Latent Effects

Latent effect observations for an additional 72 h past the 96 h test period (total observational period of 168 h; Figure 5-13) were incorporated into acute standard and pulsed exposure tests with the mysid shrimp as a growing body of research demonstrates that post-exposure effects are important to consider when evaluating effects associated with contaminant pulses. For acute exposures, researchers have found latent mortality in several species, including fish and several crustacean groups, that can be delayed up to several days, sometimes weeks (Brent and Herricks 1998, Reinert et al. 2002, Diamond et al. 2006, Hoang et al. 2007c, Angel et al. 2010, Gordon et al. 2012).

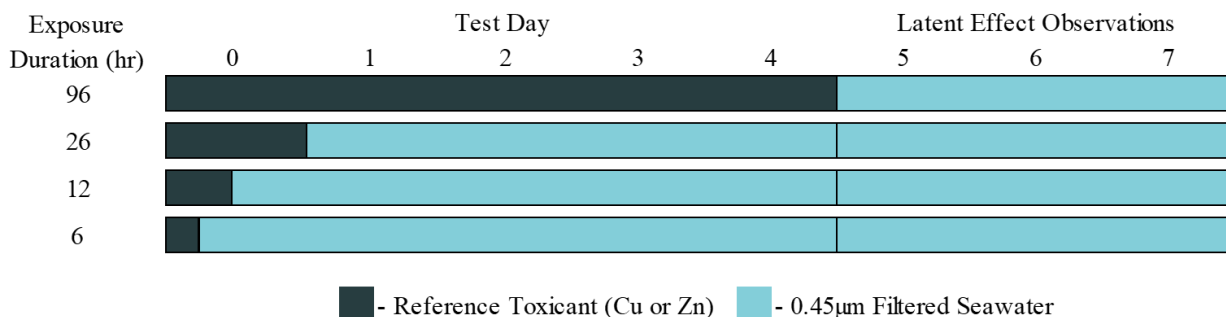


Figure 5-13. Diagram of latent effects experiment with mysid shrimp. Following the standard 96 h test duration, replicate chambers are refreshed with laboratory control and daily survival observations continue until 168 h.

Table 5-3. *Mysid* survival toxicity - laboratory test specifications.

Test organism	<i>Americamysis bahia</i> (mysid shrimp)
Test organism source	Aquatic Research Organisms, Hampton, NH or Aquatic BioSystems, Fort Collins, CO
Test endpoints	Survival
Test solution renewal	at 48 h (standard continuous exposures)
Feeding	Feed 40 newly hatched <i>Artemia</i> nauplii per larvae twice daily, morning and evening
Test Chamber size/type	400 mL Plastic Cups (metal spikes and stormwater); 400 mL or similar glass beakers for spiked tests with the organic chemical
Test solution volume	200 – 250 mL (dependent on exposure chamber volume)
Test temperature	20 ± 1 °C
Test salinity	32 ± 2 ppt
Light quality	Ambient laboratory illumination
Light intensity	10-20 $\mu\text{E}/\text{m}^2/\text{s}$ (Ambient laboratory levels)
Photoperiod	16 h light/ 8 h dark
Aeration	None, unless DO concentrations fall below 4.0 mg/L, then aerate all chambers.
No. of organisms per chamber	5
Age of test organism	1-5 days; 24 h range in size
No. of replicates	5 for spiked exposures, 6 for stormwater tests
Dilution water	Filtered (0.45 μm) natural seawater (FSW)
Test duration	Exposure for 6, 12, & 26 h followed by transfer to clean 0.45 μm filtered seawater (FSW) for remainder of the 96-h test period
Test acceptability criteria	$\geq 90\%$ survival in controls (required) PMSD < 26% recommended; Control CV – 8% recommended (EPA 2000b)
Reference toxicant	Copper sulfate or copper chloride (for lab control charts and test QA) Spiked pulsed exposure tests to include Copper, Zinc, and Bifenthrin
Test protocol	EPA 821/R-02/012

Table 5-4. *Hyalella* survival toxicity - laboratory test specifications.

Test organism	<i>Hyalella azteca</i> (amphipod)
Test organism source	Aquatic Research Organisms, Hampton, NH, or Aquatic BioSystems, Fort Collins, CO
Test endpoints	Survival
Test solution renewal	48 h (standard continuous exposures)
Feeding	Feed 0.5 mL YTC food mix at 48-hours. Organisms should also be fed prior to test initiation
Test Chamber size/type	400 mL Plastic cups (metal spikes and stormwater); 400 mL or similar glass beakers for spiked tests with the organic chemical
Test solution volume	250 mL
Test temperature	23 ± 1 °C
Light quality	Ambient laboratory illumination
Light intensity	10-20 µE/m ² /s (Ambient laboratory levels)
Photoperiod	16 h light/ 8 h dark
Aeration	None, unless DO concentrations fall below 4.0 mg/L, then aerate all chambers.
No. of organisms per chamber	5
Age of test organism	7-14 days; 1-2 d range in size
No. of replicates	5 for spiked exposures, 6 for stormwater tests
Dilution water	Moderately hard water prepared in accordance with EPA protocols
Test duration	Exposure for 6, 12, & 26 h followed by transfer to clean laboratory control water for remainder of the 96-h test period
Test acceptability criteria	≥ 90% survival in controls
Reference toxicant	Copper sulfate or copper chloride (for lab control charts and test QA) Spiked pulsed exposure tests to include Copper, Zinc, and Bifenthrin
Test protocol	EPA 821/R-02/012

Table 5-5. *Ceriodaphnia* survival toxicity - laboratory test specifications.

Test organism	<i>Ceriodaphnia dubia</i> (water flea)
Test organism source	In-house culture
Test endpoints	Survival
Test solution renewal	None
Feeding	Feed YCT and <i>Selenastrum</i> while holding prior to the test; newly released young should have food available a minimum of 2 h prior to use in a test
Test Chamber size/type	30 mL Plastic Cups
Test solution volume	20 mL
Test temperature	25 ± 1 °C
Light quality	Ambient laboratory illumination
Light intensity	10-20 µE/m ² /s (Ambient laboratory levels)
Photoperiod	16 h light/ 8 h dark
Aeration	None, unless DO concentrations fall below 4.0 mg/L, then aerate all chambers.
No. of organisms per chamber	5
Age of test organism	< 24 h
No. of replicates	5 for spiked exposures, 6 for stormwater tests
Dilution water	Moderately hard water prepared in accordance with EPA protocols
Test duration	Exposure for 6, 12, & 26 h followed by transfer to clean laboratory control water for remainder of the 96-h test period
Test acceptability criteria	≥ 90% survival in controls (required) PMSD < 21% and Control CV – 11% recommended (EPA 2000b)
Reference toxicant	Copper sulfate or copper chloride (for lab control charts and test QA) Spiked pulsed exposure tests to include Copper, Zinc, and Bifenthrin
Test protocol	EPA 821/R-02/012

5.3 LABORATORY STUDY RESULTS/REFINEMENT OF PULSED EXPOSURE METHODOLOGY

The laboratory study conducted herein were designed to refine the pulsed exposure protocol for two marine species, the purple sea urchin, *Strongylocentrotus purpuratus*, and survival of the mysid shrimp, *Americamysis bahia*, and two freshwater species, the freshwater amphipod, *Hyaella azteca*, and the water flea, *Ceriodaphnia dubia*.

This study investigated the effects of various copper, zinc or bifenthrin (a pyrethroid pesticide) concentrations, and exposure durations, on the observed toxicity for all species. These constituents were selected for evaluations as these are commonly elevated constituents in urban stormwater, typical examples of episodic discharge, and often have been identified as the primary cause of observed toxicity during routine monitoring in several studies that have evaluated toxic responses in wet weather runoff from predominantly impervious roadways and industrial facilities (Katz et al.,

2006; Kayhanian et al., 2008). The species and methods selected for this study are standard EPA-approved protocols, commonly incorporated into monitoring programs, and include both acute and short-term chronic endpoints (USEPA 1995; 2002a). The specific objectives of this study included: 1) establishing baseline sensitivity data for pulsed exposures of copper, zinc, or bifenthrin, independently, for all species relative to standard continuous exposures; 2) assessment of latent effects following pulsed exposures for *A. bahia* and *C. dubia*; and 3) examining embryo sensitivity at different times post fertilization for purple sea urchins to assess whether exposure initiation time might be important to consider when using a short-term pulsed exposure for this species and sensitive endpoint.

Table 5-6 describes the number and type of toxicity tests that were conducted as part of Performance Objective 3. All test dilutions were prepared by volumetric additions of reagent grade stock solutions into dilution waters. All stock solutions and test concentrations were sub-sampled for verification.

All statistical analyses used verified copper, zinc and bifenthrin concentration values. Median effective (EC₅₀) or median lethal (LC₅₀) concentrations for each pulsed exposure regime were calculated using CETIS (Tidepool Scientific, LLC., v1.9.2.8). To determine statistical differences ($\alpha=0.05$) among the pulse treatments for each contaminant and species, one-factor analyses of variance (ANOVAs) were conducted using mean EC₅₀/LC₅₀ as the dependent factor and pulse duration (6, 12, 26, 96 h) as the independent factor. Data from the *S. purpuratus* zinc tests were log-transformed as they failed to meet test assumptions of normality and homoscedasticity. All other data met test assumptions, so no transformations were performed. Pairwise comparisons for significant ($\alpha=0.05$) independent factors were conducted using Tukey multiple comparisons. All ANOVA and post-hoc analyses were completed using SYSTAT 13 (San Jose, CA).

Results from these efforts are also documented in Colvin et al. 2021a, Karic et al. 2022 and in the summary report prepared by the Marine Pollution Studies Laboratory (appendix B).

Table 5-6. Total number and type of toxicity test to be conducted for refinement of pulsed exposure testing with spiked stock solutions.

Species	Test Endpoint/Consideration			Total Number of Toxicity Tests
	Standard 96 h Exposure	6, 12, 26 h Pulsed Exposure	Life Stage Sensitivity	
<i>Americamysis bahia</i>	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 9 total	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 27 total	NA	36
<i>Hyalella azteca</i>	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 9 total	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 27 total	NA	36
<i>Ceriodaphnia dubia</i>	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 9 total	3 x Cu ^a 3 x Zn ^a <u>3 x Bifenthrin</u> 27 total	NA	36
<i>Strongylocentrotus purpuratus</i>	3 x Cu 3 x Zn <u>3 x Bifenthrin</u> 9 total	3 x Cu 3 x Zn <u>3 x Bifenthrin</u> 27 total	3 x Cu <u>3 x Zn</u> 24 total	60

- ^a – Latent effects were evaluated for the standard and pulsed exposures by carrying out the test for a longer period. These tests do not contribute to the total number of tests calculation. NA = not applicable.

S. PURPURATUS CHRONIC TOXICITY TESTS

Pulsed exposure methods for the chronic sea urchin embryo-larval development tests were modified from standard methods (USEPA 1995) as described in section 5.2.2 and outlined in detail by Colvin et al. (2020b). In brief, for pulsed exposures, test organisms were initially exposed to the appropriately metal or bifenthrin-spiked water for 6, 12, or 26 h. After the appropriate exposure period, the larvae were transferred to FSW. Both pulsed and static exposures were conducted concurrently, using the same batch of organisms for both exposures. At the end of the test period, larvae from pulsed exposure tests were rinsed into 20 mL scintillation vials with FWS and all tests (including static exposures) were fixed with 10% acetate buffered formalin for enumeration under an inverted microscope. Daily water quality conditions were recorded, and all applicable parameters (salinity, dissolved oxygen, pH, and temperature) met test acceptability criteria for all tests conducted.

For all tests conducted, laboratory controls met test acceptability criteria of a minimum of 80% normal embryo-larval development (USEPA 1995). For both copper and zinc pulsed exposures, there was a significant decrease in the EC₅₀ as exposure time increased (Table 5-7, $p < 0.001$). Tukey pairwise comparison results are shown in Figure 5-14 for copper and zinc, respectively. For both metals, 6 h and 12 h exposures were significantly less toxic compared to 96 h exposures ($p < 0.05$), while 26 h and 96 h exposures were not significantly different (Cu: $p = 0.23$, Zn: $p = 0.62$). Factors of difference of the EC₅₀s compared to the 96 h copper exposure were 11.0, 5.2, and 3.2 for 6, 12, and 26 h pulsed exposures, respectively. The range of these factors of difference were larger for zinc exposures, with 6, 12, and 26 h demonstrating factors of difference of 147.9, 23.8, and 1.4 compared to the 96 h exposure, respectively.

One pulsed exposure test was conducted with *S. purpuratus* with bifenthrin. While results from laboratory controls met test acceptability criteria, no significant reduction in normal development was observed for any concentration tested. A lowest observed effect concentration (LOEC) was calculated for the 24 h exposure, but this response was driven by a single replicate response of 52% normal development and is likely not biologically significant.

Table 5-7. Copper, Zinc and Bifenthrin spiked studies: Summary of Results – *S. purpuratus* embryo-larval development.

Analyte	Pulsed Exposure Duration (h)	EC ₅₀ Concentration (µg/L)	SD	Factor of Difference from Standard 96 h Exposure
Copper	6	257.4	58.3	11.0
	12	120.6	9.5	5.2
	26	75.1	10.6	3.2
	Standard (96)	23.4	1.0	-
Zinc	6	16,960.4	3,704.7	147.9
	12	2,733.1	821.1	23.8
	26	164.1	68.1	1.4
	Standard (96)	114.6	16.5	-
Bifenthrin	6	>22,000	-	0.0
	12	>22,000	-	0.0
	26	>22,000	-	0.0
	Standard (96)	>22,000	-	-

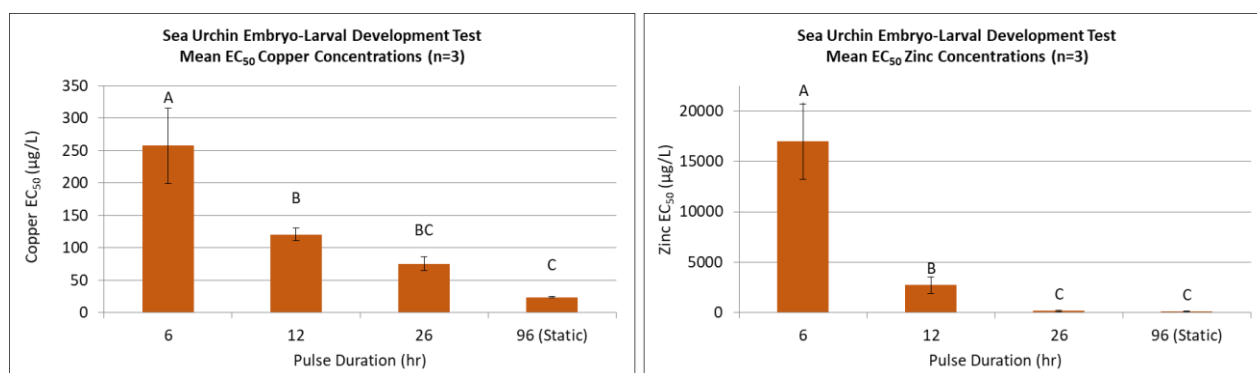


Figure 5-14. Average EC₅₀ values from n=3 replicates (mean ± standard deviation) for each pulsed exposure following embryo fertilization in *S. purpuratus* (n=2 for 6 h zinc exposure) for copper (left) and zinc (right) Letters above each bar indicate significant differences between pulsed exposures determined by Tukey multiple comparisons tests.

S. PURPURATUS CHRONIC LIFE STAGE SENSITIVITY EVALUATIONS

For the life-stage sensitivity evaluations, the 6-12 h and 12-18 h exposure period initiations had decreased normal development (lower EC₅₀ values) when compared to the 0-6 h or 18-24 h exposure periods for both copper and zinc (Table 5-8, Figure 5-15). While this trend appeared for both

contaminants, it was only statistically significant for zinc exposures ($p = 0.004$). This indicates that during the developmental period between 6-18 h, *S. purpuratus* larvae were more sensitive to these metals.

A prior study by Timourian (1968) reported a lag period of 8 to 10 h where *S. purpuratus* larvae do not uptake zinc immediately following fertilization. The mechanism for this initial protective effect is not well understood, but it has been suggested that the fertilization envelope surrounding the developing embryo acts as a barrier to divalent metals (Buznikov et al. 2007). This protective effect is especially apparent in this study for the 6 h and 12 h pulsed exposures to zinc where effects were 147.9 and 23.8 times lower in magnitude (based on EC_{50} values), respectively, compared to 96 h exposures. This lack of sensitivity of *S. purpuratus* to zinc during short pulses has been shown previously (Rosen et al. 2019). Due to the initial protective effect, it is important to consider the length of the pulse when developing pulsed exposure treatment durations to ensure that organisms are exposed when they are most sensitive. This sentiment drives the need for the testing at various developmental life stages and is important in evaluating the efficacy of a given pulsed exposure method. In this study, the 26 h and 96 h exposure treatments resulted in similar responses, suggesting that the 26 h pulse duration captured this most sensitive life stage (Figure 5-15).

Table 5-8. Copper and Zinc Spiked studies: Summary of Results – *S. purpuratus* embryo-larval development, life stage sensitivity studies.

Analyte	Timing of Pulsed Exposure Following Fertilization (h)	EC_{50} Concentration ($\mu\text{g/L}$)	SD
Copper	0-6	257.4	58.3
	6-12	165.4	14.2
	12-18	177.7	6.4
	18-24	229.9	70.9
Zinc	0-6	16960.0	3705.2
	6-12	4648.0	3092.6
	12-18	6239.1	1775.1
	18-24	16745.0	2538.5

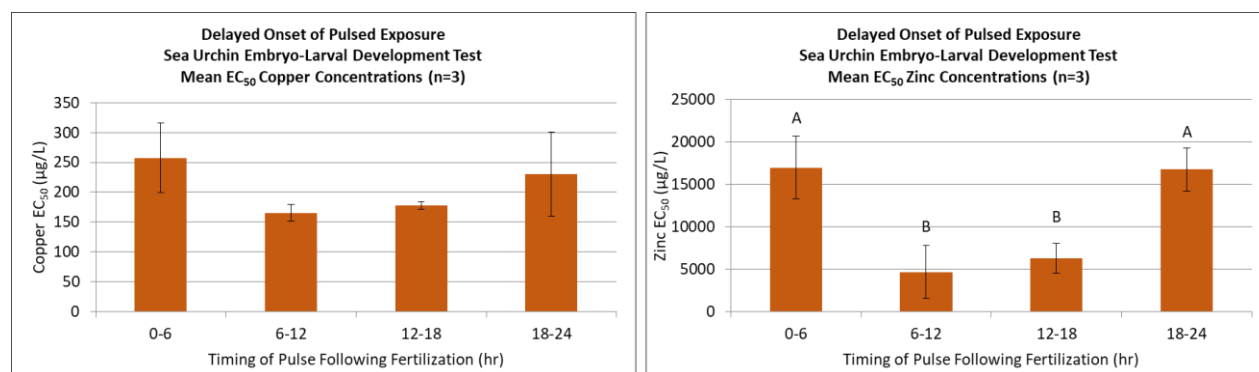


Figure 5-15. Average EC_{50} values from $n=3$ replicates (mean $\pm$ standard deviation) for each pulsed exposure following embryo fertilization for the life-stage sensitivity evaluation in *S. purpuratus*. Exposures to copper (left) did not show any significant differences between the 6 h pulsed exposures. Exposures to zinc (right) exhibited significant increases in sensitivity for the 6-12 h and 12-18 h exposure periods. Letters above each bar indicated significant differences between pulsed exposures determined through Tukey multiple comparisons tests.

A. BAHIA ACUTE TOXICITY TESTS

Similar to the chronic urchin exposures, all applicable parameters (controls $\geq 90\%$ survival, salinity, dissolved oxygen, pH, and temperature) met test acceptability criteria for all tests conducted.

ANOVA results indicated there were significant differences among the exposure durations (Table 5-9, Cu: $p = 0.003$; Zn: $p < 0.001$; Bifenthrin: $p = 0.01$). Tukey pair-wise comparison results are shown in Figure 5-16. For copper, the 6 h exposure was significantly less toxic compared to 26 h and 96 h exposures ($p < 0.05$), while the 12 h and 26 h were not significantly different from the 96 h ($p = 0.054$ and $p = 0.976$, respectively). For zinc, results indicated there was an increase in toxicity as exposure time increased with both the 6 h and 12 h exposures showing significant differences from the 96 h standard exposure ($p < 0.05$ and $p = 0.002$, respectively). For both metals, 26 h and 96 h exposures were not significantly different (Cu: $p = 0.976$; Zn: $p = 0.222$). For bifenthrin, the 6 h and 12 h exposures were significantly less toxic compared to the 96 h exposure ($p = 0.02$ and $p = 0.01$, respectively). The 26 h exposure was not statistically different from the 96 h exposure but showed a decrease in toxicity as the exposure time decreased.

Copper factors of difference from the 96 h exposure for *A. bahia* for 6, 12, 26 h were 5.3, 3.2, and 1.3, respectively. For zinc, the factor difference from the 96 h exposure for 6, 12 and 26 h exposures were 11.1, 5.7, and 2.7, respectively. Similar to *S. purpuratus*, zinc resulted in a larger range of these factors of difference, but this effect was greatly enhanced in the *S. purpuratus* exposures. Factors of difference from the 96 h exposure for bifenthrin for 6, 12 and 26 h exposures were 2.9, 3.0, and 2.2, respectively.

Table 5-9. Copper, Zinc and Bifenthrin Spiked Studies: Summary of results – *A. bahia* survival.

Analyte	Pulsed Exposure Duration (h)	LC ₅₀ Concentration (µg/L)	SD	Factor of Difference from Standard 96 h Exposure
Copper	6	1006.7	147.7	5.3
	12	613.3	273.3	3.2
	26	242.9	33.3	1.3
	Standard (96)	190.4	49.7	-
Zinc	6	5464.0	798.3	11.1
	12	2818.0	532.0	5.7
	26	1333.3	126.3	2.7
	Standard (96)	491.0	15.6	-
Bifenthrin	6	9.1	0.0	2.9
	12	9.3	2.4	3.0
	26	6.8	1.8	2.2
	Standard (96)	3.1	1.3	-

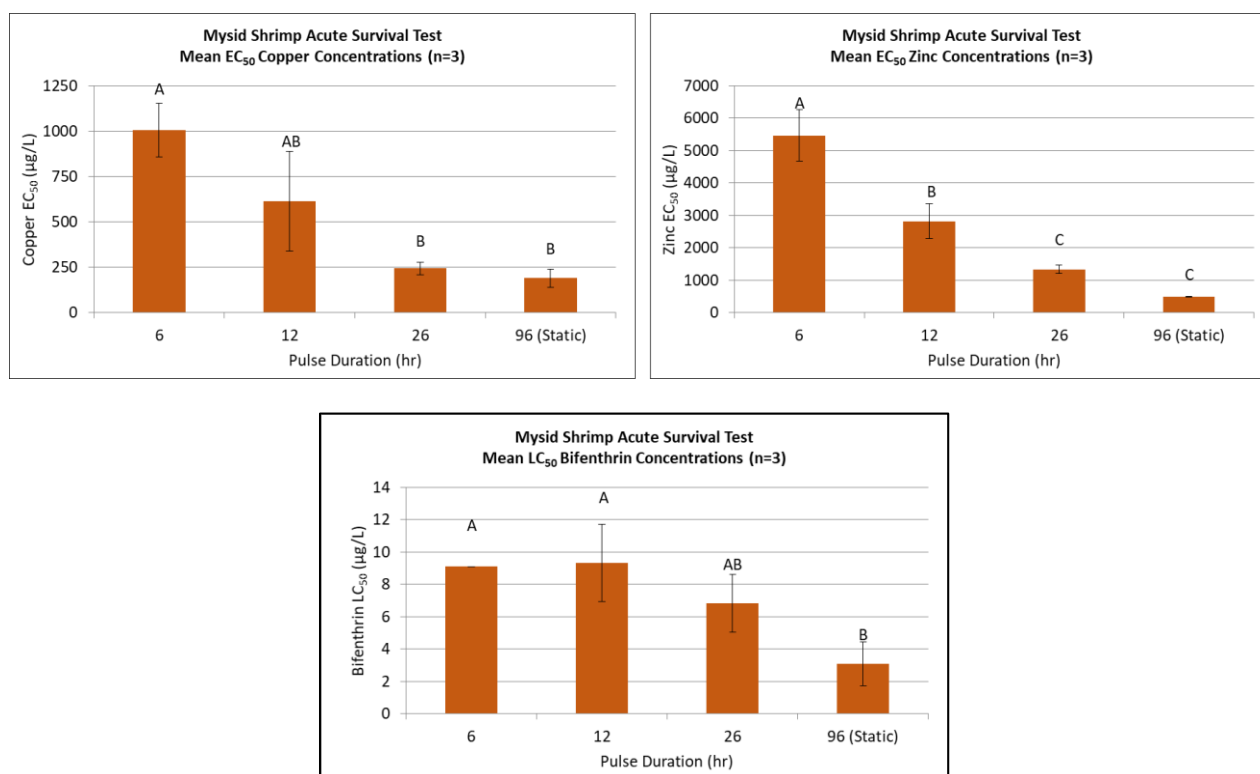


Figure 5-16. Average LC₅₀ values from n=3 replicates (mean ± standard deviation) for each pulsed exposure in *A. bahia*. Letters above each bar indicate significant differences between pulsed exposures determined through Tukey multiple comparisons tests.

A. BAHIA LATENT EFFECT EVALUATIONS

Latent effect observations were only conducted for the metals evaluated and not bifenthrin. Latent effects were apparent in both copper and zinc pulsed exposures, where survival, as indicated by the

mean LC₅₀, continued to decrease after organisms had been transferred into clean seawater (Table 5-10, Figure 5-17). This effect was statistically significant for 12 h pulsed exposures to copper ($p = 0.011$) and trending for the 6 h ($p = 0.195$) and 26 h pulsed exposure ($p = 0.054$). No significant difference in survival was observed in the standard exposure regime between the 96 h and 168 h point estimate calculations ($p = 0.342$). For the zinc exposures, the 6 h, 12 h and 26 h pulsed exposures all showed significant differences in LC₅₀ values following transfer of the organism to FSW (6 h: $p < 0.001$, 12 h: $p = 0.001$, 26 h $p = 0.002$). There was also a significant difference observed between the 96 h and 168 h point estimate calculations for the zinc standard exposures ($p = 0.003$).

The results observed corroborate with trends seen in crustaceans in several studies with pulsed metal exposures (e.g., Diamond 2006, Hoang et al. 2007 a, b, c, Angel et al. 2010). In the case of *A. bahia*, metals will accumulate in the integument, continuing to deleteriously affect the organism even if they are no longer directly in contact with contaminated water (Gordon et al. 2012). Current WET test methods typically do not allow for a post-exposure observation period, which does not account for latent mortality and possibly underestimates metal toxicity. The length of the post-exposure observation period to adequately capture latent mortality is dependent on species and pulse duration (Diamond 2006, Angel et al. 2010). In the present study, three days of observation were included past the standard WET methods 96 h exposure time period for *A. bahia*, which is similar to other studies which found latency periods up to 96 h post-exposure (Diamond 2006, Hoang et al. 2007 a, b, c, Angel et al. 2010). It should be noted that although no statistically significant differences were observed, survival continued to decrease between the 96 h and 168 h time periods for all pulsed exposures. This is an important finding because current WET testing methods require up to a 96 h continuous static exposure. Failure to account for latent mortality can result in underestimating toxicity for effluent discharges, especially as current WET methodologies that do not currently have a post-exposure observation time (Brent and Herricks 1998, Burton et al. 2000, Diamond et al. 2006). As such, the presented 96 h pulsed exposure method (placing the organisms in FSW after exposure period) incorporates a post-exposure observation period. Further, as demonstrated through the lack of significant difference between the calculated LC₅₀ at the 96 h and 168 h time points (Figure 5-17), a 72-90 h post exposure period is sufficient to capture latent mortality after 6-26 h of exposure to copper or zinc for *A. bahia*.

Table 5-10. Copper and Zinc spiked studies: Summary of results – *A. bahia* survival, latent effects studies.

Analyte	Time Point for LC ₅₀ Calculation	Pulsed Exposure Duration (h)	LC ₅₀ Concentration (µg/L)	SD
Copper	Post Pulsed Exposure	6	1333.3	57.7
		12	1333.3	57.7
		26	712.4	366.9
		Standard (96)	190.4	49.7
	96 h	6	1104.5	198.9
		12	613.3	273.3
		26	242.9	33.3
		Standard (96)	190.4	49.7
	168 h	6	1055.4	219.4
		12	591.8	272.3
		26	231.6	34.3
		Standard (96)	154.8	28.7
Zinc	Post Pulsed Exposure	6	13000.0	0.0
		12	11003.3	3458.3
		26	4697.3	1706.0
		Standard (96)	491.0	15.6
	96 h	6	5464.0	798.3
		12	2818.0	532.0
		26	1333.3	126.3
		Standard (96)	491.0	15.6
	168 h	6	5380.0	934.5
		12	2624.3	433.8
		26	1283.0	136.4
		Standard (96)	360.9	31.9

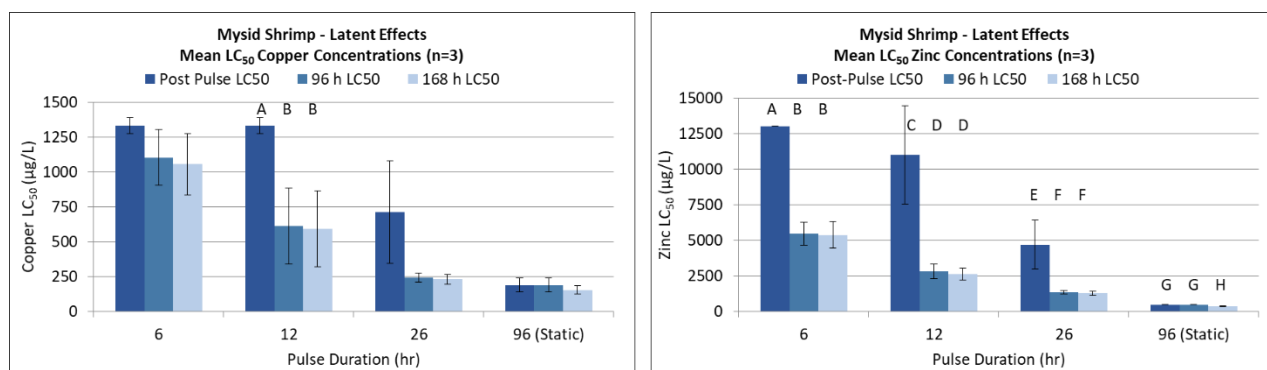


Figure 5-17. Average LC₅₀ values from n=3 replicates (mean $\pm$ standard deviation) for each pulsed exposure in *A. bahia*. LC₅₀s were calculated one of three ways: immediately after pulsed exposure (left bars), at 96 h after test initiation (middle bars) and 168 h after test initiation (right bars). Exposures to copper (left) showed a significant difference in the 12 h pulsed exposure, where latent effects were present following the 12 h exposure until the 96 h exposure time-point. Exposures to zinc (right) showed a significant difference in the 6, 12, and 26 h pulsed exposures, where latent effects were present following the initial exposure until the 96 h exposure time-point. Letters above each bar indicate significant differences between pulsed exposures determined through Tukey multiple comparisons tests, when such differences were observed.

H. AZTECA ACUTE TOXICITY TESTS

All applicable parameters (controls $\geq$ 90% survival, salinity, dissolved oxygen, pH, and temperature) met test acceptability criteria for all tests conducted.

ANOVA results indicated there were significant differences among the exposure durations for the copper and bifenthrin tests (Cu: $p = 0.004$; bifenthrin: $p = 0.004$), but not for zinc ($p = 0.23$; Table 5-11). Tukey pair-wise comparison results are shown in Figure 5-18. For copper, the 6 h exposure was significantly less toxic compared to 26 h and 96 h exposures ($p = 0.04$ and $p = 0.004$, respectively). The 12 h exposure was also significantly different from the 96 h standard exposure ($p = 0.02$), while 26 h exposure was not significantly different from the 96 h ($p = 0.392$). For zinc, results indicated there was an increase in toxicity as exposure time increased as shown in Figure 5-18. However, no significant differences among or between the pulsed exposures were observed (ANOVA: $p = 0.23$). This is likely due to the possible outlier data from the 13 May 2020 data set. For bifenthrin, only the 6 h exposure was significantly less toxic compared to the 26 h and 96 h exposures ($p = 0.02$ and $p = 0.002$, respectively). All other pair-wise comparisons were non-significant, although the trend of increasing toxicity with increasing exposure duration is observed.

Copper factors of difference from the 96 h exposure for *A. bahia* for 6, 12, 26 h were 4.2, 3.3 and 2.1, respectively. For zinc, the factor difference from the 96 h exposure for 6, 12 and 26 h exposures were 9.1, 3.3 and 1.6, respectively. Factors of difference from the 96 h exposure for bifenthrin for 6, 12 and 26 h exposures were 3.3, 2.1, and 1.7, respectively.

Table 5-11. Copper, Zinc and Bifenthrin spiked studies: Summary of results – *H. azteca* survival.

Analyte	Pulsed Exposure Duration (h)	LC ₅₀ Concentration (µg/L)	SD	Factor of Difference from Standard 96 h Exposure
Copper	6	2039.67	627.7	4.2
	12	1622.33	176.2	3.3
	26	995.80	300.0	2.1
	Standard (96)	484.53	200.9	-
Zinc	6	8115.67	8336.7	9.1
	12	2893.67	2353.3	3.3
	26	1420.00	631.0	1.6
	Standard (96)	887.47	334.3	-
Bifenthrin	6	15.7	4.3	3.3
	12	10.2	1.4	2.1
	26	7.9	0.8	1.7
	Standard (96)	4.8	1.7	-

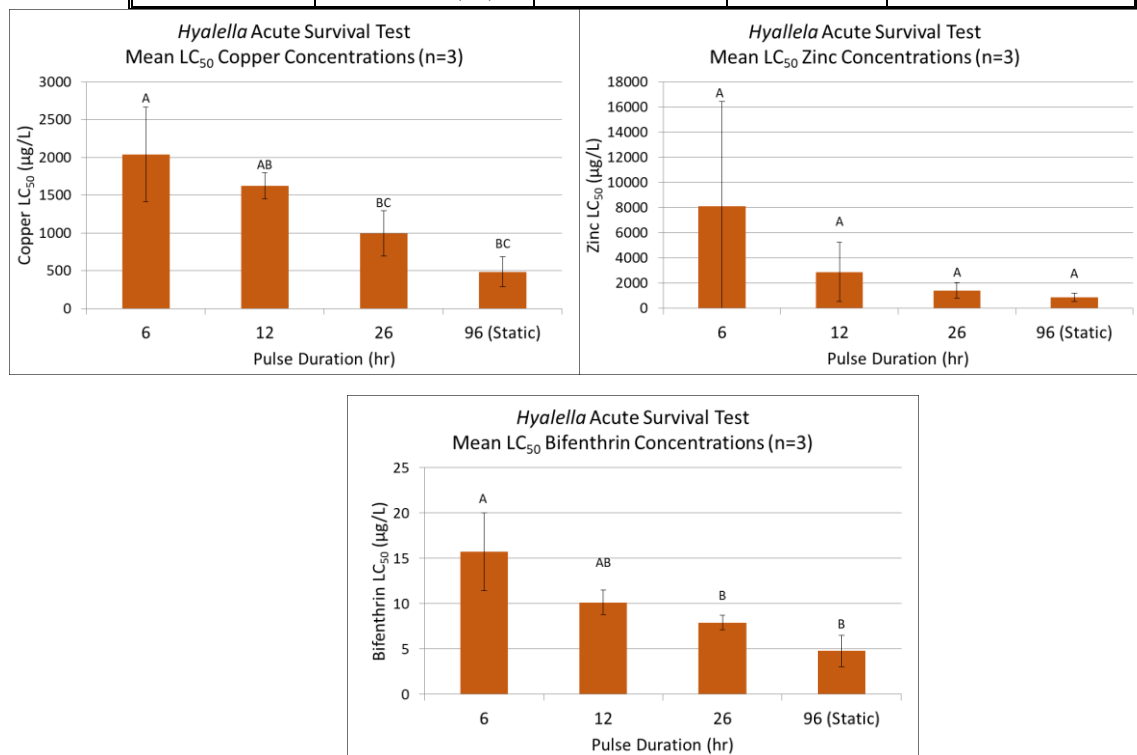


Figure 5-18. Average LC₅₀ values from n=3 replicates (mean ± standard deviation) for each pulsed exposure in *H. azteca*. Letters above each bar indicate significant differences between pulsed exposures determined through Tukey multiple comparisons tests.

C. DUBIA ACUTE TOXICITY TESTS

All applicable parameters (controls ≥ 90% survival, salinity, dissolved oxygen, pH, and temperature) met test acceptability criteria for all tests conducted for the *C. dubia* efforts.

ANOVA results indicated there were no significant differences among the exposure durations for the copper or zinc tests (Cu: $p = 0.74$; Zn: $p = 0.08$; Table 5-12). The pesticide bifenthrin, however, did show a significant difference among the exposure durations ($p = 0.0009$). Tukey pair-wise comparison results are shown in Figure 5-19. For bifenthrin, the 6 h exposure was significantly less toxic compared to the 26 h and 96 h exposures ($p = 0.003$ and $p = 0.001$, respectively). The 12 h exposure was also significantly different to the 26 h and 96 h exposures ($p = 0.03$ and $p = 0.01$, respectively). The 26 h and 96 h bifenthrin exposure durations were not significantly different from one another ($p = 0.79$).

Copper factors of difference from the 96 h exposure for *A. bahia* for 6, 12, 26 h were 1.3, 1.2 and 1.0, respectively. For zinc, the factor difference from the 96 h exposure for 6, 12 and 26 h exposures were 2.7, 2.0 and 1.5, respectively. Factors of difference from the 96 h exposure for bifenthrin for 6, 12 and 26 h exposures were 10.1, 7.6, and 2.4, respectively.

Table 5-12. Copper, Zinc and Bifenthrin spiked studies: Summary of results – *C. dubia* survival.

Analyte	Pulsed Exposure Duration (h)	LC ₅₀ Concentration (µg/L)	SD	Factor of Difference from Standard 96 h Exposure
Copper	6	25.67	8.3	1.3
	12	24.36	9.6	1.2
	26	20.61	7.6	1.0
	Standard (96)	19.98	1.4	-
Zinc	6	726.13	254.2	2.7
	12	531.23	253.7	2.0
	26	409.70	71.3	1.5
	Standard (96)	271.83	29.2	-
Bifenthrin	6	276.67	0.0	10.1
	12	206.67	56.9	7.6
	26	65.33	9.7	2.4
	Standard (96)	27.33	3.5	-

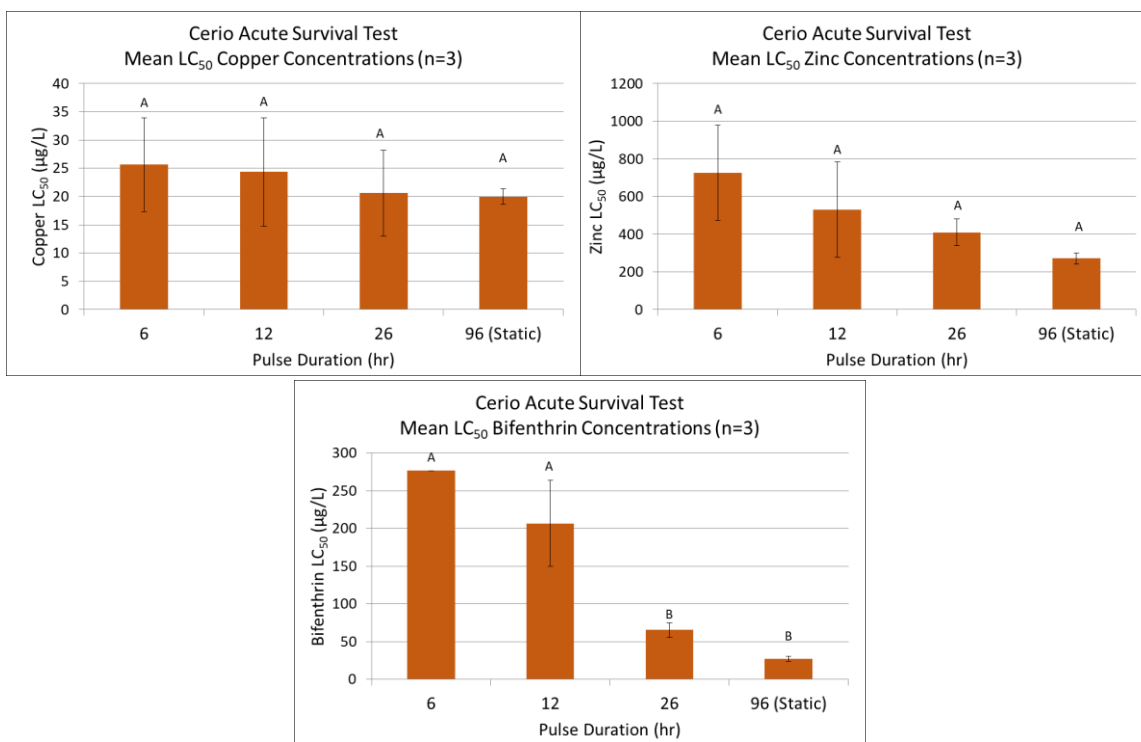


Figure 5-19. Average LC₅₀ values from n=3 replicates (mean ± standard deviation) for each pulsed exposure in *C. dubia*. Letters above each bar indicate significant differences between pulsed exposures determined through Tukey multiple comparisons tests.

5.4 FIELD TESTING/ VALIDATION OF PULSED EXPOSURE METHODOLOGY

Stormwater and/or episodic industrial discharge samples from DoD installations were evaluated using both standard and pulsed exposure laboratory methods to ensure representativeness and protectiveness of actual site conditions during dynamic runoff events. Efforts at all demonstration sites were made to collect representative water samples consistent with the Navy industrial NPDES permit to collect a grab sample during the first 4 h of runoff. Table 5-13 describes the number and type of toxicity tests that were conducted as part of Task 4 for this project.

Table 5-13. Total number and type of toxicity tests conducted for validation of pulsed exposure testing with field collected samples.

Site Name	Stations Sampled	Sample Type	Species/ Endpoint	# of Tests	# of Sampling Events	Exposure Durations Tested	Total Toxicity Tests
NBSD	Pier 13, OF39	Stormwater	Mysid acute; Urchin chronic	2	2	Standard (96 h), 6, 12, 26 h Pulsed	32
NBC	OF 9, OF111	Stormwater	Mysid acute; Urchin chronic	2	2	Standard (96 h), 6, 12, 26 h Pulsed	32
NBPL	OF 28	Stormwater	Mysid acute; Urchin chronic	2	2	Standard (96 h), 6, 12, 26 h Pulsed	16
PSNS	OF18ab, OF19, OF21, OF96	Drydock Discharge	Urchin Chronic	2	2	Standard (96 h), 24 h Pulsed	10
NSBNL	OF6, OF15, OF17, OF22A, OF27	Stormwater	<i>Ceriodaphnia</i> acute	1	1	Standard (96 h), 6, 12, 26 h Pulsed	20

OF = Outfall

Toxicity test methods followed established protocols (U.S. EPA 1995; 2002) as well as the modified pulsed exposure methodology as summarized in Standard Operating Procedures for Pulsed Exposure Methodology (Colvin et al. 2020b). Standard water quality measurements (DO, temperature, conductivity, and pH) were monitored as per protocol requirements. In brief, test organisms were exposed to the test solutions for modified pulsed exposure durations (i.e., 6, 12, or 26 hours). At the end of this time period, test organisms were transferred into clean laboratory water for the remainder of the test period. For all tests, standard exposure duration testing was conducted concurrently to allow for direct comparison of results. Statistical analyses were performed against the respective salt, brine control or receiving water control, as appropriate, for each group and pulse

duration. For *in situ* exposures, statistical comparisons were made against the SEA Ring deployed at the reference site. Tests for Significant Toxicity (TST) analyses (USEPA 2010) for both the purple sea urchin embryo-larval development tests and mysid shrimp survival tests examine whether the results of a given sample relative to its respective control differs by an *a priori* prescribed amount rather than whether they are the same, as in traditional hypothesis testing. For the purple sea urchin test, the *a priori* critical percent difference is set at 25%. TST analyses for the mysid survival tests have an *a priori* critical percent difference set at 20%. TST analyses were conducted on the highest concentration tested for each sample versus the appropriate control (USEPA 2010). Full statistical analyses, bench and field datasheets are provided in Appendix B.

5.4.1 Puget Sound Naval Shipyard (PSNS)

Dry dock discharges from PSNS were collected during two sampling events on 27 Feb 2018 and 26 Jun 2018 using leveraged support from NESDI project #547 and the PSNS ENVironmental inVESTment's (ENVVEST's) Ambient Monitoring Program. Two and three industrial effluent outfall (OF) samples were collected on 27 Feb 2018 and 27 Jun 2018, respectively (Figure 5-20). Samples were collected in high-density polyethylene (HDPE) cubitainers under the direction of NIWC Pacific personnel. All samples were hand carried to the NIWC Pacific Bioassay Laboratory where testing was conducted. Sample collection and receipt times are summarized in Table 5-14.

Table 5-14. Puget Sound Naval Shipyard: Sample collection and receipt times.

Project ENVVEST Ambient Monitoring Event	Sample/ Station ID	Type	Sample Collection Date/Time	Sample Receipt Date/Time
AMB25	OF18	Composite	2/27/2018 1015	2/28/2018 0945
	OF19	Composite	2/27/2018 0920	
AMB26	OF96	Grab	6/26/2018 1145	6/27/2018 0900
	OF19	Grab	6/26/2018 1315	
	OF21	Composite	6/26/2018 1230	

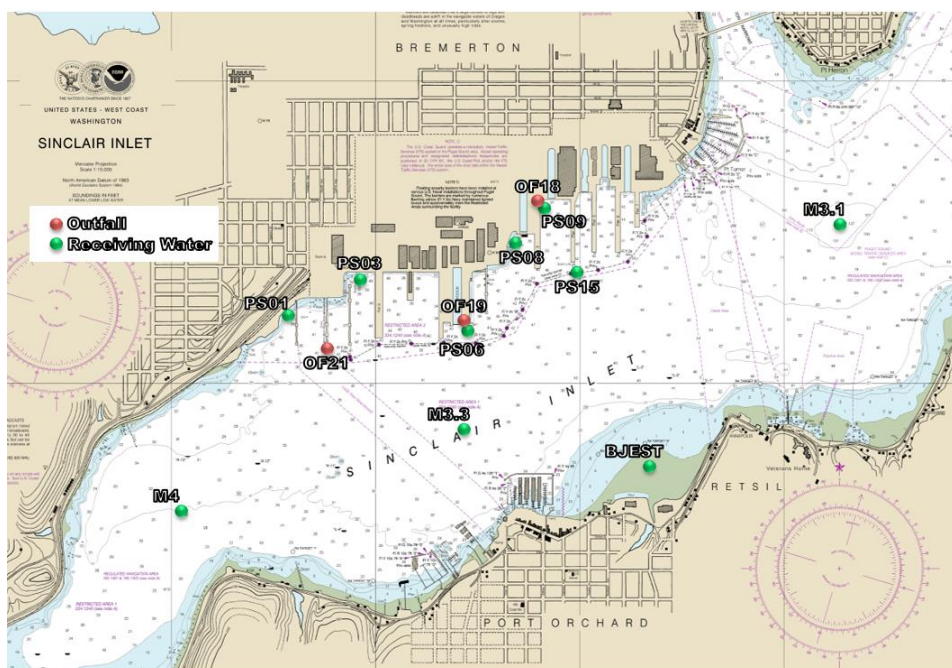


Figure 5-20. Dry dock discharge outfall effluent (red circles) and receiving water (green circles) stations sampled for toxicity testing for the Puget Sound Naval Shipyard NPDES compliance monitoring. OF=outfall.

Chronic toxicity tests were conducted on all samples in Table 5-14 with the purple sea urchin, *S. purpuratus*. Samples from AMB25 were tested at 50 and 100% concentration and samples from AMB26 were tested at 25, 50 and 100% concentration. The dry dock effluents discharged approximately 15 mins every hour. Over the course of four days, the discharge duration totaled approximately 24 h. Therefore, the pulsed exposure regime tested for these samples was a 24 h exposure to effluent followed by transfer to clean filtered seawater. Samples were also tested under standard static 96 h exposures.

For all samples tested for both monitoring events, all test acceptability criteria were met, and all water quality parameters measured for all tests were within the recommended range for the duration of each test performed. For the AMB25 monitoring effort, no significant reduction in embryo-larval development was observed for either the pulsed or standard exposure regimes (Table 5-15). For the AMB26 monitoring effort initial comparison analyses showed that the effluent sample OF96 showed a significant difference compared to the control for the static exposure. However, the Test for Significant Toxicity (TST) analysis was conducted to examine whether the results of the sample relative to its respective control differs by an *a priori* prescribed amount rather than whether they are the same, as in traditional hypothesis testing. For the purple sea urchin test, the *a priori* critical percent difference is set at 25%. Using these methods, the static OF96 sample was deemed non-toxic.

Table 5-15. Puget Sound Naval Shipyard: Summary of results – *S. purpuratus* embryo-larval development.

Project ENVVEST Ambient Monitoring Event	Station/ Sample ID	Sample Exposure Duration (h)	Mean Normal Development @ 100% Concentration (%)	SD	TST Toxicity Determination
AMB25	Control	24	81.6	10.5	-
		Standard (96)	93.8	1.9	-
	OF18	24	95.0	1.6	Non-toxic
		Standard (96)	93.8	1.5	Non-toxic
	OF19	24	97.2	1.9	Non-toxic
		Standard (96)	98.2	1.1	Non-toxic
AMB26	Control*	24	94.4	1.8	-
		Standard (96)	91.2	1.9	-
	OF96	24	92.0	4.1	Non-toxic
		Standard (96)	80.6^a	5.6	Non-toxic
	OF19	24	92.4	5.1	Non-toxic
		Standard (96)	86.8	2.7	Non-toxic
	OF21	24	87.8	7.4	Non-toxic
		Standard (96)	91.0	4.2	Non-toxic

*Mean percent normal development of three lab controls (one paired with each sample).

Value in **BOLD** indicates statistically significant from the control using a student's one-tailed t-test, but not different using the EPA TST approach.

5.4.2 Naval Region Southwest – Naval Base San Diego, Point Loma and Coronado

End-of-pipe stormwater samples were collected from two sites on 1 Jan 2018, five sites on 31 Jan 2019 and from three sites on 15 May 2019 at Naval Bases in San Diego, CA (Figure 5-21). In addition, a reference sample from Pier 169 at NIWC Pacific was collected during each event. Receiving water samples were collected from each site prior to and during each storm event in 2019. All sampling collection efforts were conducted under the direction of the ESTCP project team. Samples were collected in LDPE cubitainers and couriered to the NIWC Pacific Bioassay Laboratory for toxicity testing. Sample collection and receipt times as well as water quality conditions measured upon receipt at the NIWC Pacific bioassay laboratory are summarized in Table 5-16 through Table 5-27. Discrete water samples filtered to 0.45-µm and acidified as appropriate for each analysis were analyzed for total suspended solids, total copper and zinc, polycyclic aromatic hydrocarbons (PAHs), and dissolved organic carbon (DOC). All analyses were performed by Weck Laboratories, Inc. of Industry City, CA, except for DOC, which was performed by McCampbell Analytical, Inc. of Pittsburg, CA.

Acute and chronic toxicity tests were conducted on all samples using the mysid shrimp, *Americamysis bahia*, and the purple sea urchin, *Strongylocentrotus purpuratus*, respectively. For the 2018 effort, samples were tested at 10, 50 and 100% concentrations and 2019 samples were tested at 25, 50 and 100% concentrations. All samples were tested using the pulsed exposure regimes were 6, 12, or 26 h followed by transfer to site specific receiving water along with standard 96 h static exposures. Artificial sea salts were added to increase the salinity of all stormwater samples for toxicity testing. The receiving water samples tested in 2019 were tested at 100% concentration only.

Standard water quality measurements (DO, temperature, conductivity, and pH) were monitored as per protocol requirements. A concurrent reference toxicant test using Cu was conducted as a quality control measure to assess the health of the organisms and technical performance of the method.

For the two wet season efforts in 2019, *in situ* deployments were made using the SEA Ring as a platform for exposing the same species selected for laboratory exposures as well as house HOBO water quality loggers and passive sampling devices (DGTs – Diffusive Gradients in Thin Films) for characterization of water quality conditions while organisms were deployed. Field demonstrations verified that the pulsed methodologies are representative and protective of actual site conditions during dynamic runoff events.

SEA Rings were deployed <24 h prior to a qualifying storm event and recovered 96 h later. SEA Rings were deployed in front of outfalls at Pier 13 (Jan and May efforts) and OF09 (Jan effort only) at NBSD and NAB, respectively, near the surface, at approximately 3m depth and at the sediment surface (Figure 5-22). Each SEA Ring dedicated six replicate chambers for mysid shrimp (*Americamysis bahia*) and four replicate chambers for the purple sea urchin embryos (*Strongylocentrotus purpuratus*). For the mysid, 10 organisms per replicate resulted in 60 organisms per SEA Ring. For the purple sea urchin, three replicate drum per chamber resulted in a total of 12 replicates per SEA Ring. The survival of the mysid shrimp and the normal development of the sea urchin embryos was assessed upon recovery of the SEA Rings after 96 h and compared to the concurrent standard and pulsed exposures in the laboratory.

Hourly precipitation data was downloaded from <https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USW00023188/detail>. For the Jan 2018 and Jan 2019 efforts, more detailed precipitation data was obtained from NBSD site-specific rain gauges allowing for precipitation observations by the minute.



Figure 5-21. SEA Ring and associated monitoring equipment deployment sites for *in situ* toxicity testing validation tests and collection of grab stormwater and receiving water samples. OF = Outfall.

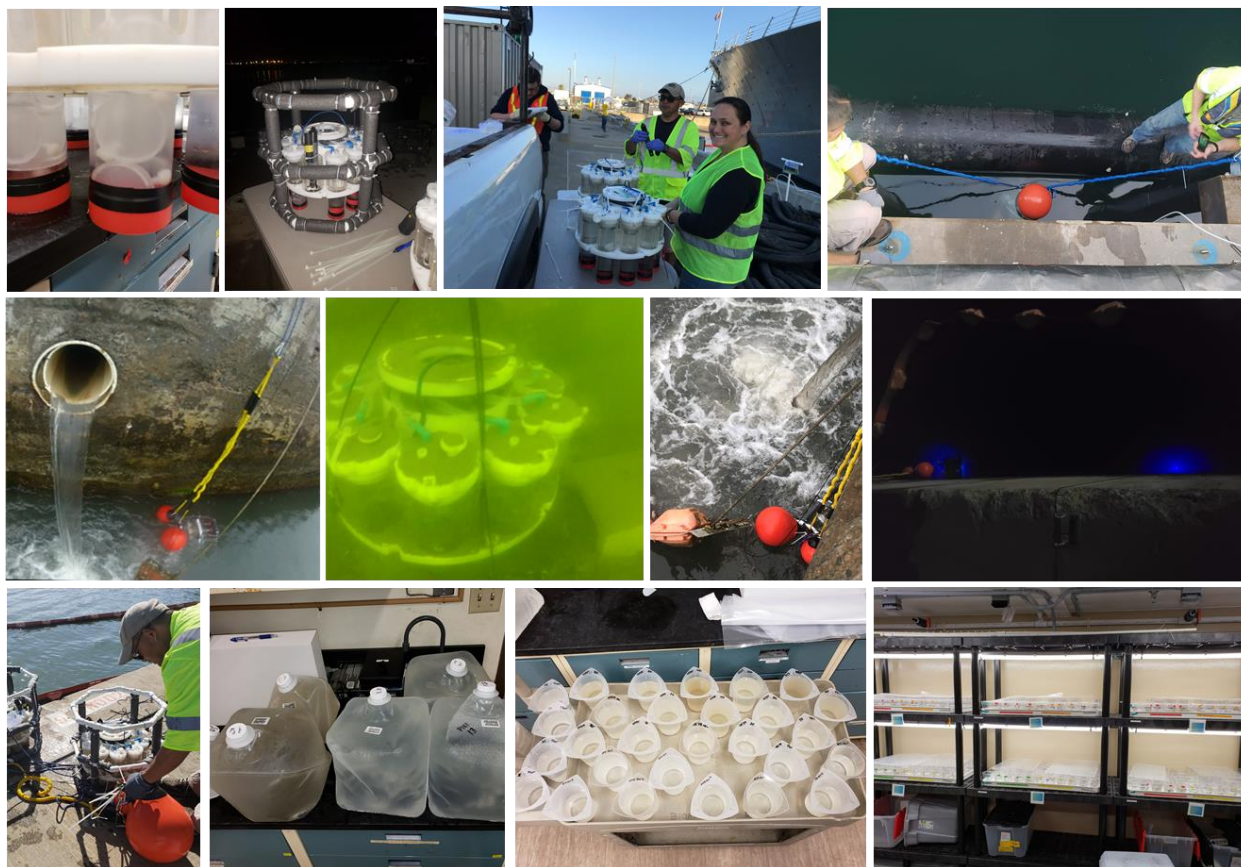


Figure 5-22. Representative photos of stormwater *in situ* deployment and recovery efforts. From left to right, Top row: embryo drums sub-chamber within exposure chamber on SEA Ring; SEA Ring preparation for deployment at NAB-OF09 with protective cage surrounding unit; SEA Ring preparation for deployment at NBSD-P13; and deployment of SEA Ring with buoy and bungee cords at NBSD-P13.

Middle row: Near surface SEA Ring at NAB-OF09; SEA Ring deployed at sediment surface at NAB-OF09; stormwater discharge from NAB-OF09 and deployed SEA Ring; and indicator lights from three SEA Rings deployed at NAB-OF09.

Bottom row: Recovery of SEA Rings from NAB-OF09; stormwater samples and receiving water samples collected from NBSD-P13; all stormwater samples poured out for laboratory check-in procedures; and set up of echinoderm embryo-larvae development for laboratory-based pulsed exposures.

For all samples tested for the three monitoring events, all test acceptability criteria were met, and all water quality parameters measured for all tests were within the recommended range for the duration of each test performed, with exception of high dissolved oxygen levels for several urchin tests. While the DO levels were high, water saturation level never rose above $100\% \pm 10\%$ and were deemed acceptable. Additionally, all reference toxicant tests that were performed concurrently met

test acceptability criteria and were within two standard deviations of the historical mean, indicating sensitivity to copper was consistent with that historically observed for these organisms (urchin: Figure 5-23; mysid: Figure 5-24).

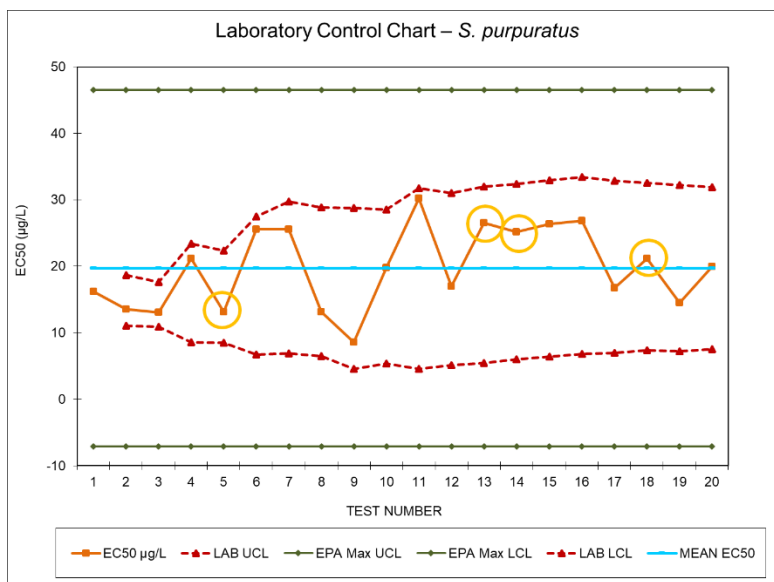


Figure 5-23. Laboratory control chart for reference toxicant tests performed using *S. purpuratus*. Yellow circles indicate the tests that were conducted concurrently to the demonstration efforts.

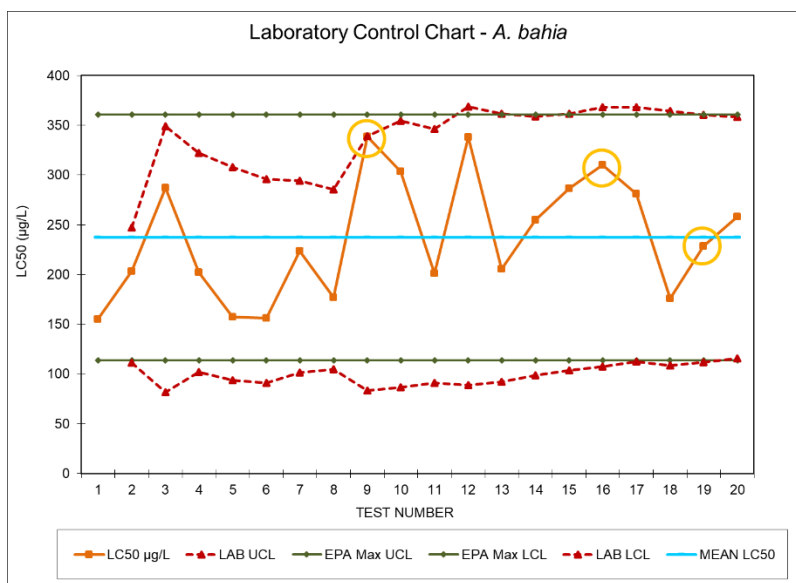


Figure 5-24. Laboratory control chart for reference toxicant tests performed using *A. bahia*. Yellow circles indicate the tests that were conducted concurrently to the demonstration efforts.

Storm Event 9 Jan 2018

Test material collection and receipt times from the 9 Jan 2018 storm event are summarized in Table 5-16. Water quality parameters upon sample receipt at NIWC Pacific are summarized in Table 5-17 and in Appendix B. No precipitation was observed in the seven days preceding the sample collection on 9 Jan 2018 and rainfall during this event totaled 1.31” and rainfall was detected for approximately 127 minutes during a single primary pulse of rain.

Table 5-16. Navy Region Southwest: Sample description, collection and receipt times for Jan 2018.

Sample/ Station ID	Matrix/Description	Type	Sample Collection Date/Time	Sample Receipt Date/Time
NBSD-P10	Pier 10, surface stormwater runoff	Grab	1/09/2018 0836	1/9/2018 1718
NBSD-OF73	Surface stormwater runoff discharging to OF73	Grab	1/09/2018 0625	

NBSD – Naval Base San Diego

Table 5-17. Water quality parameters measured upon receipt at NIWC Pacific for Jan 2018.

Sample/ Station ID	Temp (°C)	Dissolved Oxygen (mg/L)	pH (units)	Salinity (ppt)
NBSD-P10	3.3	8.8	8.04	NR
NBSD-OF73	3.2	8.8	8.10	NR

NBSD – Naval Base San Diego

For the Jan 2018 effort, the results show greater frequency of sea urchin embryo abnormality with increasing effluent exposure with duration for both samples tested as indicated by the TST analyses that show toxicity (Table 5-18). For the mysid tests, while there were no statistically significant results of toxicity, there appears to be an increasing trend of mortality with increasing exposure time for both samples tested (Table 5-19). There was an apparent hit for toxicity for the Pier-10 sample for the 12 h exposure. However, since both the 6 h and 26 h exposures are non-toxic at the 100% concentration tested, this incidence of toxicity is potentially an outlier.

Table 5-18. Navy Region Southwest: Summary of results from Jan 2018 – *S. purpuratus* embryo-larval development.

Station/ Sample ID	Sample Exposure Duration (h)	Mean Normal Development @ 100% Concentration (%)	SD
NBSD-P10	6	97.0	2.0
	12	97.2	1.3
	26	54.4	6.5
	Standard (96)	49.4	3.5
NBSD-OF73	6	96.2	1.3
	12	97.8	1.3
	26	8.8	3.4
	Standard (96)	0.0	0.0

Value in **BOLD** indicates statistically significant from the control using the EPA TST approach.

Table 5-19. Navy Region Southwest: Summary of results from Jan 2018 – *A. bahia* survival.

Station/ Sample ID	Sample Exposure Duration (h)	Mean Survival @ 100% Concentration (%)	SD
NBSD-P10	6	93.3	10.3
	12	73.3	16.3
	26	96.7	8.2
	Standard (96)	86.7	16.3
NBSD-OF73	6	96.7	8.2
	12	100.0	0.0
	26	83.3	10.3
	Standard (96)	86.7	10.3

Value in **BOLD** indicates statistically significant from the control using the EPA TST approach.

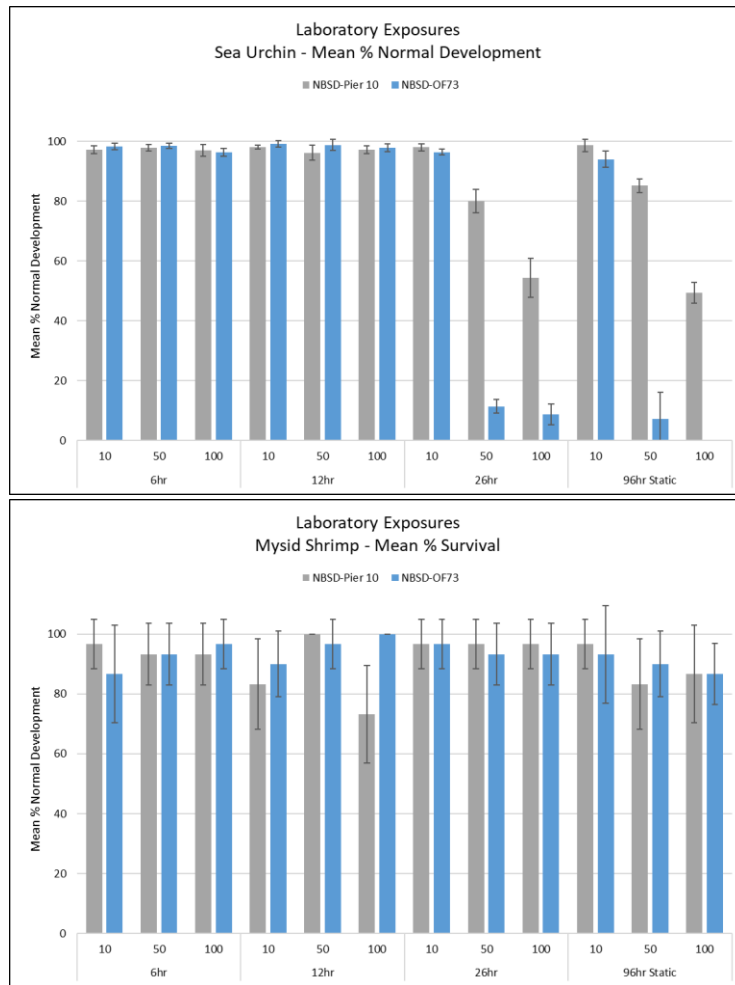


Figure 5-25. Summary of toxicity results with the purple urchin (top) and mysid shrimp (bottom) for laboratory-only exposures for Jan 2018: NBSD-Pier10 and NBSD-OF73.

Storm Event 31 Jan 2019

Test material collection and receipt times from the 31 Jan 2019 storm event are summarized in Table 5-20. Water quality parameters upon sample receipt at NIWC Pacific are summarized in Table 5-21 and in Appendix B. No precipitation was observed in the seven days preceding the sample collection on 31 Jan 2019. The pulse of rain that first occurred on 31 Jan 2019 resulted in 0.62” with rainfall being detected for 73 min over course of 7.5 h. During the full deployment period, 2.37” of rainfall was observed with intermittent rain occurring for an additional 82 min for a total of 155 min of detected rainfall during the 4 d period. Figure 5-26 shows water quality conditions as observed by water quality HOB0 loggers that were co-deployed with SEA Rings at P13 at NBSD and OF09 at NAB. Loggers were deployed on the surface and the mid-depth SEA Rings. The sensor that was deployed on the SEA Ring at OF09 was intermittently exposed to air during the deployment and thus the readings from that sensor were unable to be interpreted and not included herein.

Table 5-20. Navy Region Southwest: Sample description, collection and receipt times for Jan 2019.

Sample/ Station ID	Matrix/Description	Type	Sample Collection Date/Time	Sample Receipt Date/Time
NBSD-P13	Pier 13, surface stormwater runoff	Grab	1/31/2019 1530	1/31/2019 1800
NBSD-P13-RW	Receiving water adjacent to Pier 13, collected before storm event	Grab	1/30/2019 1645	
NBSD-P13-RW-DS	Receiving water adjacent to Pier 13, collected during storm event	Grab	1/31/2019 1725	
NBSD-OF39	Mole Pier, surface stormwater runoff discharging to OF39	Grab	1/31/2019 1700	
NBSD-OF39-RW	Receiving water adjacent to Mole Pier at OF39, collected before storm event	Grab	1/31/2019 1200	
NBSD-OF39-RW- DS	Receiving water adjacent to Mole Pier at OF39, collected during storm event	Grab	1/31/2019 1700	
NAB-OF09	Amphibious Base, surface stormwater runoff discharging to OF09	Grab	1/31/2019 1500	
NAB-OF09-RW	Receiving water adjacent to OF09, collected before storm event	Grab	1/30/2019 1900	
NAB-OF09-RW-DS	Receiving water adjacent to OF09, collected during storm event	Grab	1/31/2019 1500	
NAB-OF111	Amphibious Base, surface stormwater runoff discharging to OF111	Grab	1/31/2019 1530	
NAB-O111-RW	Receiving water adjacent to OF111, collected before storm event	Grab	1/31/2019 1300	
NAB-OF111-RW- DS	Receiving water adjacent to OF111, collected during storm event	Grab	1/31/2019 1530	
NBPL-OF28	Sierra Pier, surface stormwater runoff discharging to OF28	Grab	1/31/2019 1425	
NBPL-OF28-RW- DS	Receiving water adjacent to Sierra Pier at OF28, collected during storm	Grab	1/31/2019 1530	
REF	Reference sample adjacent to NIWC Pacific Pier	Grab	2/1/2019 1425	

NBSD – Naval Base San Diego; NAB – Naval Amphibious Base, Coronado; NBPL – Naval Base Point Loma; REF – Reference Station.

Table 5-21. Water quality parameters measured upon receipt at NIWC Pacific for Jan 2019.

Sample/ Station ID	Temp (°C)	Dissolved Oxygen (mg/L)	pH (units)	Salinity (ppt)
NBSD-P13	2.0	9.1	6.52	1.14
NBSD-P13-RW	4.3	8.9	6.55	31.2
NBSD-P13-RW-DS	2.0	9.3	7.68	23.9
NBSD-OF39	1.6	9.0	8.87	0.02
NBSD-OF39-RW	2.3	8.8	7.98	30.0
NBSD-OF39-RW-DS	3.1	9.0	7.58	22.6
NAB-OF09	4.6	9.0	8.12	0.17
NAB-OF09-RW	5.1	5.1	7.74	31.7
NAB-OF09-RW-DS	3.2	9.2	7.93	28.4
NAB-OF111	3.7	9.1	9.00	0.08
NAB-O111-RW	3.4	9.1	7.97	30.2
NAB-OF111-RW-DS	2.2	9.2	7.99	26.8
NBPL-OF28	12.2	9.0	8.27	0.45
NBPL-OF28-RW-DS	15.0	9.5	7.64	31.0

NBSD – Naval Base San Diego; NAB – Naval Amphibious Base, Coronado; NBPL – Naval Base Point Loma; REF – Reference Station.

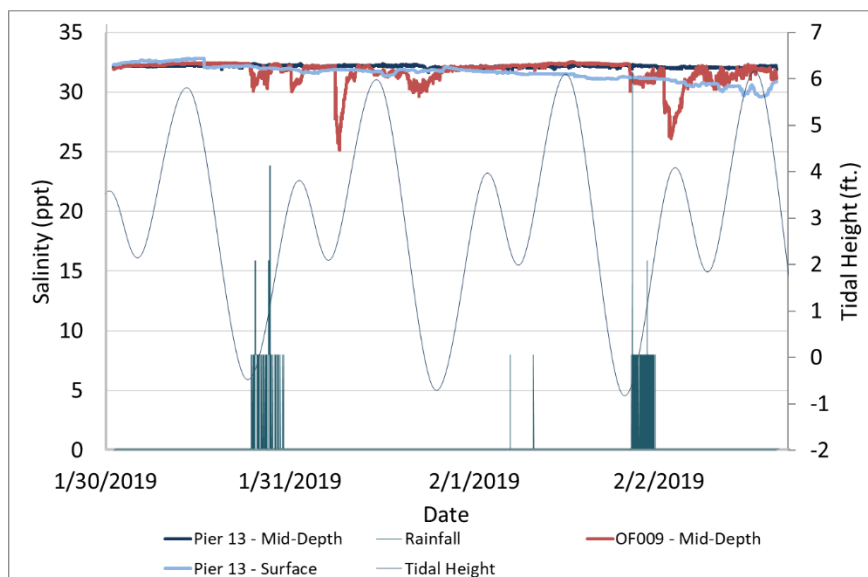


Figure 5-26. Summary of salinity, tidal height, and hourly rainfall observed during the January 2019 field effort in San Diego.

Three SEA Rings were deployed each at the P13 site at NBSD and the OF09 site at NAB on 30 Jan 2019. An additional SEA Ring was deployed at the NIWC Pacific pier as a reference site. SEA Rings were recovered from both locations on 3 Feb 2019 and organisms removed from the exposure chambers and evaluated. Over the course of the SEA Ring deployment, organisms were exposed to

ambient conditions for approximately 18 to 24 h prior to the start of rainfall from the initial pulse of rain arriving on 31 Jan 2019. A second pulse of rain occurred on the third day of deployment exposing the organisms to a second flush of stormwater runoff. Tidal cycles throughout the deployment were fairly extreme for the San Diego region (spring tides), ranging from - 0.84' to + 6.1' (Figure 5-26). A strong freshwater signal was observed for the SEA Ring located at the mid-depth location at OF09 at Naval Amphibious Base, Coronado. However, little to no signal was observed at the NBSD P13 deployment location at either depth.

Laboratory and In Situ Toxicity Results. Sample collection, deployment and recovery of SEA Rings and test organisms was successful at all targeted locations. Toxicity results for both laboratory-based exposures and *in situ* exposures are summarized in Table 5-22 through Table 5-26 and shown in Figure 5-27 through Figure 5-29.

General trends for both species show greater frequency of sea urchin embryo abnormality and juvenile mysid mortality with increasing effluent exposure with both duration and concentration. However, the *in situ* exposures provide basis for comparison and show that standard end-of-pipe exposures over-estimated toxicity in the receiving environment for the highly sensitive chronic sea urchin test and in several instances; also, for the less sensitive acute mysid exposure.

For the sea urchin embryo-larvae development test, all laboratory-based exposures exhibited toxicity throughout the concentration series tested. However, the receiving water samples that were collected prior to the storm event and during the storm event, both resulted in no toxicity. The *in situ* exposures in the SEA Rings also exhibited no toxicity.

For the mysid survival test, only the 26 h and static exposures for the sample collected at NBSD-P13 exhibited toxicity. All other stormwater samples and receiving water samples exhibited no toxicity. The *in situ* exposures did show some toxicity for the SEA Ring deployed at NBSD-P13, but only for the SEA Ring deployed at the sediment surface, possibly indicating deposition of materials that led to the toxicity observed (Figure 5-22). The SEA Ring deployed at NAB-OF09 also exhibited toxicity to the mysids for all SEA Ring deployment depths. The decreased survival that was observed is likely due to the depressed salinity that was observed during the storm events (Figure 5-26), as even the 100% concentration stormwater sample (salted for laboratory exposures) for the 96 h static exposure did not elicit a toxic response.

A study by Weston (2011) found that a reduced salinity to 10 ppt or less for a period of 6 h affected survival of *A. bahia* with few to no survivors following exposure to 6 ppt for the same timeframe. Salinity tolerance studies conducted for the City of San Diego have found that the marine kelp shrimp, *Holmesimysis costata*, is very sensitive to brief reductions in salinity, resulting in zero percent acute survival following exposure to a salinity of 20 ppt for only 30 min (AMEC 2015). The AMEC study (2015) found that sea urchin embryos were less sensitive to reduced salinities, which is also supported by the lack of toxic response observed in this current study for *in situ* exposures.

Table 5-22. Navy Region Southwest: Summary of results from Jan 2019 – *S. purpuratus* embryo-larval development.

Station/ Sample ID	Sample Exposure Duration (h)	Mean Normal Development @ 100% Concentration (%)	SD
NBSD-P13	RW-Pre	92.6	3.4
	RW-DS	83.8	5.8
	6	0.0	0.0
	12	0.0	0.0
	26	0.0	0.0
	Standard (96)	0.0	0.0
NBSD-OF39	RW-Pre	97.0	2.2
	RW-DS	97.2	2.2
	6	0.0	0.0
	12	0.0	0.0
	26	0.0	0.0
	Standard (96)	0.0	0.0
NAB-OF09	RW-Pre	100.0	0.0
	RW-DS	99.6	0.9
	6	19.0	5.6
	12	0.0	0.0
	26	0.0	0.0
	Standard (96)	nt	-
NAB-OF111	RW-Pre	94.2	5.2
	RW-DS	91.9	3.5
	6	1.4	2.2
	12	0.4	0.9
	26	0.0	0.0
	Standard (96)	0.0	0.0
NBPL-OF28	RW-Pre	94.8	2.8
	RW-DS	89.8	12.0
	6	24.1	21.4
	12	0.0	0.0
	26	2.0	4.5
	Standard (96)	0.0	0.0

RW-Pre – Receiving Water collected adjacent to sampling site prior to the onset of discharge. RW-DS – Receiving Water collected adjacent to the sampling site during active discharge. Value in **BOLD** indicates statistically significant from the control using the EPA TST approach. nt – not tested due to technician error.

Table 5-23. Navy Region Southwest: Summary of results from *in situ* deployment Jan 2019 – *S. purpuratus* embryo-larval development.

Station/ Sample ID	SEA Ring Position in Water Column	Mean Normal Development @ 100% Concentration (%)	SD
Reference	Top	97.6	1.1
NBSD-P13	Top	97.3	1.9
	Middle	96.3	3.2
	Sediment	98.1	1.4
NAB-OF09	Top	92.5	5.7
	Middle	88.2	6.8
	Sediment	94.4	3.0

Table 5-24. Navy Region Southwest: Summary of results from Jan 2019 – *A. bahia* survival.

Station/ Sample ID	Sample Exposure Duration (h)	Mean Survival @ 100% Concentration (%)	SD
NBSD-P13	RW-Pre	100	0
	RW-DS	96.7	8.2
	6	93.3	10.3
	12	96.7	8.2
	26	80.0	25.3
	Standard (96)	5.0	10.0
NBSD-OF39	RW-Pre	95.0	10.0
	RW-DS	96.7	8.2
	6	96.7	8.2
	12	93.3	16.3
	26	100	0
	Standard (96)	95.0	10.0
NAB-OF09	RW-Pre	100	0
	RW-DS	96.7	8.2
	6	100	0
	12	100	0
	26	93.3	10.3
	Standard (96)	95.0	10.0
NAB-OF111	RW-Pre	100	0
	RW-DS	96.7	8.2
	6	90.0	16.7
	12	96.7	8.2
	26	100	0
	Standard (96)	100	0
NBPL-OF28	RW-Pre	100	0
	RW-DS	96.8	8.2
	6	93.3	10.3
	12	100	0
	26	93.3	10.3
	Standard (96)	100	0

RW-Pre – Receiving Water collected adjacent to sampling site prior to the onset of discharge – 96 h static exposure. RW-DS – Receiving Water collected adjacent to the sampling site during active discharge – 96 h static exposure. Value in **BOLD** indicates statistically significant from the control using the EPA TST approach.

Table 5-25. Navy Region Southwest: Summary of results from *in situ* deployment Jan 2019 – *A. bahia* survival.

Station/ Sample ID	SEA Ring Position in Water Column	Mean Survival @ 100% Concentration (%)	SD
Reference	Top	85.0	15.2
NBSD-P13	Top	91.7	9.8
	Middle	85.0	16.4
	Sediment	78.3	22.3
NAB-OF09	Top	75.0	10.5
	Middle	56.7	18.6
	Sediment	75.0	15.2

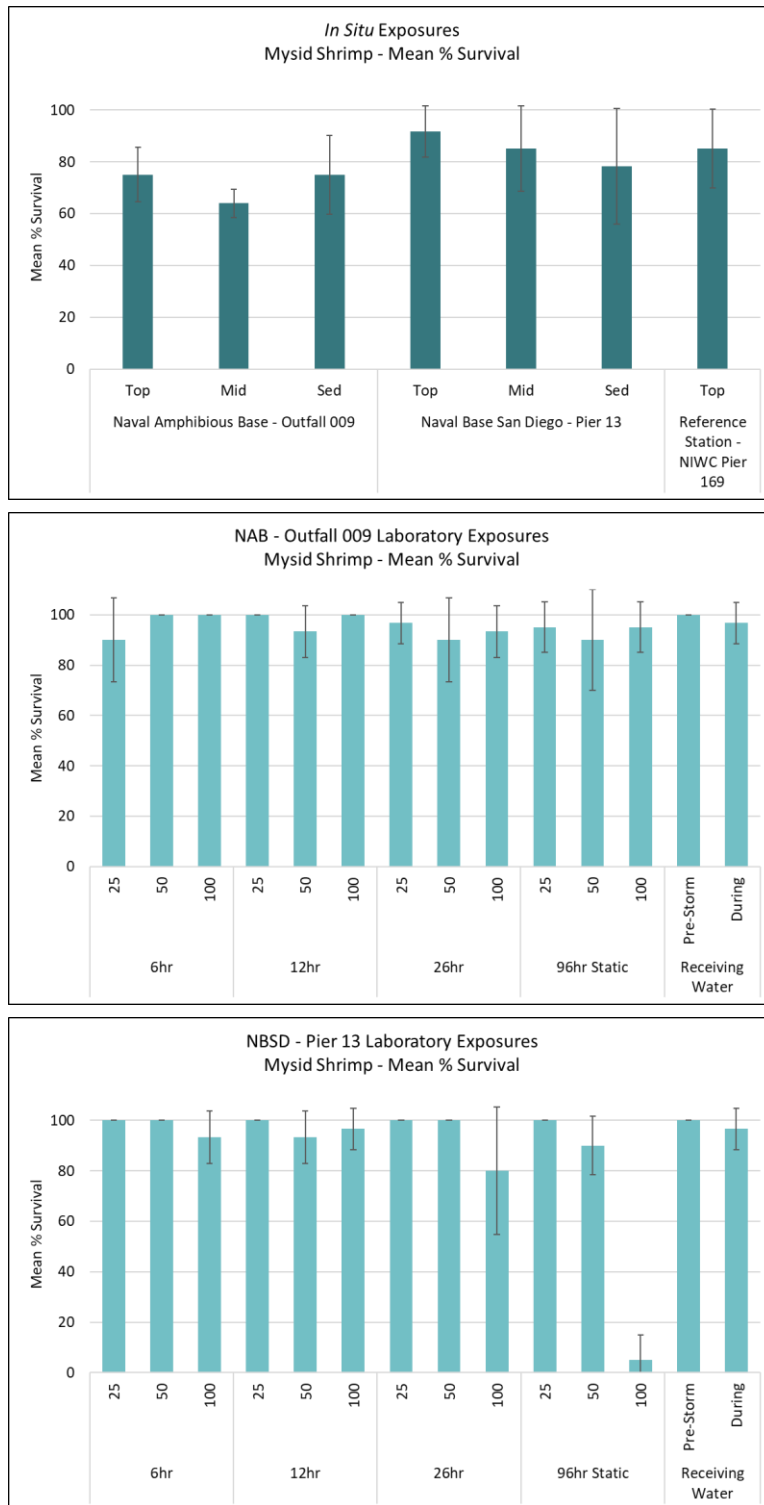


Figure 5-27. Summary of toxicity results with the mysid shrimp (*A. bahia*) for both *in situ* and laboratory-based exposures for Jan 2019. NBSD – Naval Base San Diego; NAB – Naval Amphibious Base, Coronado.

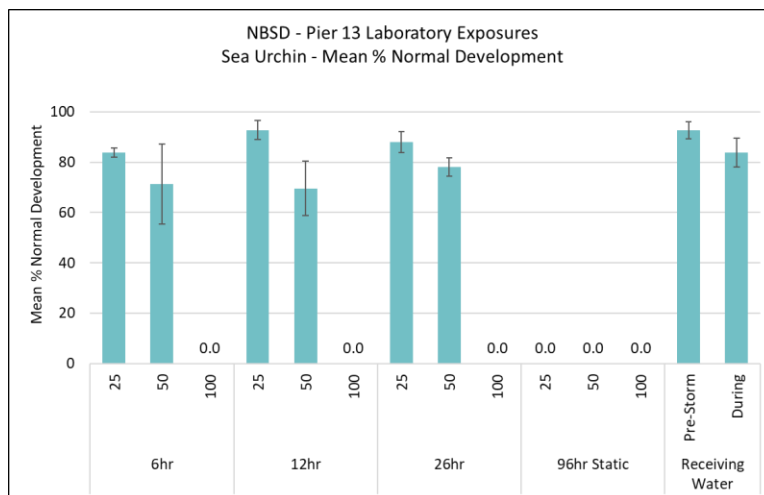
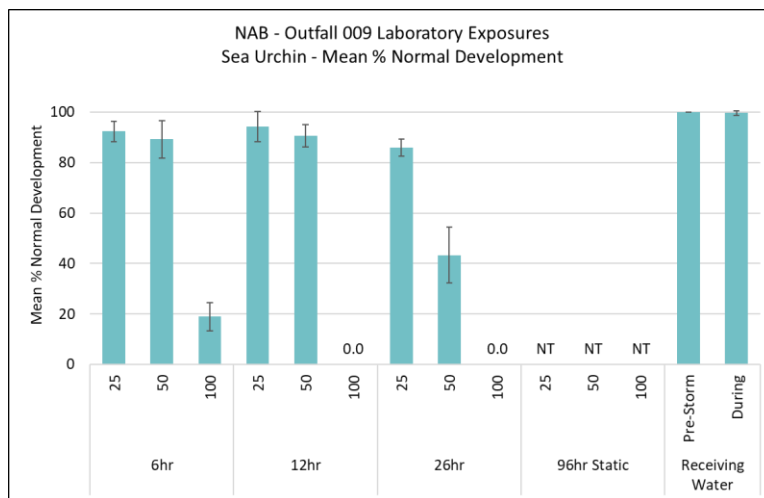
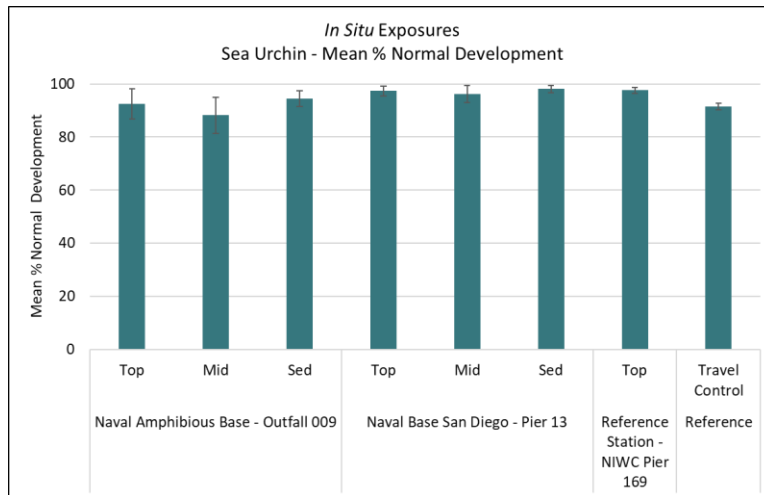


Figure 5-28. Summary of toxicity results with the purple urchin (*S. purpuratus*) for both *in situ* and laboratory-based exposures for Jan 2019. NBSD – Naval Base San Diego; NAB – Naval Amphibious Base, Coronado. NT – Not tested.

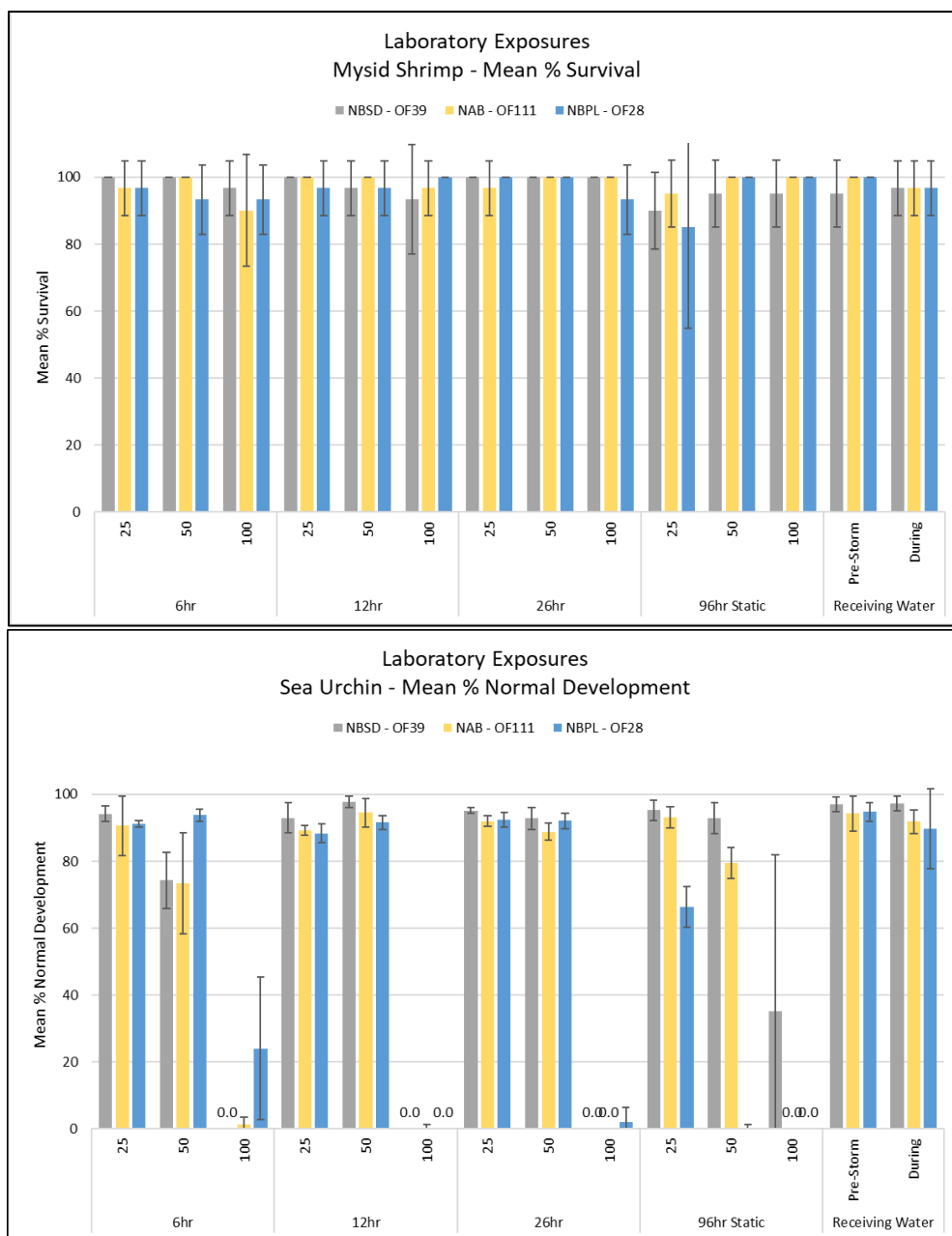


Figure 5-29. Summary of toxicity results with mysid shrimp (top) and the purple urchin (bottom) for laboratory-only exposures for the 31 Jan 2019 storm event: NBSD-OF39, NAB-OF111 and NBPL-OF28.

Analytical and DGT Chemistry. As described previously, a limited suite of analytical measurements was performed to assess the magnitude of influence for previously identified constituents of primary concern in stormwater runoff to San Diego Bay; e.g., copper, zinc, and PAHs. Total suspended solids and DOC were also analyzed for a select few samples. A complete summary of these measurements is provided in Appendix B. Note, that while analytical chemistry results presented in Appendix B state total copper and total zinc analyses were conducted, the samples were in fact filtered to 0.45µm and results reflect dissolved concentrations of metals in the samples. Measured concentrations of dissolved copper and zinc in the stormwater outfall samples

ranges from 12 to 190 µg/L and 140 to 290 µg/L, respectively. Measured concentrations of dissolved copper in the receiving water samples collected during the storm ranged from non-detect to 14 µg/L and dissolved zinc concentrations in the receiving waters collected during the storm were all non-detect. Dissolved copper and zinc collected from the reference site were both non-detect.

Time-weighted average labile concentrations of trace metals were measured from the DGT devices mounted on both the inside and outside of exposure chambers on the SEA Ring and are shown in Figure 5-30. Paired two sample t-tests for Cu and Zn show no significant difference between concentrations measures inside or outside exposure chambers (Cu: $p = 0.655$; Zn: $p = 0.569$), giving confidence that the exposure conditions inside the exposure chambers were similar to those outside the exposure chambers.

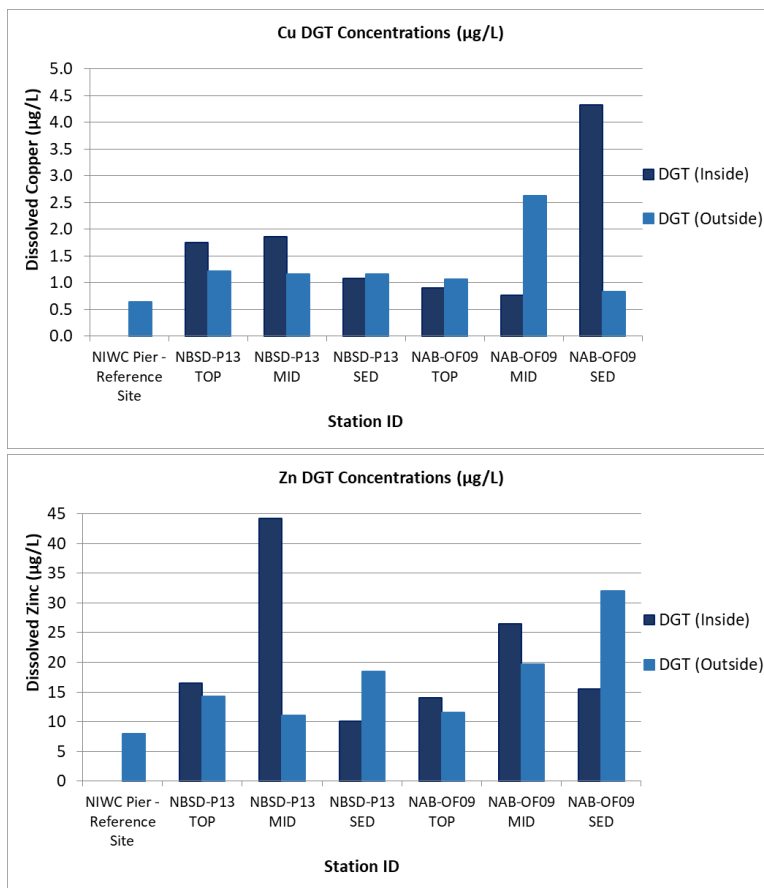


Figure 5-30. Copper (upper) and zinc (lower) concentrations measured in DGTs inside and outside the exposure chambers from the 31 Jan 2019 storm event.

Storm Event 15 May 2019

Test material collection and receipt times from the 15 May 2019 storm event are summarized in Table 5-26. Water quality parameters upon sample receipt at NIWC Pacific are summarized in Table 5-27 and in Appendix B. No precipitation was observed in the three days preceding the sample collection on 16 Jan 2019. A total of 0.18” of rainfall was observed during the SEA Ring deployment period with an initial pulse of rain on 16 May 2019 of approximately 0.02”

(<https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USW00023188/detail>).

Due to a loss of host data from the NBSD rain gauge, observations by the minute were unable to be obtained. Using the hourly data from the above reference NOAA data-site, the first pulse of rain on 16 May resulted in detectable rainfall during 1 h and trace detections for 2 h. Hourly detections of rainfall for 17, 18 and 19 May were 2, 0 and 11 h, respectively.

Salinity conditions observed *in situ* ranged from 31.2 to 32.9 ppt and averaged 32.7 ppt over the course of the deployment period.

Table 5-26. Navy Region Southwest: Sample description, collection and receipt times for May 2019.

Sample/ Station ID	Matrix/Description	Type	Sample Collection Date/Time	Sample Receipt Date/Time
NBSD-P13	Pier 13, surface stormwater runoff	Grab	5/16/2019 1145	5/16/2019 1500
NBSD-P13- RW	Receiving water adjacent to Pier 13, collected before storm event	Grab	5/15/2019 1230	5/15/2019 1500
NBSD-P13- RW-DS	Receiving water adjacent to Pier 13, collected during storm event	Grab	5/16/2019 1230	5/16/2019 1500
NBSD-OF39	Mole Pier, surface stormwater runoff discharging to OF39	Grab	5/16/2019 1204	5/16/2019 1500
NBSD- OF39-RW	Receiving water adjacent to Mole Pier at OF39, collected before storm event	Grab	5/15/2019 1700	5/16/2019 1500
NBSD- OF39-RW- DS	Receiving water adjacent to Mole Pier at OF39, collected during storm event	Grab	5/16/2019 1210	5/16/2019 1500
NBPL-OF28	Sierra Pier, surface stormwater runoff discharging to OF28	Grab	5/16/2019 1100	5/16/2019 1500
NBPL- OF28-RW- DS	Receiving water adjacent to Sierra Pier at OF28, collected during storm event	Grab	5/16/2019 1230	5/16/2019 1500
REF	Reference sample adjacent to NIWC Pacific Pier	Grab	5/16/2019 1710	5/16/2019 1500

NBSD – Naval Base San Diego; NBPL – Naval Amphibious Base, Coronado; REF – Reference Station.

Table 5-27. Water quality parameters measured upon receipt at NIWC Pacific for May 2019.

Sample/ Station ID	Temp (°C)	Dissolved Oxygen (mg/L)	pH (units)	Salinity (ppt)
NBSD-P13	22.7	8.4	7.23	1.85
NBSD-P13- RW	18.8	8.2	7.86	33.1
NBSD-P13- RW-DS	18.6	8.3	7.94	32.7
NBSD-OF39	22.7	8.2	8.50	31.2
NBSD-OF39- RW	17.1	8.4	7.83	32.6
NBSD-OF39- RW-DS	17.2	8.3	8.00	32.1
NBPL-OF28	19.1	8.3	8.72	0.0
NBPL-OF28- RW-DS	18.2	8.7	7.86	32.5

NBSD – Naval Base San Diego; NBPL – Naval Amphibious Base, Coronado; REF – Reference Station.

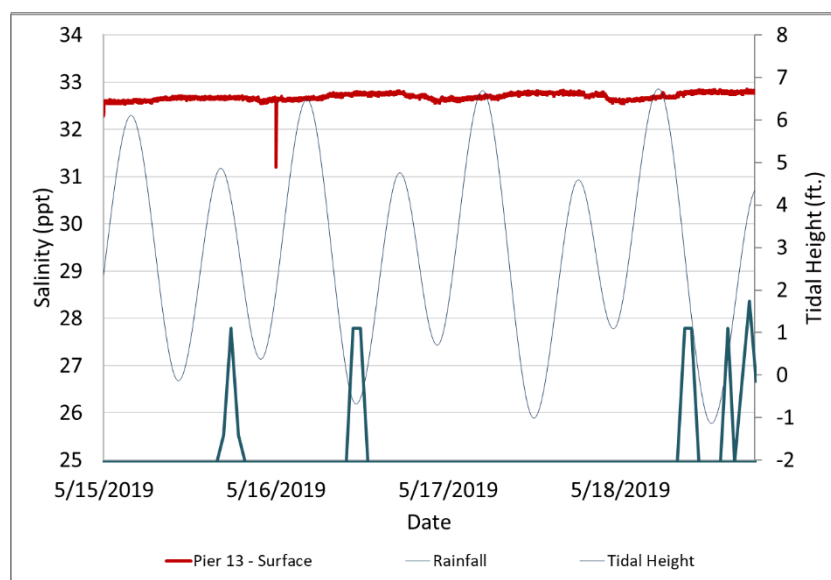


Figure 5-31. Summary of salinity, tidal height, and hourly rainfall observed during the May 2019 field effort in San Diego.

For May 2019 efforts, three SEA Rings were deployed at the P13 site at NBSD on 15 May 2019. A fourth SEA Ring was deployed at the NIWC Pacific pier as a reference site. SEA Rings were recovered from both locations on 19 May 2019 and organisms removed from the exposure chambers and evaluated. Deployed organisms were exposed to ambient conditions for approximately 16-18 h prior to the start of rainfall from the initial pulse of rain arriving on 16 May 2019. Tidal cycles

throughout the deployment were fairly extreme for the San Diego region (spring tides), ranging from -1.12' to + 6.7' (Figure 5-31). A minor freshwater signal was observed for the SEA Ring located at the surface location at P13 at Naval Base San Diego.

Laboratory and In Situ Toxicity Results. Sample collection, deployment and recovery of SEA Rings and test organisms were successful at all targeted locations. Toxicity results for both laboratory-based exposures and *in situ* exposures are summarized in Table 5-28 through Table 5-31 and shown in Figure 5-32 through Figure 5-34.

As with the previous effort, the *in situ* exposures showed that the standard end-of-pipe exposures over-estimated toxicity in the receiving environment for the highly sensitive chronic sea urchin test.

The laboratory-based sea urchin toxicity results showed increasing embryo abnormality with increasing effluent exposure with both duration and concentration. All samples exhibited 100% reduction in normal development under the standard 96 h static exposures using the TST analysis. The 26 h pulsed exposure also exhibited toxic responses for samples NBSD-OF39 and NBPL-OF28 using the TST analysis. However, *in situ* SEA Ring results as well as the receiving water samples collected before and during the storm event showed high incidence of normal embryo development.

For the mysid survival test, only the 100% concentration static exposure for sample NBSD-P13 exhibited toxicity. While the mean percent survival for NBSD-P13 was the same as the mean percent survival for NBSD-OF39, both were 85%, only NBSD-P13 was determined to be significantly toxic using the TST analysis. Each sample was compared statistically against their respective pre-storm receiving water sample. The NBSD-P13 and NBSD-OF39 pre-storm receiving water samples resulted in 95 and 90% survival, respectively. Due to the slightly reduced, yet acceptable, result for NBSD-OF39, the comparative analysis conducted with the TST led NBSD-P13 to be statistically significant and not NBSD-OF39. Similar to the Jan 2019 deployment, the SEA Ring deployed at the sediment surface at NBSD-P13 showed toxicity where the other depths did not show any toxicity; possibly indicating deposition of materials that led to the toxicity observed.

Table 5-28. Navy Region Southwest: Summary of results from May 2019 – *S. purpuratus* embryo-larval development.

Station/ Sample ID	Sample Exposure Duration (h)	Mean Normal Development @ 100% Concentration (%)	SD
NBSD-P13	RW-Pre	89.8	5.9
	RW-DS	88.0	3.0
	6	92.2	1.6
	12	93.4	2.9
	26	71.2	4.4
	Standard (96)	0.0	0.0
NBSD-OF39	RW-Pre	95.6	1.1
	RW-DS	89.6	3.0
	6	89.8	3.1
	12	92.8	3.1
	26	17.8	7.2
	Standard (96)	0.0	0.0
NBPL-OF28	RW-Pre	91.4	2.1
	RW-DS	86.8	2.8
	6	94.6	1.1
	12	89.0	4.0
	26	59.4	8.5
	Standard (96)	0.0	0.0

RW-Pre – Receiving Water collected adjacent to sampling site prior to the onset of discharge. RW-DS – Receiving Water collected adjacent to the sampling site during active discharge. Value in **BOLD** indicates statistically significant from the control using the EPA TST approach.

Table 5-29. Navy Region Southwest: Summary of results from *in situ* deployment May 2019 – *S. purpuratus* embryo-larval development.

Station/ Sample ID	SEA Ring Position in Water Column	Mean Normal Development @ 100% Concentration (%)	SD
Reference	Top	93.4	5.6
NBSD-P13	Top	93.9	6.0
	Middle	92.5	9.5
	Sediment	94.3	3.8

Table 5-30. Navy Region Southwest: Summary of results from May 2019 – *A. bahia* survival.

Station/ Sample ID	Sample Exposure Duration (h)	Mean Survival @ 100% Concentration (%)	SD
NBSD-P13	RW-Pre	95.0	10.0
	RW-DS	90.0	11.5
	6	95.0	10.0
	12	100	0.0
	26	95.0	10.0
	Standard (96)	85.0	19.1
NBSD-OF39	RW-Pre	90.0	20.0
	RW-DS	100.0	0.0
	6	95.0	10.0
	12	95.0	10.0
	26	90.0	20.0
	Standard (96)	85.0	19.1
NBPL-OF28	RW-Pre	90.0	11.5
	RW-DS	95.0	10.0
	6	95.0	10.0
	12	100	0.0
	26	90.0	11.5
	Standard (96)	90.0	11.5

RW-Pre – Receiving Water collected adjacent to sampling site prior to the onset of discharge – 96 h static exposure. RW-DS – Receiving Water collected adjacent to the sampling site during active discharge – 96 h static exposure. Value in **BOLD** indicates statistically significant from the control using the EPA TST approach.

Table 5-31. Navy Region Southwest: Summary of results from *in Situ* deployment May 2019 – *A. bahia* survival.

Station/ Sample ID	SEA Ring Position in Water Column	Mean Survival @ 100% Concentration (%)	SD
Reference	Top	98.3	4.1
NBSD-P13	Top	98.3	4.1
	Middle	96.7	5.2
	Sediment	81.7	16.0

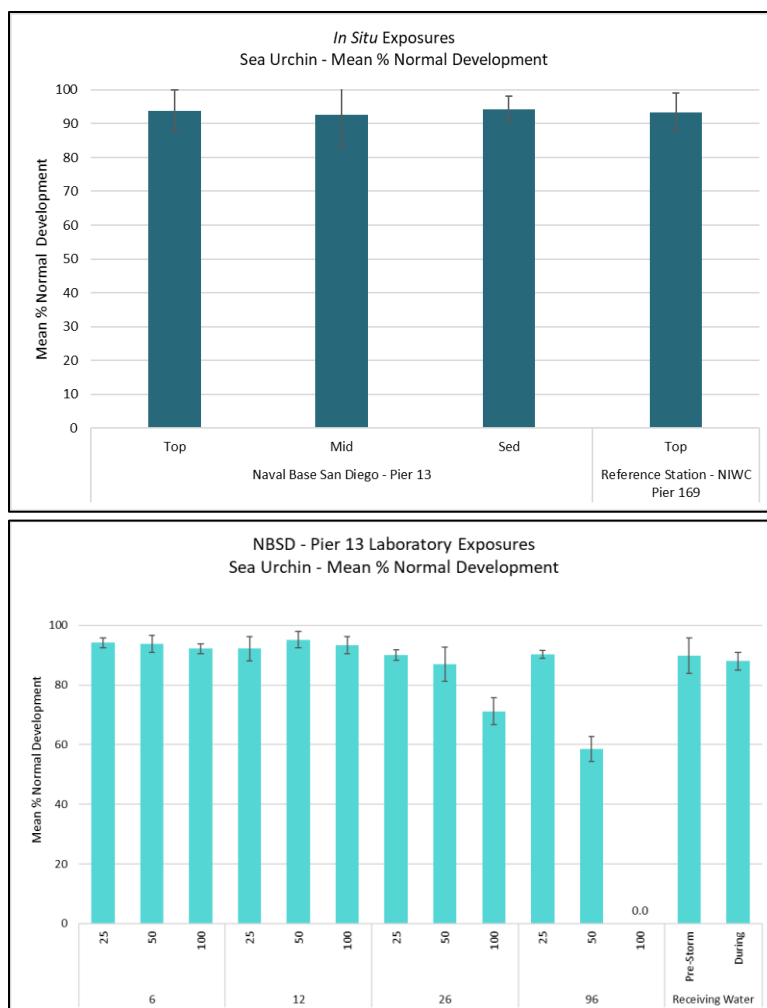


Figure 5-32. Summary of toxicity results with the purple urchin (*S. purpuratus*) for both *in situ* and laboratory-based exposures for May 2019. NBSD – Naval Base San Diego.

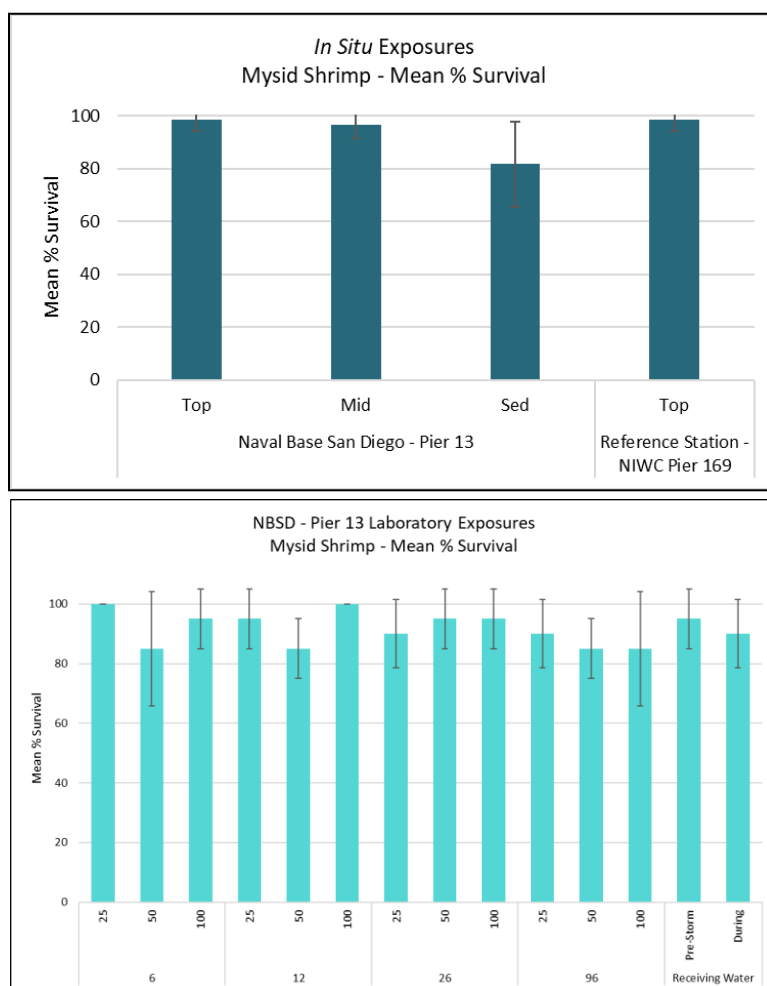


Figure 5-33. Summary of toxicity results with the mysid shrimp (*A. bahia*) for both *in situ* and laboratory-based exposures for May 2019. NBSD – Naval Base San Diego.

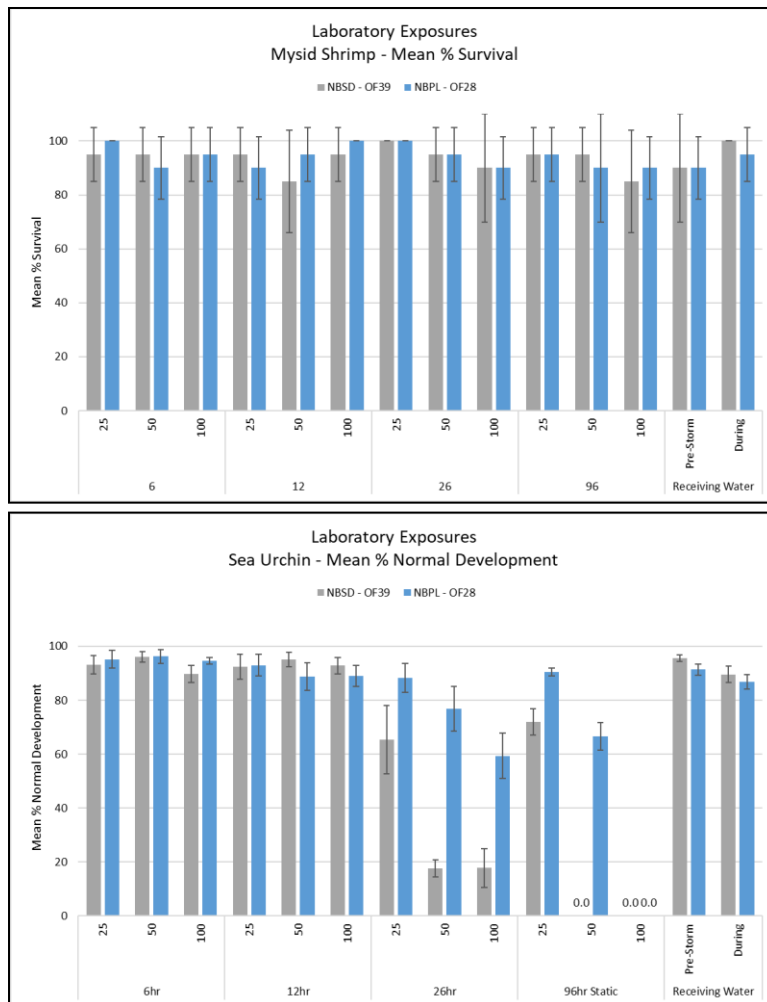


Figure 5-34. Summary of toxicity results with mysid shrimp (top) and the purple urchin (bottom) for laboratory-only exposures for May 2019: NBSD-OF39 and NBPL-OF28.

Analytical and DGT Chemistry. A complete summary of these measurements is provided in Appendix B. Note that while analytical chemistry results presented in Appendix B state total copper and total zinc analyses were conducted, the samples were in fact filtered to 0.45µm and results reflect dissolved concentrations of metals in the samples. Measured concentrations of dissolved copper and zinc in the stormwater outfall samples ranges from 76 to 96 µg/L and 140 to 310 µg/L, respectively. Measured concentrations of dissolved copper and zinc in the receiving water samples collected during the storm as well as from the reference site were all non-detect.

Time-weighted average labile concentrations of trace metals were measured from the DGT devices mounted on both the inside and outside of exposure chambers on the SEA Ring and are shown in Figure 5-35. Paired two sample t-tests for Cu and Zn show no significant difference between concentrations measures inside or outside exposure chambers (Cu: $p = 0.373$; Zn: $p = 0.783$), giving confidence that the exposure conditions inside the exposure chambers were similar to those outside the exposure chambers.

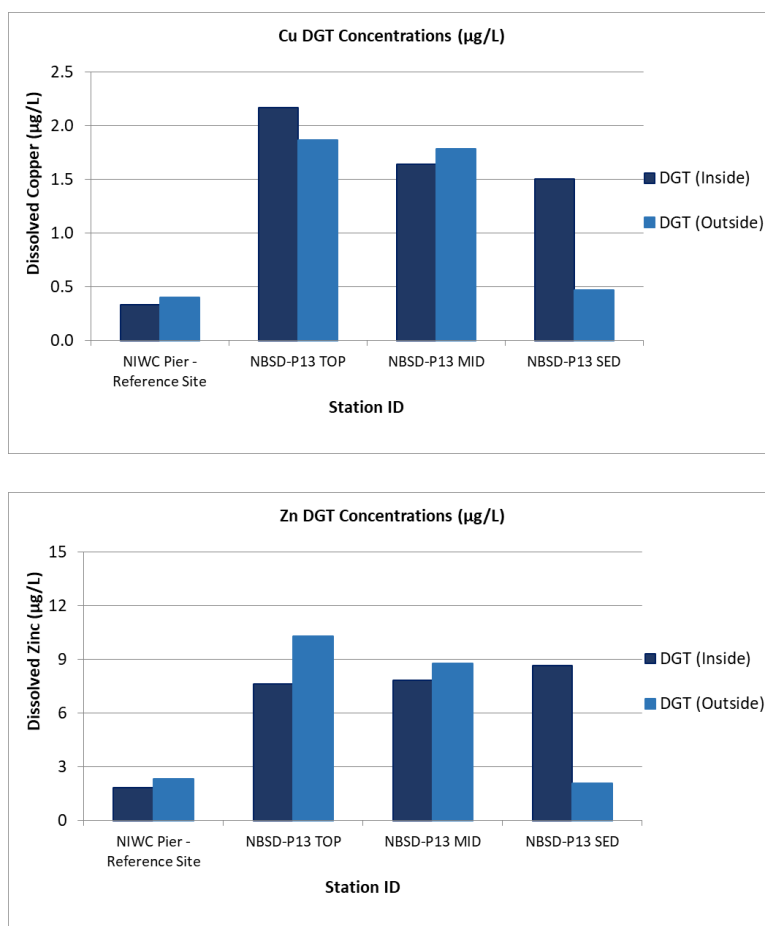


Figure 5-35. Copper (upper) and zinc (lower) concentrations measured in DGTs inside and outside the exposure chambers.

Pulsed Exposure and In Situ Demonstration Conclusion

This research determined that shorter pulsed exposures (6 h and 12 h) require significantly higher metal (copper or zinc) concentrations in order to yield similar toxic responses in both *A. bahia* and *S. purpuratus* when compared to standard, static 96 h exposures. This is consistent with several other studies in the literature using metals as the contaminant (Diamond et al. 2006, Hoang et al. 2007 a, b, Angel et al. 2010, Stransky et al. 2015, Rosen et al. 2019). This suggests that adverse effects on an organism are highly dependent on duration of exposure and is indicative of the need for alternative methods to evaluate episodic exposures. The results of this study demonstrated that current WET methods, which require static exposures between two to seven days depending on species and endpoints, may overestimate the toxicity of episodic discharges, thus minimizing their intended value towards estimating potential adverse impacts to systems receiving such discharges. However, toxicity of 26 h pulsed exposures were not statistically different when compared to 96 h exposures for both species and contaminants. This may indicate that uptake by the organism has reached a toxic threshold for these endpoints after only a 26 h exposure (Diamond et al. 2006).

Understanding the impacts of stormwater runoff on marine receiving water environments is a serious challenge using existing standard laboratory-based methods, with *in situ* assessment providing a much more realistic and defensible approach. When compared to current WET test methods, pulsed exposures are a feasible approach to assess short-term exposure conditions, are

sufficiently conservative, and account for latent effects that may be occurring. For the chronic toxicity method with *S. purpuratus*, exposure duration and timing of exposure are as critical testing conditions as is the exposure concentration when evaluating toxicity.

In situ validation was performed in San Diego during two storm events during Southern California's wet weather season of October 2018 through March 2019. Deployment of SEA Rings and associated monitoring equipment occurred at three locations in San Diego Bay concurrently during the stormwater collection events referenced above. *In situ* deployments were conducted at NBSD at Pier 13, NBC in front of industrial OF09, and at NIWC Pacific near the mouth of San Diego Bay (Figure 5- 21). Pier 13 at NBSD is at the southern end of the naval installation with a drainage area that includes mixed industrial uses of ship repair and maintenance, material storage and several parking lots. Outfall 9 at NBC includes a small surrounding industrialized watershed with paved parking lots, several buildings, and a baseball field. At the time of these field and lab efforts (October 2018 through March 2019), this outfall has a recent history of failing end-of-pipe acute toxicity tests in January and February 2018 and was under an accelerated monitoring program with the development and implementation of a Toxicity Reduction Evaluation (TRE) Plan. The location at NIWC Pacific provided a historically monitored "reference" location off of a pier near the mouth of San Diego Bay for comparison purposes. Table 5-32 below, provides a timeline for field and laboratory activities for the week prior to field deployment and sample collections up through the day of equipment retrieval and termination of laboratory-based toxicity exposures.

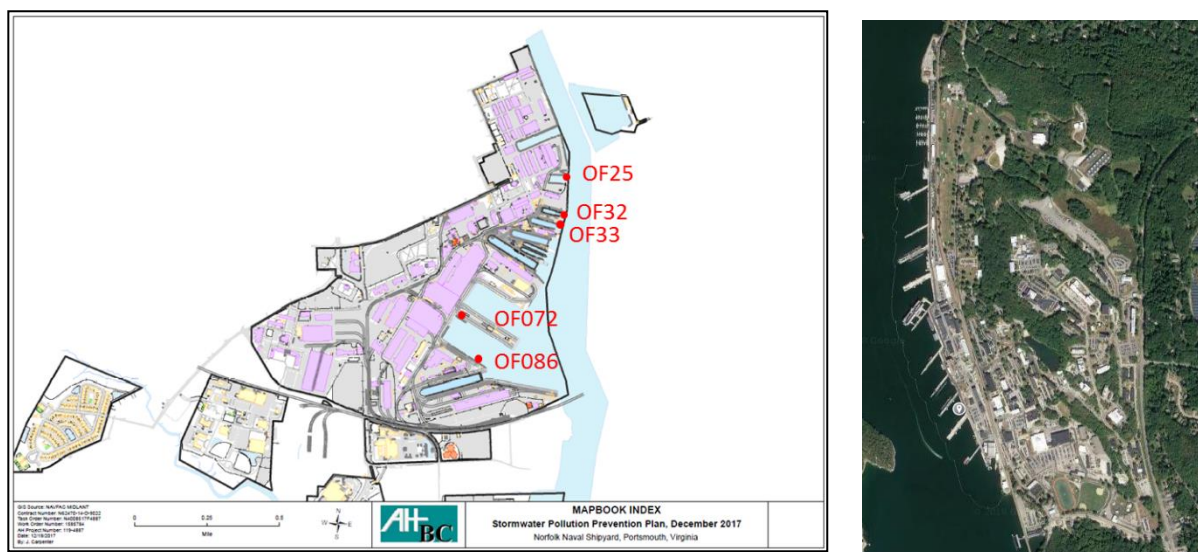


Figure 5-36. Stormwater outfall effluent stations sampled for toxicity testing for the Norfolk Naval Shipyard NPDES compliance monitoring (left) and Naval Submarine Base New London (right).

Table 5-32. Schedule of laboratory and field activities for field validation efforts.

Day	Field Tasks	Laboratory Tasks
2-7 days prior to storm/discharge event	○ Order animals, acclimate in the lab ○ Prepare datasheets, laboratory glassware and dilution waters ○ Pre-label sample collection equipment and bottles for analytical chemistry	
-1	○ Prepare SEA Rings and sondes ○ Measure pre-storm water quality ○ Observe site, take photos	○ Continue acclimation of organisms ○ Prepare necessary dilution waters
0	○ Prepare urchin embryos, and inoculate embryo drums ○ Load embryo drums and mysid shrimp into the SEA Rings ○ Set anchors, buoys, and lead lines ○ Mount data sondes onto SEA Rings and deploy ○ Measure water quality ○ Collect grab stormwater samples for laboratory exposures ○ Collect receiving water samples for pulsed exposure transfers ○ Observe site, take photos	○ Calibrate meters ○ Maintain organisms prior to initiations ○ Check in field collected samples ○ Prepare dilutions ○ Prepare concurrent reference toxicant dilutions ○ Collect subsamples for analytical chemistry ○ Measure water quality prior to initiations ○ Initiate toxicity tests ○ Perform 6-h pulsed exposure transfers ○ Perform 12-h pulsed exposure transfers
1	○ Observe site, take photos ○ Measure water quality	○ Daily water quality measurements and counts as required ○ Perform 26-h pulsed exposure transfers
2	○ Observe site, take photos ○ Measure water quality	○ Daily water quality measurements and counts as required ○ Perform standard exposure 48-h water renewals
3	○ Observe site, take photos ○ Measure water quality ○ Prepare vials to preserve urchin embryos	○ Daily water quality measurements and counts as required
4	○ Measure water quality ○ Retrieve SEA Rings, anchors and buoys from each site ○ Count mysids ○ Download water quality data from sondes ○ Download pump rate data from SEA Rings ○ Observe site, take photos	○ Water quality measurements and counts as required ○ Terminate all toxicity tests as appropriate

5.4.3 Naval Submarine Base New London

Similar to PSNS, coordination with shipyard staff at the Naval Submarine Base New London (NSBNL) facilitated the collection and shipment of stormwater samples following a qualifying event to the Wood Environment and Infrastructure Solutions, Inc. Bioassay Laboratory for subsequent standard and pulsed exposure toxicity testing using the freshwater daphnid, *Ceriodaphnia dubia*.

Test material consisted of five outfall samples collected from NSBNL. The specific outfalls at NSBNL that are targeted for sample collections during the 2018-2019 wet season are shown in Figure 4-12. On January 24, 2019, approximately 1.54" of rain fell in the Groton, CT, area (NOAA, 2019). Stormwater samples were collected in low-density polyethylene (LDPE) cubitainers under the direction of NSBNL staff. All samples were shipped FedEx priority overnight to the NIWC Pacific Bioassay Laboratory. Samples were then couriered to the Wood Toxicology Laboratory for testing. Sample collection and receipt times are summarized in Table 5-33. Water quality parameters of the samples including pH, dissolved oxygen (DO), conductivity and temperature were measured upon receipt at the laboratory prior to testing (Table 5-34).

Discrete water samples for each effluent sample were analyzed for total suspended solids, dissolved copper and zinc, polycyclic aromatic hydrocarbons (PAHs), and dissolved organic carbon (DOC). All analyses were performed by Weck Laboratories, Inc. of Industry City, CA, except for DOC, which was performed by McCampbell Analytical, Inc. of Pittsburg, CA.

Table 5-33. Sample description, collection and receipt times (Pacific Time Zone).

Sample/ Station ID	Matrix/Description	Type	Sample Collection Date/Time	Sample Receipt Date/Time
NSBNL-O-6	Stormwater sample discharged from Outfall 6 at Building 535 CB C775	Grab	1/24/2019 08:50	1/25/2019 11:35
NSBNL-O-15	Stormwater sample discharged from Outfall 15 at ARDM-4 CB C764	Grab	1/24/2019 09:29	1/25/2019 11:35
NSBNL-O-17	Stormwater sample discharged from Outfall 17 at Building 174 CB C759	Grab	1/24/2019 09:47	1/25/2019 11:35
NSBNL-O-22A	Stormwater sample discharged from Outfall 22A at Building 88 MC C530	Grab	1/24/2019 10:38	1/25/2019 11:35
NSBNL-O-27	Stormwater sample discharged from Outfall 27 at Building 38 MH C520	Grab	1/24/2019 10:18	1/25/2019 11:35

Table 5-34. Water quality parameters measured upon receipt at Wood Laboratory.

Station/ Sample ID	Temp (°C)	Dissolved Oxygen (mg/L)	pH (units)	Conductivity (µS/cm²)	Alkalinity (mg/L)	Hardness (mg/L)	Total Chlorine (mg/L)
NSBNL-O-6	3.0	11.4	5.6	2,298	12	42	0.03
NSBNL-O-15	4.0	11.6	6.4	1,171	14	35	<0.02
NSBNL-O-17	3.5	11.5	6.3	4,023	6	30	<0.02
NSBNL-O- 22A	5.0	11.3	6.6	839	7	20	<0.02
NSBNL-O-27	4.0	11.9	6.5	2,340	8	31	<0.02

All toxicity tests were conducted at the Wood Toxicology Laboratory in San Diego, CA. The freshwater daphnid tests utilized moderately hard water created from a mixture of Perrier® mineral water and deionized water (USEPA 2002). Toxicity test methods followed established protocols (USEPA 2002) as well as pulsed exposure methods (Colvin et al. 2020b) and summarized in Table 5-5. In brief, test organisms were exposed to the stormwater solutions for 6, 12, or 26 hours. At the end of this time period, test organisms were transferred into clean laboratory water for the remainder of the test period. All samples were tested at 25, 50 and 100% concentration. Standard water quality measurements (DO, temperature, conductivity, and pH) were monitored as per protocol requirements. For all tests, standard exposure duration testing was conducted concurrently to allow for direct comparison of results. The median lethal concentration (LC₅₀) was determined for each pulsed and standard exposure duration with the CETIS statistical software. A concurrent reference toxicant test using Cu was conducted as a quality control measure to assess the health of the organisms and technical performance of the method.

Each pulse duration tested had two separate controls as the stormwater samples were tested in two groups with the samples sharing a lab control. The laboratory control #1 for the first group of samples for the 6 h and 26 h pulse did not meet test acceptability criteria for mean percent survival of 90%. The laboratory control #2 for the second group of samples did meet test acceptability criteria and therefore statistical analyses were performed against laboratory control #2 for the 6 h and 26 h pulse durations. All other controls met test acceptability criteria for mean percent survival of 90%. The first group included samples NSBNL-O-6 and NSBNL-O-15. The second group included NSBNL-O-17, NSBNL-O-22A and NSBNL-O-27. A summary of results is shown in Table 5-35 through Table 5-37.

Sample NSBNL-O-6 showed complete mortality across all concentrations tested for all exposure regimes. Sample NSBNL-O-15 showed a significant decrease in survival for the 25% concentration for the 26 h pulse for the 24 h and 48 h test endpoints (Table 5-35). However, at the 24 h test endpoint, while the decrease was statistically significant, a 90% survival rate in an effluent sample is typically deemed non-toxic following the Test for Significant Toxicity (TST) analysis where, for the cerio survival tests, a percent difference of ≤ 10 is considered passing. (USEPA 2010). Since the highest concentration tested (100%) showed no significant decrease in survival, the sample is deemed non-toxic for the 26 h pulsed exposure at the 24 h test endpoint.

Median lethal concentrations (LC₅₀) were calculated and are shown in Table 5-38. For sites NSBNL-O-15, NSBNL-O-22A and NSBNL-O-27, the LC₅₀ values were estimated due to no dose response was elicited in any of the pulsed or standard exposure durations. For NSBNL-O-6, the LC₅₀ values were also estimated because mortality was observed across all concentrations and pulsed exposures tested. NSBNL-O-17 had LC₅₀ values calculated and generally decreased with increasing

exposure duration, indicating increased sensitivity. However, the 26 h pulse for NSBNL-O-17 did not fall in this pattern. Test initiations were grouped by pulse length, and it is possible that organism sensitivity could have varied slightly among pulses.

Table 5-35. Summary of results – *Ceriodaphnia* 24 & 48 h survival: Outfalls NSBNL-O-6 & NSBNL-O-15.

Sample ID	Stormwater Exposure Duration (h)	Test Concentration (%)	Mean Survival (% alive)	
			24 h	48 h
-	6	Laboratory Control #1	70*	70*
	12		95	95
	26		85*	80*
	Standard exposure		100	90
NSBNL-O-6	6	25	0	0
		50	0	0
		100	0	0
	12	25	0	0
		50	0	0
		100	0	0
	26	25	0	0
		50	0	0
		100	0	0
	Standard exposure	25	0	0
		50	0	0
		100	0	0
NSBNL-O-15	6	25	100	100
		50	100	95
		100	100	100
	12	25	100	100
		50	100	100
		100	95	95
	26	25	90	65
		50	100	100
		100	100	100
	Standard exposure	25	95	95
		50	100	100
		100	100	95

Shaded cells indicate a statistically significant decrease compared to the control.

* Controls did not pass test acceptability criteria for these pulsed exposures. Statistical comparisons for 6 h and 26 h pulsed exposure of NSBNL-O-6 and NSBNL-O-15 were made against Laboratory Control #2.

Table 5-36. Summary of results – *Ceriodaphnia* 24 & 48 h survival: Outfalls NSBNL-O-17 & NSBNL-O-22A.

Sample ID	Stormwater Exposure Duration (h)	Test Concentration (%)	Mean Survival (% alive)	
			24 h	48 h
-	6	Laboratory Control #2	100	100
	12		100	100
	26		100	100
	Standard exposure		100	100
NSBNL-O-17	6	25	50	40
		50	10	10
		100	25	10
	12	25	20	15
		50	5	5
		100	35	0
	26	25	100	100
		50	30	30
		100	70	35
	Standard exposure	25	45	45
		50	30	5
		100	0	0
NSBNL-O-22A	6	25	95	90
		50	70	70
		100	80	80
	12	25	100	100
		50	60	60
		100	80	75
	26	25	95	95
		50	100	95
		100	95	95
	Standard exposure	25	95	95
		50	90	90
		100	80	65

Shaded cells indicate a statistically significant decrease compared to the control.

Table 5-37. Summary of results – *Ceriodaphnia* 24 & 48 h survival: Outfalls NSBNL-O-27.

Sample ID	Stormwater Exposure Duration (h)	Test Concentration (%)	Mean Survival (% alive)	
			24 h	48 h
-	6	Laboratory Control #2	100	100
	12		100	100
	26		100	100
	Standard exposure		100	100
NSBNL-O-27	6	25	95	95
		50	95	95
		100	95	90
	12	25	100	100
		50	95	95
		100	100	100
	26	25	100	100
		50	100	100
		100	95	90
	Standard exposure	25	100	95
		50	100	100
		100	95	85

Shaded cells indicate a statistically significant decrease compared to the control.

Table 5-38. Summary of statistical results – *Ceriodaphnia* survival.

Station/ Sample ID	Stormwater Exposure Duration (h)	LC ₅₀ (% sample)	
		24 h	48 h
NSBNL-O-6	6	< 12.5	< 12.5
	12	< 12.5	< 12.5
	26	< 12.5	< 12.5
	standard	< 12.5	< 12.5
NSBNL-O-15	6	> 100	> 100
	12	> 100	> 100
	26	> 100	> 100
	standard	> 100	> 100
NSBNL-O-17	6	25.0	20.8
	12	15.6	14.7
	26	70.7	41.8
	standard	22.7	22.7
NSBNL-O-22A	6	> 100	> 100
	12	> 100	> 100
	26	> 100	> 100
	standard	> 100	> 100
NSBNL-O-27	6	> 100	> 100
	12	> 100	> 100
	26	> 100	> 100
	standard	> 100	> 100

Analytical Chemistry. A summary of the chemistry results for water samples collected are shown in Table 5-39. All data, QA summaries, data qualifiers and sample chain-of-custody forms reported by the laboratory are provided in Appendix C. All quality control (QC) parameters were within the established control limits. All laboratory blanks were non-detects. Laboratory spikes, matrix spikes and matrix spike duplicates all had appropriate percent recoveries for each analyte prepared.

Table 5-39. Analytical chemistry results.

Station ID	Total Suspended Solids (mg/L)	Dissolved Copper (µg/L)	Dissolved Zinc (µg/L)	Total PAHs (µg/L)	Dissolved Organic Carbon (mg/L)
NSBNL-O-6	11	640	1300	ND	4.6
NSBNL-O-15	160	ND	67	ND	3.6
NSBNL-O-17	100	ND	440	ND	2.3
NSBNL-O-22A	11	ND	130	0.20	1.6
NSBNL-O-27	62	ND	ND	24.01	2.3

* ND – Not detected at Method Reporting Limit (MRL). MRLs: TSS – 5mg/L (SM2540D); Cu – 0.010 mg/L, Zn – 0.050 mg/L (EPA 200.7); PAH – 0.20 µg/L (EPA 8270C SIM); DOC – 0.7 mg/L (SM5310B).

Note: Analytical chemistry reports metals data as total, however, samples were filtered to 0.45µm for dissolved metal analysis and are reported as such.

Discussion. The results of this study showed that modifying standard WET methods is a feasible approach to assess short-term exposure conditions.

Table 5-40 approximates the time-averaged concentrations of Cu and Zn that the organisms were exposed to test organisms during the shortest pulsed exposure duration of 6 h. For example, for NSBNL-O-6, the dissolved Cu concentration for the whole effluent samples was 640 µg/L. Organisms were exposed to this sample for 6 h and then transferred to clean laboratory water for the remainder of the 24 and 48 h test periods, would have been exposed on average to 160 and 80 µg/L Cu, respectively. For the lowest concentration tested (i.e., 25%), the time-averaged concentration would be 40 and 20 µg/L Cu for the 24 and 48 h test periods, respectively. These concentrations are within the historic range of the LC₅₀s generated from this lab expected for healthy organisms (11.5 – 45.9 µg/L) and therefore, the response observed for this sample is not unexpected. An EPA Ecotox database (<https://cfpub.epa.gov/ecotox/>) search reports that LC₅₀ concentrations of Zn range from 100 to 540 µg/L for this species. Similarly, to the Cu, the Zn concentrations in the NSBNL-O-6 were sufficiently high enough to elicit a response to the *Ceriodaphnia*, even at the reduced exposure times.

Table 5-40. Approximate Time-Average Concentrations for 6 h Pulsed Exposures.

Station ID	Dissolved Copper (µg/L)	Approximate Time Averaged Concentration (µg/L)		Dissolved Zinc (µg/L)	Approximate Time Averaged Concentration (µg/L)	
		24 h Endpoint	48 h Endpoint		24 h Endpoint	48 h Endpoint
NSBNL-O-6	640	160	80	1300	325	163
NSBNL-O-15	ND	-	-	67	16.8	8.4
NSBNL-O-17	ND	-	-	440	110	55
NSBNL-O-22A	ND	-	-	130	32.5	16.3
NSBNL-O-27	ND	-	-	ND	-	-

The laboratory pulsed testing procedures were conducted under conditions that reflected realistic stormwater conditions found at the end-of-pipe at the New London facility. Rainfall on 24 January 2019, began at approximately 06:00 local time and no further precipitation was recorded after 11:00 (Wunderground.com). The range of exposure durations tested in the laboratory were appropriate, if not overly-conservative, relative to the duration of rainfall that fell during this particular event. This simple modification of the standard WET procedure provides a reasonable logistical change that could easily be implemented in future testing.

Although the test procedures focused on exposure conditions likely to occur at the end-of-pipe, those conditions are still conservative in comparison to actual exposures that occur once the stormwater is discharged to, and mixes with, receiving waters.

5.5 INTERLABORATORY CALIBRATION STUDY

The objective of the Interlaboratory Calibration Study (ICS) was to assess the ability of the pulsed exposure methodology to provide consistent and defensible data for monitoring of episodic discharges and is fully described in Colvin et al. (2021b). The organization, implementation and performance goal objectives of the study was comparable to that performed by EPA's interlaboratory calibration study of standardized freshwater and marine WET methods conducted in 1999-2000 (USEPA 2001).

The specific goals in conducting the pulsed toxicity exposure Interlaboratory Calibration Study were:

1. Evaluate the ability of laboratories to successfully execute the pulsed exposure toxicity methods for each species (completion rate);
2. Evaluate the rate at which laboratories detected toxicity when evaluating non-toxic samples (false positive rate);
3. Characterize the interlaboratory variability of the three modified pulsed exposure test methods for each species through the comparison of coefficients of variation (CVs) for median lethal effect and median sublethal effect (LC₅₀ and EC₅₀) concentration values and ranges for no observed effect concentration (NOEC) values and 95% confidence intervals.
4. Assess repeatability by comparing results obtained for both pulsed and static exposures in blind duplicate samples.

The following three WET methods that were modified for pulsed exposures and included in the ICS were:

- Cladoceran, *Ceriodaphnia dubia*, acute test (*Ceriodaphnia* acute)
- Mysid, *Americamysis bahia*, acute test (Mysid acute)
- Purple Sea Urchin, *Strongylocentrotus purpuratus*, larval development/short-term chronic (Purple Sea urchin chronic)

The following study design was followed for conducting this effort and described in brief in the subsections below:

- Laboratory Procurement;
- Sample Preparation;
- Preliminary Testing;
- Sample Distribution;
- Interlaboratory Calibration Study Testing;
- Data Review and Analysis.

5.5.1 Laboratory Procurement

National or state-certified toxicology laboratories for the species tested were sought out to participate in the intercalibration study. The parent list of laboratories and primary contacts were derived from those that have participated in the North America Society of Environmental Toxicology and Chemistry (SETAC) Aquatic Toxicity Testing Interest Group (ATTIG) during an initial meeting at the North America National SETAC conference in Toronto, Canada in 2019 and thereafter. A total of 14 laboratories in the United States and one laboratory in Canada were contacted via phone calls

and email. Of those contacted a total of 10 of laboratories in the United States and the one laboratory in Canada expressed interest in participation.

After confirming those laboratories that were interested in the study a request for qualifications (RFQ) and costs package was sent out to each laboratory. A third-party contractor, Edmond Scientific Company, managed the RFQ coordination and cost negotiations with each interested laboratory.

Upon receiving laboratory qualification packages, a team of scientists from NIWC Pacific, Wood, and Edmond Scientific independently reviewed each submittal and scored each laboratory using a rubric as shown in Table 5-41 and Table 5-42. A goal for the program was to include a minimum of five and up to seven qualified laboratories for each test procedure. All selected laboratories were paid to participate in the study, which limited the total number of participating laboratories possible for this program.

Table 5-41. Pulsed exposure participating laboratory scoring criteria.

Technical Capability (50 points)			
Experience/Capability (20 points)	Submittal Completeness (20 points)	Comprehension/Workplan reflects understanding of SOP (10 points)	
Staff Qualifications (40 points)			
Qualifications of Proposed Lead? (20 points)	Qualifications of Proposed Supporting Staff? (10 points)	Overall Years of Experience? (5 points)	Staff Redundancy (COVID-19 considerations)? (5 points)
Past Performance (10 points)			
Within 5 years? Y/N	Relevant Experience? (5 points)	Number of Citations/ Presentations Related to Pulsed Exposures (5 points)	

Table 5-42. Scoring rubric for each sub-category.

20 points	10 points	5 points	
15-20	8-10	5	Far Exceeds Requirements - Excellent
10-15	6-8	4	Exceed Requirements - Very Good
5-10	4-6	3	Meets Requirements - Acceptable
0-5	0-4	1-2	Does Not Meet Requirements - Unacceptable

After selection of participant laboratories several meetings were held specific to each test species to summarize the pulsed exposure method in person and to cover any questions, concerns, or clarifications needed in the SOPs that were provided along with the RFQ. A training video that was developed by NIWC Pacific and Wood was also played showing various acceptable methods that

may be used to transfer test organism to clean laboratory water after the initial pulsed exposure. Following meetings and follow up communications to clarify any outstanding items, a revised set of final SOPs were provided and redistributed to all participating laboratories for reference. Finally, testing dates were selected that would best accommodate the multiple laboratory schedules. Each participating laboratory was assigned a random number to keep the identity of the laboratory associated with each dataset blind throughout the study including all data analysis and reporting efforts.

5.5.2 Sample Preparation

For each test method, four samples were prepared in bulk in precleaned and rinsed 151 L (40 gallon) HDPE carboys by the project team at the Wood Bioassay Laboratory. The sample types are listed below:

1. A blank sample – a negative control consisted of laboratory water obtained from the referee laboratory appropriate for a given test method;
2. A non-toxic stormwater sample – collected from the San Diego region (salted with Crystal Sea Marine Mix © as appropriate for the marine species a given test methods);
3. A toxic stormwater sample – this sample was spiked with copper sulfate to ensure a consistent measurable toxic effect (salted with Crystal Sea Marine Mix© as appropriate for the marine species test methods);
4. A copper-spiked sample – a positive control prepared using referee laboratory water spiked with copper sulfate at a concentration that resulted in a consistent measurable dose response for the particular species;
5. A fifth duplicate sample was sent to each laboratory for a total of five samples tested. This duplicate was the positive control for every laboratory (Sample 4).

The stormwater sample was collected on 6 April 2020 from a public roadway located in central San Diego (4360 Morena Blvd.; approximately 200L of stormwater runoff was collected by the project team. The concentration of dissolved organic carbon (DOC) was 5.5 mg/L. Concentrations of total and dissolved Cu were 7.4 and 5.8 µg/L, respectively (Weck Analytical Laboratory, USEPA Method 200.8).

All sample types were subsampled at the time of sample distribution for water quality characterization (i.e., salinity, conductivity, dissolved oxygen [DO], temperature, pH). Samples were sent to Weck Analytical Laboratory for measurements of dissolved copper (USEPA method 200.8).

The laboratory control water that was used in the freshwater studies consisted of diluted mineral water (DMW) prepared by the referee laboratory, Wood Bioassay Laboratory. The laboratory control water that was used in the marine studies consisted of seawater collected from Scripps Institution of Oceanography (SIO), referred hereafter as filtered seawater (FSW). Raw seawater at SIO is collected from an intake near the end of the 1084-foot Ellen Browning Scripps Memorial Pier in La Jolla, CA, and is pulled through a series of large sand filters prior to distribution points.

Spiked, or positive control, samples were prepared by the volumetric addition of 1000 µg/L Cu stock solution to a targeted concentration as was determined by the studies performed described in section 5.3. Water quality characterization of freshwater and marine samples are shown in Table 5-43 through Table 5-45.

Table 5-43. Water quality characterization of freshwater samples.

Sample #/ Type	DO (mg/L)	pH (pH units)	Conductivity (µS/cm)	Alkalinity (CaCO ₃ /L)	Hardness (CaCO ₃ /L)	Targeted Nominal Cu (µg/L)	Dissolved Cu (µg/L)
1 Negative Control	9.6	7.71	209	76	91	0.0	1.6
2 Non-toxic Stormwater	9.8	6.58	54	62	26	0.0	7.5
3 Toxic Stormwater	10.6	7.61	400	79	150	200	170
4 Positive Control	9.6	8.15	209	68	88	100	95
5 Duplicate Sample	9.6	8.11	191	65	87	100	88

Table 5-44. Water quality characterization of marine samples – Mysid (*Americamysis*) acute test.

Sample #/ Type	DO (mg/L)	pH (pH units)	Salinity (ppt)	Targeted Nominal Cu (µg/L)	Dissolved Cu (µg/L)
1 Negative Control	8.5	7.92	32.1	0.0	5.2
2 Non-toxic Stormwater	9.3	7.79	29.8	0.0	ND
3 Toxic Stormwater	9.8	7.69	29.4	1600	1000
4 Positive Control	9.0	7.90	32.0	800	580
5 Duplicate Sample	9.0	7.86	32.3	800	580

ND – Non-detect at method reporting limit of 5.0 µg/L.

Table 5-45. Water quality characterization of marine samples – Purple Sea Urchin chronic test.

Sample #/ Type	DO (mg/L)	pH (pH units)	Salinity (ppt)	Targeted Nominal Cu (µg/L)	Dissolved Cu (µg/L)
1 Negative Control	8.6	7.98	32.5	0	10
2 Non-toxic Stormwater	8.9	7.98	29.6	0	ND
3 Toxic Stormwater	8.5	7.90	31.0	400	390
4 Positive Control	9.2	7.96	32.5	200	210
5 Duplicate Sample	8.8	7.96	32.1	200	230

ND – Non-detect at method reporting limit of 10.0 µg/L.

5.5.3 Preliminary Testing

Preliminary testing of all samples used in the Pulsed ICS were conducted by the referee laboratory, Wood Bioassay Laboratory. Testing was conducted to confirm control and nontoxic stormwater samples were stable and could achieve control acceptability criteria, and that copper-spiked laboratory water and stormwater resulted in a sufficient repeatable toxic dose response for each sample for use in the Pulsed ICS. Previous testing (Rosen et al. 2019; results from section 5.3) has shown a decrease in the sensitivity for pulsed exposures relative to standard, static exposures. Thus, preliminary testing included both standard continuous exposures and the modified and pulsed exposure method for each sample and method combination.

Laboratory control water (negative control), appropriate for each species, was tested to ensure a lack of toxicity and the stormwater sample that was collected for this study was tested to determine relative toxicity, if any, for each species using the pulsed exposure methodology. The stormwater sample exhibited no toxicity for any of the species evaluated for either the pulsed or standard methods. Both the laboratory control water and the stormwater sample were then spiked with copper sulfate (CuSO₄) specific for each species to create the positive control sample and the toxic stormwater sample. Spiked concentrations for both samples were based on analytical chemistry properties and historical results from previous spiking studies (Rosen et al. 2019; results from section 5.3).

Definitive testing of the spiked samples was then conducted for each species to ensure a desired dose response for use in the ICS. For the freshwater testing, Sample #3: the toxic stormwater sample, definitive testing determined that the first preparation of the sample was more toxic than desired and thus a 25% dilution of the sample was made through the addition of DMW. This too was determined to be result in more toxic dose response than desired and thus a second 25% dilution was made with DMW for a final nominal concentration of approximately 200 µg/L Cu. Final definitive testing

resulted in a dose response that was acceptable, and the sample was deemed ready for distribution to participant laboratories for the ICS.

No further sample manipulations were required for the samples prepared for the mysid acute testing as preliminary testing showed that acceptable dose responses were observed for each sample tested. For the samples prepared for the purple sea urchin short-term chronic tests, Sample #3: the toxic stormwater sample, a 50% dilution of the sample was conducted following the first round of preliminary testing showed that the sample was more toxic than desired. The targeted nominal concentration was then 400 µg/L Cu. The second round of definitive testing with the purple urchins resulted in an appropriate dose response and the sample was deemed ready for distribution to participant laboratories.

5.5.4 Sample Distribution

Laboratories participating in the ICS each received five blind samples, one of which was a duplicate of the spiked laboratory water sample (#4). For each method tested, the sample ID number was randomized so that participant laboratories would not be aware of potential response if they were involved with multiple methods. Bulk test samples were distributed to appropriately sized LDPE cubitainers that were rinsed with sample water three times prior to the final fill. All cubitainers were capped with zero head space and placed in coolers lined with heavy duty plastic bags. Wet ice was added thoroughly to surround all samples and plastic bags were then secured by a knot or zip tie. Plastic bags were used to minimize potential leakage of wet ice and samples during shipment. Chain-of-custody forms were placed in large Ziploc bags and laid on top of the samples within the coolers. Coolers were then secured with shipping tape and pre-printed shipping labels secured to the top of each cooler. Coolers were labeled by the random number that was assigned to each Laboratory. All coolers were shipped priority overnight via FedEx to laboratories located outside the San Diego region. Laboratories co-located in the vicinity of San Diego; CA received coolers via hand-delivery by NIWC Pacific personnel. Referee laboratories, which were located in San Diego, CA, kept samples in coolers overnight and “received” the samples on the following day to mimic overnight shipment.

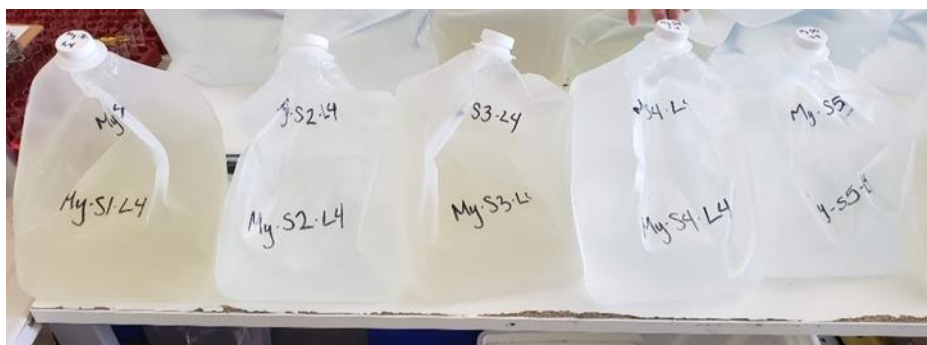


Figure 5-37. Sample preparation for one of the mysid shrimp testing dates.

Americamysis bahia were provided to each participant laboratory by the project team by Aquatic Biosystems, Inc. (ABS, Fort Collins, CO). In order to meet the quantities necessary for all laboratories to test the samples with a similar batch of organisms, testing was broken into two testing weeks with mysid shrimp shipped priority overnight from ABS to each participant laboratory on the Wednesday of each testing week. Four laboratories, including a referee laboratory, participated in the first round of testing with mysids shipped on 8 July 2020. Five laboratories, including a referee laboratory, participated in the second round of testing with mysids shipped on 15 July 2020. For each

round of testing, mysids were in the age range of two to three days old upon shipment. Mysids were used for test initiations on the day they were received, when they were three to four days old.

Purple sea urchins, *Strongylocentrotus purpuratus*, were provided along with the samples to each of the laboratories participating in the short-term chronic testing. Purple sea urchins were field collected from kelp beds located offshore of Point Loma, CA, on 23 July 2020 and held in flow-through seawater tanks at the NIWC Pacific Bioassay Laboratory. On the Wednesday of the testing week, purple sea urchins were brought to the Wood Bioassay Laboratory in coolers with blue ice and seawater dampened clean rags. Approximately 18 randomly selected urchins were packaged in plastic shoe box containers with blue ice and seawater dampened clean rags to minimize jostling in transit (Figure 5-38). These boxes with the test organisms were placed in one of the two coolers exterior to the plastic bags filled with the samples and wet ice that were sent to each participant laboratory.



Figure 5-38. Packaging of purple sea urchins for the ICS effort.

Ceriodaphnia used for ICS efforts were provided by each laboratory from in-house organism cultures.

5.5.5 Interlaboratory Calibration Study Testing

For each of the five samples, including the duplicate sample, laboratories were expected to conduct toxicity testing using species-specific pulsed exposure methods using a 26 h exposure duration to each sample followed by transfer to laboratory control water. Each sample was tested using a 5 concentration dilution series (e.g., 6.25, 12.5, 25, 50 & 100% concentration of whole sample). Each sample was also tested under standard static exposure conditions with 6.25 & 100% concentrations. Samples were to be tested alongside a laboratory control that was prepared in accordance with EPA WET protocols following specific methods employed by each individual participant laboratory. In addition, concurrent reference toxicant tests were to be performed by each laboratory according to internal procedures employed by each individual participant laboratory.

5.5.6 Data Review and Analysis

Within three weeks of test termination, laboratories were requested to submit their testing results to the ESTCP project team using an Excel-based Electronic Data Deliverable (EDD) that was provided by the project team. Fields of entry included water quality information of the samples upon receipt and daily water quality measurements during the course of the toxicity tests (i.e., DO, pH, salinity or conductivity and pH). Information on the testing dates/times and individual replicate performance was documented in the EDD as well. To assist laboratories that may have questions about methods or how to input data into the provided EDD, the project team provided open support hours via MS Teams on a weekly basis. A brief report was also requested of each laboratory following testing to document the test acceptability of their methods and whether their reference toxicant test met internal quality control requirements. EDD submissions were received from all participant laboratories where tests were completed.

For the *Ceriodaphnia* acute method, two laboratories were unable to complete testing due to complications with the in-house organism cultures that resulted in the tests failing to meet test acceptability criteria (TAC) for the controls. Data for the *Ceriodaphnia* acute method was excluded for a third laboratory due to initiation issues. The reported dose responses (e.g., lack of dose responses) from this laboratory were considered extreme outliers and warranted discussions with the laboratory to investigate any issues that may have been encountered. It was determined that a new employee at the laboratory added an excessive amount of culture water along with the organisms that diluted the samples and resulted in the lack of dose responses that were reported. As a result of these deviations, interlaboratory calibration data for the *Ceriodaphnia* acute method included results from six laboratories and the referee laboratory.

For the mysid acute method, data from nine laboratories was received. Data from a single laboratory was excluded due to a lack of dose response observed in the reported results for the spiked “toxic” samples. Discussions with the laboratory failed to provide a resolution and a lack of confidence in the data reported led the project team to exclude this dataset. As a result, interlaboratory calibration data for the acute mysid acute method included results from six laboratories and two referee laboratories.

For the short-term chronic sea urchin embryo development method, data from eight laboratories was received. Data from a single laboratory was excluded due to the concurrent reference toxicant test failing to meet the internal TAC for that laboratory. This rendered accompanying tests invalid and thus the data were excluded. As a result, interlaboratory calibration data for the chronic purple sea urchin test method included results from five laboratories and two referee laboratories.

Once data were deemed appropriate and acceptable, the project team conducted statistical analyses using CETIS Analytical Software to generate the effect concentrations for each test for each species. The Test for Significant Toxicity (TST; USEPA 2010) was the statistical method used for comparisons for both standard exposure testing where point estimates were not calculatable (due to only 6.25 & 100% concentrations being tested), and for the 100% concentration of the pulsed exposures.

Valid data from all tests were used in the determination of the objectives of this effort as shown below:

1. Evaluate the ability of laboratories to successfully execute the pulsed exposure toxicity methods for each species (completion rate);
2. Evaluate the rate at which laboratories detected toxicity when evaluating non-toxic samples (false positive rate);

3. Characterize the interlaboratory variability of the three modified pulsed exposure test methods for each species through the comparison of coefficients of variation (CVs) for the LC₅₀ and EC₅₀ values and ranges for NOEC values and 95% confidence intervals.

Referee laboratory results were included in determinations of successful test completion rates, false positive rates, and precision for methods. The decision to include referee results was made because testing was conducted concurrently to the participant laboratories and the identity of the samples were blinded to the referee laboratories until completion of the testing.

For samples that elicited a dose response, point estimates (i.e., LC₅₀ and EC₅₀) were calculated and the NOEC and LOEC determined. The coefficient of variation (CV) was also determined for each test method, sample and endpoint evaluated as well as for the 6.25 and 100% concentrations for both the pulsed exposure and standard method.

The full detailed report of the Interlaboratory Calibration Study is provided in Colvin et al. 2021b. In brief, all laboratories that participated in the study successfully completed both pulsed and standard test methods and met standard EPA laboratory test acceptability criteria including control performance. Summaries for the specific metric are described below.

Successful Test Completion Rate

The test completion rates calculated for each test method were evaluated as the percentage of tests successfully initiated and terminated meeting all TAC. Completion rates were 100% for all methods for both the pulsed exposure and the standard methods as summarized in Table 5-46 reflecting a very successful outcome for the study.

Table 5-46. Successful test completion rates for test methods evaluated in the pulsed exposure toxicity interlaboratory calibration study.

Test Method	Exposure Duration	N	No. of Invalid Tests	Successful Test Completion Rate (%)
<i>Ceriodaphnia</i> acute	26 h	35	0	100
	Standard/ 96 h	35	0	100
<i>Americamysis</i> acute	26 h	40	0	100
	Standard/ 96 h	40	0	100
<i>Strongylocentrotus</i> chronic	26 h	35	0	100
	Standard/ 96 h	35	0	100

False Positive Rate

The false positive rate was calculated as the number of valid test results that indicated a deleterious effect when in fact there should be none (i.e., a statistical effect in an undiluted blank/control sample). Table 5-47 summarizes the false positive rates observed for all test methods for both pulsed exposure and standard exposures. For both static and pulsed exposures, the false positive rate for the *Ceriodaphnia* acute method was 7.12%. The false positive results for the *Ceriodaphnia* were

observed only for the non-toxic stormwater sample. There were no false positive results for the laboratory blank/control water for either standard static or pulsed exposures.

False positive rates were 0% for the both the mysid acute and purple sea urchin chronic test methods for both sample types and for both exposure scenarios.

Table 5-47. False positive rates for test methods evaluated in the Pulsed Exposure Toxicity Interlaboratory Calibration Study.

Test Method	Exposure Duration	N	False Positive Rate (%)		
			Sample 1 Negative Control	Sample 2 Non-toxic Stormwater	TOTAL
<i>Ceriodaphnia</i> acute	26 h	14	0.00	14.3	7.12
	Standard/ 96 h	14	0.00	14.3	7.12
Mysid acute	26 h	16	0.00	0.00	0.00
	Standard/ 96 h	16	0.00	0.00	0.00
Purple sea urchin chronic	26 h	14	0.00	0.00	0.00
	Standard/ 96 h	14	0.00	0.00	0.00

Precision

The precision of test methods evaluated in the Pulsed Exposure Interlaboratory Calibration Study was estimated by calculating a CV for point estimates (i.e., LC₅₀s and EC₅₀s) for samples that elicited a toxic response. Additionally, to compare the pulsed exposure method to standard continuous exposure methods, the CVs of the 6.25 and 100% concentrations were calculated for each sample. CVs calculated for each test method and for each exposure scenario are summarized in Table 5-48.

Variability observed in the Pulsed Exposure Interlaboratory Calibration Study averaged 67.2 and 22.6% for LC₅₀ values for the *Ceriodaphnia* and mysid acute tests for all sample types. Variability averaged 55.5% for EC₅₀ values for the short-term chronic tests for all sample types.

For comparison, USEPA (2001) reported multi-laboratory precision CVs for acute WET test methods that ranged from 20.0 to 38.5% and averaged 28.4%. Previous CVs reported from USEPA (1988) were 22-167% (with a weighted mean of 50%) for acute WET methods testing reference toxicants.

Table 5-48. Mean precision estimates (CVs) for test methods evaluated in the Pulsed Exposure Toxicity Interlaboratory Calibration Study.

Test Method	Exposure Duration	CV* (%)	
		LC ₅₀ or EC ₅₀	6.25% Concentration
<i>Ceriodaphnia</i> acute	26 h	67.2	28.7
	Standard/ 96 h	--	39.0
Mysid acute	26 h	28.7	6.6
	Standard/ 96 h	--	5.7
Purple sea urchin chronic	26 h	55.4	7.5
	Standard/ 96 h	--	26.1

*CVs presented are based on total variance and averaged across sample types.

Repeatability

Test repeatability for both pulsed and static exposures was evaluated by comparing toxic response endpoints (TST results and point estimate LC₅₀s/EC₅₀s) between the two duplicate positive control copper-spiked laboratory control waters (Samples 4 and 5). Results for the duplicate samples were found to be all very comparable both within each laboratory and between laboratories. All laboratories identified these two samples as toxic using the TST approach. Furthermore, between laboratories the pooled mean LC₅₀/EC₅₀ values for the pulsed exposure tests for the two duplicate samples were all not statistically different from each other for all three test species. Intra-laboratory repeatability was calculated by looking at the relative percent differences (RPD) between the duplicate samples for each laboratory and method tested (Table 5-49). Mean RPDs for the methods tested were all below 20%.

Table 5-49. Intra-laboratory relative percent difference (%) for duplicates samples.

Laboratory ID	<i>Ceriodaphnia</i> Acute	Mysid Acute	Purple Sea Urchin Chronic
1	4.3	34.4	13.0
2	5.4	27.6	4.8
3	-	11.6	17.2
6	3.4	37.1	-
7	18.4	2.2	3.0
8	23.0	-	-
9	-	1.7	27.3
10	0	-	-
11	-	1.2	54.0
12	13.8	36.0	1.5
AVERAGE	9.8	19.0	17.3

6. PERFORMANCE ASSESSMENT

6.1 QUANTITATIVE PERFORMANCE OBJECTIVES

6.1.1 Performance Objective #1: Historical Rainfall Analysis and Literature Review

The goal of this performance objective had two parts. The first was a summary document that details analyses conducted on historical rainfall data at numerous locations across the United States in order to develop site-specific recommendations for exposure durations for laboratory toxicity testing specifically for assessment of stormwater discharges. This part of the performance objective was met with the publication of a technical document entitled *Long-term Rainfall Analysis at Select DoD-relevant Sites for the Determination of Appropriate Toxicity Exposure Durations*. This document was made publicly available through Defense Technical Information Center (DTIC; Colvin et al. 2020a).

The document details analyses conducted on historical rainfall data at numerous locations across the United States in order to develop site-specific recommendations for exposure durations for laboratory toxicity testing specifically for assessment of stormwater discharges. If applied properly, a modification to generate more realistic exposure conditions for toxicity testing will still provide an appropriate level of protection, particularly given that the exposure at the end-of-pipe is further reduced once it mixes in the receiving environment. As described in section 5.2.1, rainfall duration was used as a proxy for discharge duration in areas where most of the drainage basin feeding the discharge consists of impervious surfaces, a common characteristic at most industrial sites. Therefore, using rainfall data, digital data sets covering up to a 20-year period were used at 21 locations (selected based on their proximity to DoD Installations) to determine total rainfall duration for any given rolling 24 h period and 96 h continuous basis at the onset of a qualifying rain event. For the 96 h analysis, an antecedent dry period of at least 12 h was a requirement prior to the summing of rainfall duration during the subsequent 96 h period. Additionally, a total of 0.05” cumulative rainfall must be observed prior to the summing of rainfall duration to account for surface saturation to allow for runoff to occur. These requirements were made to be in concordance with typical stormwater collection requirements for standard regulatory monitoring. The mean, standard deviation and the 50th, 75th and 95th percentile of rainfall duration was calculated for each location. Results from these analyses are shown in Table 6-1 and Table 6-2.

Table 6-1. Rainfall analysis results over a 24 h period.

Location	Region	Average	SD	50th Percentile	75th Percentile	95th Percentile
Chicago, IL	Central	4.5	3.9	3	6	13
Louisville, KY	Central	4.9	4.2	4	7	14
Detroit, MI	East North Central	4.5	3.8	3	6	13
St. Paul, MN	East North Central	4.2	3.7	3	6	12
Boston, MA	Northeast	5.5	4.7	4	8	15
Seattle, WA	Northwest	6.1	4.7	5	9	16
Anchorage, AK	Other	5.0	4.3	4	7	14
Honolulu, HI	Pacific	2.7	2.7	2	3	8
Piti, Guam	Pacific	4.4	3.7	3	5	12
Corpus Christi, TX	South	3.5	3.2	2	4	10
New Orleans, LA	South	3.9	3.4	3	5	11
Charleston, NC	Southeast	4.1	3.6	3	6	12
Jacksonville, FL	Southeast	3.8	3.5	3	5	11
Norfolk, VA	Southeast	4.8	4.1	3	7	14
Pensacola, FL	Southeast	4.0	3.5	3	5	11
Tucson, AZ	Southwest	3.2	2.9	2	4	9
Paso Robles, CA	West	4.8	4.1	3	7	13

Table 6-2. Rainfall analysis results over a 96 H period following onset of qualifying storm.

Location	Region	Average	SD	50th Percentile	75th Percentile	95th Percentile
Chicago, IL	Central	9.1	7.3	7	13	24
Louisville, KY	Central	11.2	8.9	9	16	29
Detroit, MI	East North Central	9.3	7.4	7	13	24
St. Paul, MN	East North Central	9.0	7.7	7	13	24
Boston, MA	Northeast	10.9	9.2	9	15	28
Seattle, WA	Northwest	18.2	13.7	15	26	45
Anchorage, AK	Other	11.6	9.7	9	17	32
Honolulu, HI	Pacific	7.1	7.7	4	9	24
Piti, Guam	Pacific	15.0	11.2	13	20	35
Corpus Christi, TX	South	7.7	7.4	5	11	22
New Orleans, LA	South	8.6	8.5	6	11	23
Charleston, NC	Southeast	9.1	7.7	7	12	25
Jacksonville, FL	Southeast	9.4	8.9	7	12	28
Norfolk, VA	Southeast	9.9	8.5	8	14	26
Pensacola, FL	Southeast	8.7	7.6	7	12	23
Tucson, AZ	Southwest	6.1	5.6	5	8	18
Paso Robles, CA	West	10.7	10.2	8	15	34
San Diego, CA	West	8.9	8.6	6	12	26
San Francisco, CA	West	16.0	12.1	13	23	39
Great Falls, MT	West North Central	9.0	9.3	6	12	29
Rapid City, SD	West North Central	9.0	9.1	6	12	27
GRAND MEAN (hrs)		10.2	8.9	7.8	14.1	27.9

The second aspect of this performance objective was also met in the publication of a technical report entitled *Approaches for Evaluation of Episodic Discharges: A Review and Recommendations for Toxicity Testing Compliance Monitoring*. This document was made publicly available through DTIC (Colvin et al. 2020c). The literature review covers numerous topic areas related to pulsed exposures

and are outlined below. In brief, the document includes a discussion of the advantages of pulsed toxicity methodologies, and various considerations for designing pulsed exposure testing protocols. In addition, there is a brief discussion of potential options to incorporate pulsed exposure testing methods into regulatory frameworks.

- Applications for Pulsed Exposure Toxicity Testing and Alternative Considerations
- Advantages of Pulsed Toxicity Testing
 - Increased environmental realism
 - Inclusion of a post-exposure period
- Considerations of Pulsed Exposures
 - Concentration, duration and magnitude
 - Frequency
 - Latent effects
 - Organism age at initiation
 - Toxicant mixtures
 - Endpoint selection
- Considerations for Regulatory Acceptance
 - Time-Averaged Concentration
 - Median-Effect Time Criteria

6.1.2 Performance Objective #2: Pulsed Protocol Development and Demonstration

The goal for this performance objective was to build on preliminary work conducted by NIWC Pacific (Rosen et al. 2019), which resulted in recommendations to verify repeatability and reproducibility through multiple tests for each species and test regime, expand species and contaminant lists, consider the timing of onset of pulses with respect to species life stage, identify the most sensitive life stage for pulsed exposures, and examine potential for latent effects.

Through lab-based exposures, this study evaluated two marine species, *S. purpuratus* and *A. bahia*, and two freshwater species, *Hyalella azteca* and *Ceriodaphnia dubia*, all of which are important and common species for toxicity testing (USEPA 1995, USEPA 2002a, b, c; Figure 6-1). Each species was tested in triplicate for each exposure duration (standard, 6 h, 12 h and 96 h) using Copper (Cu), Zinc (Zn) or Bifenthrin-spiked test solutions. This resulted in at least 96 toxicity tests. A SOP was generated for all species included in this performance objective and published as a technical document through the NIWC Pacific Publications Office and made publicly available through DTIC (Colvin et al. 2020b).



Figure 6-1. Toxicity test species from left to right: the purple sea urchin *Strongylocentrotus purpuratus* (with larval development endpoint shown), the mysid shrimp *Americamysis bahia*, the freshwater amphipod *Hyalella azteca*, and the water flea *Ceriodaphnia dubia*.

All data generated from lab-based exposures had individual test acceptability criteria and objectives met for all toxicity tests performed. Test acceptability criteria for each test endpoint were:

- *Strongylocentrotus purpuratus* chronic embryo-larval development tests: minimum 80% survival in controls;
- *Americamysis bahia* acute survival tests: minimum 90% survival in controls;
- *Hyalella azteca* acute survival tests: minimum 90% survival in controls;
- *Ceriodaphnia dubia* acute survival tests: minimum 90% survival in controls.

All water quality parameters (dissolved oxygen, pH, salinity/conductivity, and temperature) were maintained within recommended ranges for the duration of all toxicity tests performed (USEPA 1995, 2002a, b, c; Table 5-3 through Table 5- 2). Results from the marine species evaluations were published in the *Chemosphere Journal* (Colvin et al. 2021a). Preliminary freshwater efforts conducted by Loyola University Chicago were also published in the journal *Environmental Toxicology and Chemistry* (Karic et al. 2022), but not incorporated into the summary of results reported in section 5.3 as methods were not consistent. Pulsed exposures efforts by Loyola University were conducted in series using different batches of organisms for the different exposure durations. Further testing to evaluate the relative responses of the freshwater organisms were conducted at the Wood Environment and Infrastructure Solutions, Inc. Bioassay Laboratory where 3 rounds of testing were conducted evaluating the multiple pulsed exposures methods concurrently (i.e., 6, 12 and 26 h pulsed and standard exposures tested concurrently with the same batch of organisms).

6.1.3 Performance Objective #3: Pulsed Exposure Protocol Validation

Laboratory evaluations of stormwater and/or episodic industrial discharges from three DoD facilities were tested for toxicity using the methods developed and demonstrated in the previous performance objective including standard exposure methods for appropriate comparison. Table 5-13 shows the number and types of toxicity tests conducted as part of this performance objecting. Concurrent *in situ* toxicity testing with the marine species in the adjacent receiving waters was conducted at NBSD and NBC during two storm events in San Diego using techniques demonstrated previously (e.g., SEA Ring under ESTCP #ER-201130; Rosen et al. 2012, Rosen et al. 2017). *In situ* testing during each event was performed at multiple depths covering a gradient away from the primary end-of-pipe source and one representative location was positioned outside the influence of the discharge as a receiving water “reference” location for comparison purposes. End-of-pipe sampling locations included the sites at NBSD, NBC and NBPL in addition to sites at Puget Sound Naval Shipyard (PSNS) and Naval Submarine Base New London (NSBNL).

Data generated from laboratory-based and *in situ*-based exposures was met for all toxicity tests performed.

- Navy Region Southwest (NBSD, NBC and NBPL) –
 - Jan 2018: 2 samples
 - Laboratory-based effort using purple sea urchin and mysid shrimp
 - Jan 2019: 15 samples
 - *In situ* and laboratory-based effort using purple sea urchin and mysid shrimp
 - May 2019: 9 samples
 - *In situ* and laboratory-based effort using purple sea urchin and mysid shrimp
- Puget Sound Naval Shipyard –
 - Laboratory-based effort using purple sea urchin
 - Feb 2018: 2 samples

- Jun 2018: 3 samples
 - Jan 2021: 1 sample
 - Apr 2021: 1 sample
 - Dec 2021: 1 sample
- Navy Submarine Base New London –
 - Jan 2019: 5 samples
 - Laboratory-based effort using *Ceriodaphnia dubia*

All water quality parameters (dissolved oxygen, pH, salinity/conductivity, and temperature) were maintained within recommended ranges for the duration of all laboratory-based toxicity tests performed (USEPA 1995, 2002a, b, c).

A goal for the project was to show statistically similar results between the laboratory pulsed exposure methods and *in situ* exposure methods indicating an appropriate realistic exposure regime in the lab (i.e., using a student's t-test with unequal variances, p-values ≥ 0.05). Provided no confounding influences *in situ* (e.g., low salinity), effects in the laboratory should be no less than that observed in the field to ensure protectiveness of the lab-based methods. *In situ* exposures were conducted only for the Naval installations in San Diego, CA, and are discussed below.

For the sea urchin embryo-larvae development test, all laboratory-based exposures exhibited toxicity throughout the concentration series tested. However, the receiving water samples that were collected prior to the storm event and during the storm event, both resulted in no toxicity. The *in situ* exposures in the SEA Rings also exhibited no toxicity for all depths deployed. This directly shows that the typical end-of-pipe sample that is used for regulatory monitoring purposes is overestimating the impacts to the receiving environment.

For the mysid survival test, only the 26 h and static exposures for the sample collected at NBSD-P13 exhibited toxicity. All other stormwater samples and receiving water samples exhibited no toxicity. The *in situ* exposures did show some toxicity for the SEA Ring deployed at NBSD-P13, but only for the SEA Ring deployed at the sediment surface, possibly indicating deposition of materials that led to the toxicity observed. The SEA Ring deployed at NAB-OF09 also exhibited toxicity to the mysids for all SEA Ring deployment depths. The decreased survival that was observed is likely due to the depressed salinity that was observed during the storm events, as even the 100% concentration stormwater sample (salted for laboratory exposures) for the 96 h static exposure did not elicit a toxic response. A study by Weston (2011) found that a reduced salinity to 10 ppt or less for a period of 6 hours affected survival of *A. bahia* with few to no survivors following exposure to 6 ppt for the same timeframe. Salinity tolerance studies conducted for the City of San Diego have found that the marine kelp shrimp, *Holmesimysis costata*, is very sensitive to brief reductions in salinity, resulting in zero percent acute survival following exposure to a salinity of 20 ppt for only 30 minutes (AMEC 2015). The AMEC study (2015) found that sea urchin embryos were less sensitive to reduced salinities, which is also supported by the lack of toxic response observed in this current study for *in situ* exposures.

General trends for both species show greater frequency of sea urchin embryo abnormality and juvenile mysid mortality with increasing effluent exposure with both duration and concentration. However, the *in situ* exposures provide basis for comparison and show that standard end-of-pipe exposures over-estimated toxicity in the receiving environment for the highly sensitive chronic sea urchin test and in several instances; also, for the less sensitive acute mysid exposure.

6.1.4 Performance Objective #4: Interlaboratory Calibration Study

The ESTCP project team modeled the Pulsed Exposure Toxicity ICS after the USEPA's Interlaboratory Variability Study of *EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Vol. 1* (USEPA 2001) and is reported in Colvin et al. 2021b. In brief, a total of 14 laboratories in the United States and one laboratory in Canada were contacted via phone calls and email. Of those contacted a total of 10 of laboratories in the United States and the one laboratory in Canada expressed interest in participation. After confirming those laboratories that were interested in the study, a request for qualifications (RFQ) and costs package was sent out to each laboratory. A third-party contractor, Edmond Scientific Company, managed the RFQ coordination and cost negotiations with each interested laboratory. Each participating laboratory was required to prequalify for each test method by documenting their capabilities, experience and proficiency, and quality assurance processes in order to meet the needs of the study. For the final analysis and reporting, 12 laboratories were involved in the study, with 7, 8 and 7 laboratories participating in the *Ceriodaphnia*, *Mysid* and the purple sea urchin pulsed exposure test methods, respectively. A list of selected participant laboratories and species tested by each is shown in Table 6-3.

Table 6-3. Selected laboratories for the pulsed exposure interlaboratory study and species tested.

Laboratory	Location	Test Species		
		<i>Ceriodaphnia</i>	<i>Mysid</i>	Purple Sea Urchin
Aquatic Bioassay Consulting (ABC)	Ventura, CA	X	X	X
EA Engineering	Hunt Valley, MD	X	--	X
EcoAnalysts	Port Gamble, WA	X	X	X
Enthalpy	San Diego, CA	X	X	X
GEI Consultants	Denver, CO	X	--	--
Great Lakes Environmental Co (GLEC)	Columbus, OH	X	--	--
McCampbell Analytical Inc.	Pittsburg, CA	--	X	X
Nautilus Environmental Company	Burnaby, BC	X	X	X
Pacific EcoRisk	Martinez, CA	X	X	X
Tetra Tech	Owings Mills, MD	X	--	--
Naval Information Warfare Center (NIWC)A	San Diego, CA	--	X	X
Wood Environment & Infrastructure Solutions Inc. (Wood)A	San Diego, CA	X	X	X

^A – referee laboratory

For each test method, two referee laboratories were involved in the Calibration Study. The referee laboratories were responsible for preparing bulk test samples, conducting preliminary testing of the samples and distribution of the “blind” samples to the participating laboratories. The following five samples were prepared for each test method: 1) a blank control water; 2) a non-toxic stormwater sample; 3) a toxic stormwater sample; and 3) a toxic metal-spiked laboratory control sample, and 5) a blind duplicate of the metal-spiked laboratory control.

Participant laboratories were instructed to perform 26 h pulsed exposure toxicity testing on each of the five blind samples using instructions provided in a pulsed exposure toxicity testing standard operating procedure. Each sample was tested using a five-concentration dilution series (i.e., 6.25, 12.5, 25, 50 & 100% concentration of whole sample). Each blind sample was also tested under standard static exposure conditions with 6.25 & 100% concentrations. Additionally, concurrent reference toxicant tests were conducted by each laboratory for each species study they participated in. Interlaboratory Calibration testing was conducted from July to August 2020.

Two contracted laboratories that planned to initiate the daphnia tests were unable to do so properly due to a lack of suitable in-house neonates to initiate all tests at once. In addition, one participating laboratory provided questionable supporting water quality results and had insufficient documentation to verify that required test methods were followed and thus their data was excluded from the study. Once all testing was completed, participant laboratories submitted all data using an electronic data deliverable provided by the project team. The project team reviewed all data to ensure that all required information was provided. Results were then compiled, and method performance characteristics were calculated for each pulsed exposure test method.

Data from laboratories that participated in the study successfully completed both pulsed and standard test methods and met standard EPA laboratory test acceptability criteria including control performance. An overall summary of results from the Interlaboratory Calibration Study is provided in Table 6-4.

In addition to successful completion of the tests, two additional key metrics for this evaluation included the frequency of false positives (declaring a control sample toxic), and interlaboratory precision measured as the percent coefficient of variation (% CV) among labs. False positive rates for the negative control (Sample #1) were 0% for all tests conducted. Overall false positive rate, including the non-toxic stormwater sample (Sample #2), was 2.3%. Precision for the test methods was determined by comparing % CVs based on both laboratory control performance and dose response relationships measured using calculated median lethal or median effect (LC₅₀ or EC₅₀) concentration values. Confidence intervals surrounding the point estimates were also evaluated when a dose response was observed. Interlaboratory CVs of LC₅₀s ranged from 25.6 to 70.1% for acute test methods using *Ceriodaphnia* and mysid shrimp. Interlaboratory CVs of EC₅₀s for the short-term chronic tests using the purple sea urchin ranged from 50.4 to 59.2%. Furthermore, the pooled mean LC₅₀/EC₅₀ values for the pulsed exposure tests for the two duplicate samples were not statistically different from each other for all three test species indicating good repeatability for intra-laboratory performance.

For the key metric evaluating the false positive rate of the non-toxic stormwater sample (Sample #2) alone (e.g., not included with the non-toxic control) for the acute test with *Ceriodaphnia dubia*, one of the seven laboratories identified this sample to be toxic using the pulsed exposure approach based on the TST (Fail) with mean survival of 68%. This laboratory did not identify this sample to be toxic using the standard continuous method. Additionally, a different laboratory identified Sample #2 as toxic using the standard static exposure (0% survival), but non-toxic using the pulsed exposure method on the same sample (100% survival). The reason for these discrepancies remains unknown despite discussions with each laboratory and a thorough review of all supporting information (i.e., benchtop datasheets and electronically submitted results). No other laboratories identified Sample #2 as exhibiting toxicity using either standard static or pulsed exposure methods. As a result, 1 of the 7 tests using both the standard static and pulsed exposure methods resulted in a false positive, or a rate of 14.3% for Sample #2. If it were determined that the results for Sample #2 from these laboratories were not appropriate for inclusion in the meta-analyses, the false positive rate for Sample #2 would be 0% and the overall false positive rate for both the control sample (Sample #1) and Sample #2 would also be 0%.

Data from these two laboratories for the *Ceriodaphnia* acute test was reviewed for the remaining three samples tested (e.g., the toxic stormwater sample and the two toxic spiked laboratory water samples). It was decided by the project team to include all data submitted as dose responses observed for each sample were as expected. For discussion purposes, values in parentheses of Table 6-4 show the precision and repeatability results if the data from these two laboratories were excluded from the precision and repeatability analyses. For the precision analyses, the CVs are reduced, whereas the intra-laboratory relative percent difference increases slightly.

Table 6-4. Summarized test results from pulsed exposure interlaboratory calibration study.

Test Method	Exposure Duration	Successful Test Completion Rate (%)	Negative Control False Positive Rate (%)	Interlaboratory Precision (%CV)		Intra-Laboratory Relative Percent Difference (%)
				6% Sample	LC ₅₀ /EC ₅₀	
<i>Ceriodaphnia</i> acute*	26 h	100	0	35.9 (29.0)	67.2 (64.4)	9.8 (10.0)
	Standard/ 96 h	100	0	41.1 (35.6)	-	-
Mysid acute	26 h	100	0	6.6	28.7	19.0
	Standard/ 96 h	100	0	5.7	-	-
Purple sea urchin chronic	26 h	100	0	7.5	55.4	17.3
	Standard/ 96 h	100	0	26.1	-	-

*Data shown in parentheses are from alternate analyses where data from two laboratories were excluded for discussion purposes.

It should be noted that a primary objective of this ICS effort was to evaluate the ability of laboratories to successfully execute the pulsed exposure toxicity methods for each species. Although the final interpretation of the 26 h and the standard exposure for the spiked or stormwater samples tested for each species resulted in the same conclusion (i.e., samples were determined to be toxic), the intention of the study was to allow for interpretation of the point estimates generated from the data.

The application of the pulsed exposure methodology in a regulatory monitoring program is site and potentially event specific with possible alternate exposure designs where differences in results could be observed. In addition, this ICS effort used a single chemical as the spiking agent to elicit a toxic response (e.g., copper). In practical scenarios, the pulsed exposure method can be used for the evaluation of stormwaters or other episodic discharges as whole effluent samples with the potential for adverse effects associated with complex mixtures (i.e., multiple contaminants).

6.2 QUALITATIVE PERFORMANCE OBJECTIVES

6.2.1 Performance Objective #5: Establish Advisory Panel

Individuals and organizations that may have had an interest in the pulsed exposure and its method development were engaged from the early stages of the project preparation. Collaboration and consensus of a modified testing methodology and its applicability among experts, regulators and end-users were key for project success. During the proposal stage, letters of support were provided for the development and validation of the pulsed exposure method. Letters were received from:

- The San Diego Regional Water Quality Control Board – Executive Officer Dave Gibson
- Southern California Coastal Water Research Project (SCCWRP) – Steve Bay
- University of California, San Diego Scripps Institution of Oceanography – Kimberly O’Connell
- California Stormwater Quality Association (CASQA) - Geoff Brosseau, Armand Ruby

- Naval Facilities Engineering Command Southwest (NAVFAC SW) – Len Sinfeld, Jessica Palmer
- Puget Sound Naval Shipyard and Intermediate Maintenance Facility (PSNS&IMF) – Michelle Aylward

Following the award of the project, all of the individuals listed above, or representatives from their establishment, became participants on the Pulsed Exposure Advisory Panel. Table 3-3 shows the individuals that participated on the Panel. While a few participants were asked to be removed from the Panel due to retirement, individuals from the same institution or company were included on the Panel as a replacement.

Work products from the ESTCP project were shared with the Panel for review and commentary and progress presentations were given either at professional conferences as side meetings or through remote webinars/conference calls.

In November 2018, at the annual meeting for the SETAC, the project team met with the Panel in a side meeting as well as during a special session entitled “Assessment and monitoring of risks to aquatic environments from short-term (episodic) contaminant exposures”. These meetings allowed for the communication of and discussion of pulsed exposure toxicity methodologies and considerations that must be made when studying these efforts. Another side meeting was held for in-person discussions at the November 2019 SETAC meeting. Conferences and/or updates were held throughout the project either at these national conferences, via teleconference or an update brief was provided to Panel participants.

In addition to sharing project progress, the Demonstration Plan and Literature Review (Colvin et al. 2021c) were both shared with the Panel and all edits and comments were taken into consideration and incorporated into the respective final documents as appropriate.

The EPA hosted a two-day workshop in September 2019 that discussed the derivation of water quality criteria recommendations for aquatic life entitled “Frequency and Duration Experts Workshop”. Criteria derivations have not largely taken duration and frequency into account when deriving the numerical limits, while magnitude has largely driven the derivations. The goal of the workshop was to discuss and capture the state of the science for frequency and duration to provide this information to EPA for their further consideration. Two members of this ESTCP project participated in this meeting, Ms. Molly Colvin and Mr. Chris Stransky, along with several other scientists and regulators with interest in this field.

Throughout this project, it has been clear that there is a consensus among Panel members and others in the scientific and regulatory community that there is justification for an alternative method for the assessment of episodic discharges.

6.2.2 Performance Objective #6: Ease of Use of New Methodology

While we were primarily concerned with the development and demonstration of a scientifically defensible and more environmentally representative methodology for the evaluation of episodic discharges, it was not our intention to create an onerous methodology that is fiscally irresponsible with regard to labor and material expenses to do such.

A survey was provided to laboratories requesting their feedback on their participation in the inter-laboratory calibration study and implementation of the pulsed exposure methods. Four laboratories provided feedback and the primary concern was the increased labor hours that would be required for performing the pulsed exposure method. An additional concern was the one-time cost of constructing the materials needed for performing organism transfers (i.e., screen tubes). However, the labs

recognized that the cost was an appropriate investment if pulsed exposure methodologies were integrated into future monitoring efforts.

It was less than ideal to have so few survey responses, but the positive enthusiasm from the participant laboratories during planning meetings was noticeable and made for a successful ICS effort. Comments received following the ICS effort are shown below:

- “Great study! It was well organized and orchestrated by the coordinators. For the cost concerns, the time and materials associated with constructing the SWICs was fairly expensive, however, they can be re-used for future studies.”
- “Thanks for letting our lab participate!”
- “Cost concerns regarding the cost to purchase/construct/clean screens, as well as increased staff time required for renewals of mysid and urchin tests (and termination of urchin tests).”

6.2.3 Performance Objective #7: Engage Regulatory Community

The regulatory community has been and will continue to be engaged. Initially, preliminary efforts were conducted in 2016 in response to a NBSD industrial NPDES permit (Permit R9-2013-0064) condition that allowed the Navy to assess alternative approaches to evaluating stormwater discharges and propose alternative toxicity testing parameters. The results of this study showed that modifying standard WET test methods is a feasible approach to accurately assess short-term exposure conditions (Rosen et al. 2019). With the successful completion of the preliminary study, the project team sought to propose further efforts under the ESTCP Environmental Restoration pillar. With the development of the proposal, Mr. Dave Gibson, Executive Officer of the San Diego Regional Water Control Board, was engaged for their involvement with this project. He was supportive of the project proposal and provided a letter of support. Once the project was awarded, a member of his staff, Mr. Keith Yaeger, participated on the Pulsed Exposure Advisory Panel for the entire duration of the project.

Persons from the regulatory community (i.e., U.S. EPA, Office of Compliance and Office of Research and Development; Department of Ecology, WA) were also involved on the Advisory Panel (Table 3-3) and provided critical review and feedback of work products from this project.

In addition, the U.S. EPA, Region 9 Quality Assurance Branch and the California State Water Resources Control Board (SWRCB) were also engaged through the process of having the pulsed exposure toxicity method reviewed through the EPA Alternate Test Procedure (ATP) protocol. The CASWRCB had to be the entity the initially requested the ATP review by Region 9, and thus Ms. Renee Spears of CASWRCB forwarded our request for ATP review to Ms. Roseanne Sakamoto of EPA Region 9. The pulsed exposure methods will be submitted (anticipated submittal late 2022) as a modification of the existing WET methods for the acute tests using Mysid shrimp and *Ceriodaphnia*. These methods will be potentially applicable to Navy Region Southwest and the NPDES monitoring requirements that are present for the stormwater discharges at the installations.

Ongoing efforts to engage the regulatory community in the San Diego region have also been conducted by the project team through the development of draft NPDES permits for the three installations located on San Diego Bay. Throughout the permit renewal process, the appropriateness of the application of the pulsed exposure method has been communicated to the Regional Board and the pulsed exposure method is currently written into the draft permits as an alternate test procedure for monitoring of stormwater discharges. It is anticipated that the permit for the Metro San Diego Bases will be issued in early 2023.

7. COST ASSESSMENT

In order to successfully transition this methodology to end-users and laboratories for use, the costs associated with implementing the pulsed exposure methodology should be comparable to existing standard methods or at a minimum provide a greater benefit and understanding of episodic discharge impacts per unit of cost.

7.1 COST MODEL

Operational costs for performing standard toxicity test methods were compared to costs associated with conducting the pulsed exposure methodology. Typically, costs are reported on a per sample basis and are species dependent. Laboratories that participated in the baseline characterization as well as those that participated in the ICS effort provided cost estimates for performing toxicity tests. Table 7-1 shows the range and averages of costs for each methodology performed.

Table 7-1. Range of costs for performing both standard and pulsed exposure toxicity test methods.

	Species					
	<i>Ceriodaphnia dubia</i> acute		<i>Americamysis bahia</i> acute		<i>Strongylocentrotus purpuratus</i> chronic	
	Standard	Pulsed	Standard	Pulsed	Standard	Pulsed
Min	\$1,160.00	\$1,000.00	\$900.00	\$960.00	\$1,020.00	\$1,200.00
Max	\$1,700.00	\$1,500.00	\$1,120.00	\$2,000.00	\$1,600.00	\$1,400.00
Average	\$1,430.00	\$1,248.16	\$1,010.00	\$1,321.93	\$1,310.00	\$1,343.70

NOTE: This is assuming that a single pulsed exposure test is being conducted.

The cost model for performing the pulsed exposure methodology for the different species is shown below in Table 7-3. The main notable difference in costs between standard toxicity test methods and the pulsed exposure methodology is the number of labor hours that are required to conduct the transfer of organisms from the test/effluent solution into receiving or laboratory control water at the appropriate time point. Conducting the pulsed exposure method does not require a higher level of expertise than the standard methodology and typical laboratory technician rates run approximately \$80/hr. The example in the table does not consider the statistical analyses and reporting that typically accompany these tests as the data generated from these tests are identical in nature (i.e., percent normal development; percent survival). There would be potentially increased reporting efforts associated with the pulsed exposure methodology as appropriate interpretation of the results would be required on a case-by-case basis. This additional interpretation may require a higher level scientist at a higher labor-rate (i.e., project manager @ \$150/hr).

Table 7-2. Number of labor hours per test day for performing standard or pulsed exposure toxicity test methods.

	Species					
	<i>Ceriodaphnia dubia</i> acute		<i>Americamysis bahia</i> acute		<i>Strongylocentrotus purpuratus</i> chronic	
	Standard	Pulsed	Standard	Pulsed	Standard	Pulsed
Day 0 – test initiation	3	3	4	4	5	6
Day 1*	1	12 h pulse: 6 24 h pulse: 3	2	12 h pulse: 4-5 24 h pulse: 3-4	0.5	12 h pulse: 2 24 h pulse: 1
Day 2	3	1	3	2	0.5	0.5
Day 3	1	1	2	2	0.5	0.5
Day 4 – Test termination	1	1	2	2	0.5	2
TOTAL LABOR HOURS	9	9-12	13	13-14	7	10-11

*Example shown above is for a single sample toxicity test with either a 12 or 24 h pulsed exposure followed by transfer to receiving or laboratory control water.

For the *Ceriodaphnia* acute test, the labor hours are very comparable between the pulsed and standard exposure methodologies. For the standard test method, organisms are hand-transferred with pipettes to new exposure chambers at the mid-point of the test. This effort is extremely similar to the transfer of organisms under the pulsed exposure scenario, albeit the pulsed exposure transfer may not occur at 48 h, but likely will occur within the first 24 h of the test exposure. The main difference in effort is if the transfer of organisms in the pulsed exposure scenario occurs after regular business hours. For example, if tests are initiated at 13:00, a pulsed exposure transfer would require a laboratory employee to conduct the transfer at 01:00. This would increase the labor hours above the normal workday. Both method scenarios require daily microscopic evaluation of survival of the organisms and water quality measurements. Test termination is identical in terms of effort for the pulsed and standard exposure methods. The *Ceriodaphnia* test method doesn't require investment in additional specialized equipment or material for performing the pulsed exposure methodology.

For the *Americamysis* acute survival test, again, the main difference in labor is during the transfer of organisms for the pulsed exposure methodology. The potential increase in labor is mainly due to site/sample-specific scenarios that would require off-hour transfers of organisms. A considerable potential cost for performing the pulsed exposure method using the mysid shrimp would be an initial investment in the construction of scree-tubes that would facilitate the transfer of the organisms. While not required to perform the pulsed exposure method, the use of the screen-tubes expedites the organism transfer and can be faster than performing the standard water renewal that is required at 48 h for the standard methodology. Alternatively, rather than using the screen-tube, organism transfer can be performed by pouring the contents of an exposure chamber over a piece of mesh screen that would retain the mysids. The mysids can then be rinsed into a new exposure chamber containing the receiving or laboratory control water. This tactic is more time consuming than simply transferring a screen tube into a new chamber as care is needed to not lose mysids in the process of rinsing onto the screen. For both methods, daily water quality measurements and counts of survival are required and the termination of the tests are identical in level of effort.

For the purple urchin embryo-larval development test, the use of screen-tubes for the transfer of organisms is needed, so the initial investment of constructing these materials is required.

Additionally, there is more labor involved in preparing test chambers for the pulsed exposure method. A larger volume of sample (approx. 250 mL) is required per test exposure chamber to accommodate the screen tubes for the pulsed exposure method, rather than the 10 mL per scintillation vial that is used for the standard method. Setting up the exposure chambers and screen-tubes takes about an hour more than standard test initiations. For the standard exposure method, daily water quality measurements are taken in surrogate test chambers and the test chambers are untouched and thus daily labor efforts are fairly minimal until test termination. The pulsed exposure methodology also requires daily monitoring of water quality, but at the designated time, screen-tubes are rinsed and transferred into receiving or laboratory control water. While not extremely time-consuming, care is needed to ensure that the larvae are handled gently and not exposed to the air for longer than necessary. At test termination for the standard method, organisms are fixed directly in their respective exposure chambers (i.e., scintillation vial) whereas for the pulsed exposure method, larvae must be rinsed carefully from the screen-tubes into scintillation vials or similar for fixation and later microscopic evaluation. This termination step for the pulsed exposure method provides the greatest difference in labor effort relative to the standard method.

7.2 COST DRIVERS

The primary cost drivers for implementing the pulsed exposure methodology are test specific but mainly is driven by the labor differences in performing the methods. Capital investments from the construction of screen-tubes for performing transfers must be recognized but are minor and for approximately one day of labor (technician level) and less than \$250, a set of 35 screen-tubes can be built for testing. Additional one to two days of labor is needed for extensive washing and leaching of newly constructed screen-tubes to ensure any residual contaminants from the construction process are removed. These costs can be recouped over time through equipment fees passed on to the customer.

7.3 COST ANALYSIS

A cost analysis is important for any monitoring program and in the case of the pulsed exposure methodology, it can provide a more realistic assessment of conditions observed at the end-of-pipe or in the receiving environment related to episodic discharges. When paired with concurrent *in situ* deployment of SEA Rings, the site-specific multiple line-of-evidence assessments can be enhanced to better understand the impacts of episodic discharges on the beneficial uses of the receiving environments.

A cost analysis for using the SEA Rings for the assessment of water column testing, as could be used for stormwater or other episodic discharges, was conducted under ESTCP project #ER-201130. In brief, use of the SEA Rings in a field program primarily depends on site-specific logistics, operational costs and the costs of either purchasing or renting the SEA Ring equipment. Operational costs are largely controlled by labor rates for deploying and recovering the equipment, analyzing the data and reporting results. For the purposes of the pulsed exposure project demonstration, and for future implementation, only high-risk discharges and respective receiving water locations should be considered for the full multiple LOE approach, as concurrent *in situ* deployments at multiple locations would not be cost effective. An important note that was included in the cost analysis from ER-201130, was that use of the SEA Rings could be a relatively minor cost component if used as an add-on to existing monitoring programs which would benefit from incorporating the *in situ* data. On a per-sample/location basis, costs of concurrent use of SEA Rings ranges from \$7.7 to \$9.8k for a local deployment (assuming rental of the SEA Ring units; Rosen et al. 2016).

Similarly, the addition of the pulsed exposure testing to an existing monitoring program could be a minimal cost increase. Pulsed exposure testing will likely still require some degree of side-by-side testing with standard continuous exposure methods for quality assurance. However, the relatively minor increase in labor costs associated with performing pulsed exposure toxicity methods, and a concurrent deployment of organisms near the point of compliance within the receiving environment, provides more realistic information about the impacts of the monitored discharge that cannot be achieved through existing standard, continuous exposure toxicity methods. The benefit from the multiple LOE approach provides realistic data from which management actions can be based on.

8. IMPLEMENTATION ISSUES

The pulsed exposure methodology is a fairly straightforward adaptation of existing toxicity methods (US EPA 1995, 2002a, b, c) that are widely used for the evaluation of effluent samples. The data generated in the laboratory refinement studies were in line with our expectations. The only deviation from this was the pulsed exposure efforts with the purple urchin and the pyrethroid pesticide, bifenthrin. It was unexpected that we were unable to generate a dose-response with this species and chemical combination for the pulsed exposure methods or for the standard exposure method.

The project team had been using the pulsed exposure methods for a few years prior to the ICS and were very familiar with nuances associated with the methods for the different species. It was reassuring that participant laboratories were able to execute the pulsed exposure methods and made for a successful ICS effort.

The project team has been and continues to be involved in regulatory monitoring programs for several entities and the pulsed exposure method has been incorporated into some of these programs as an alternate line of evidence to evaluate risk to receiving waters from discharges. For example, pulsed exposure efforts have been implemented on stormwater monitoring from Scripps Institution of Oceanography. While these added pulsed tests are not required by the NPDES permit, the results provide added confidence that the receiving waters are being protected. In addition, pulsed exposure methods as well as field deployed DGTs have been incorporated to the 2020-2021 & 2021-2022 wet weather season stormwater monitoring programs for a high-risk industrial site at NBC. Results of this multiple LOE effort suggests that the pulsed exposure methodology is adequately protective of the receiving environment, perhaps even over-estimating the effect relative to the standard continuous exposures.

The primary issue in getting the method accepted as an alternate test method has been due to that there is no specific EPA procedure for getting a Whole Effluent Toxicity (WET) test method evaluated through the EPA's Alternate Test Procedure (ATP) process. It has also been a learning process determining the optimal way to approach getting the method submitted to the ATP process. It was discovered that the project team would be unable to submit the method for review for a nationally accepted method, but rather the pulsed method would be reviewed for regional and permit specific acceptance. Therefore, since the primary demonstrations for this project took place within Navy Region Southwest, the ATP submission will apply to the future NPDES permit that will cover the three regional bases on San Diego Bay. This will set a precedent for other end-users to request use of the pulsed exposure method for their monitoring requirements.

We expect that there will be push-back by permit-writers at some stage of the permit negotiation process where it will be perceived that the pulsed exposure methodology is not conservative enough to fully replace standard testing and won't protect the beneficial uses of the receiving environment. We have developed a potential monitoring framework where toxicity testing is one component of an overall risk evaluation. The approach is fairly site-specific and would not be appropriate for discharges to small ponds, lakes, or other water bodies that have limited assimilative capacity. However, many or most NPDES monitoring programs that require toxicity testing include end-of-pipe monitoring to larger, more dynamic water bodies (i.e., large streams, rivers, bays, harbors, and ocean). These locations will undoubtedly have rapid mixing due to flow, tides, currents, wave action where pulsed exposures approaches may be more appropriate and representative than standard static exposures. A pulsed exposure assessment can also be an added tool for areas identified as having high risk with prior documented toxicity in the outfall samples using standard continuous exposure methods. The idea is not to replace existing methods, but rather to supplement them to provide a

more representative and meaningful measure for subsequent management decisions. This is a current approach we are now implementing for select outfall locations at Navy bases discharging to San Diego Bay before making decisions on expensive treatment or control options.

An example of this framework can be seen in Figure 8-1. If a sample from a high-risk outfall has no exceedances (e.g., passes toxicity testing), then the end-user would continue with routine compliance monitoring. However, if the outfall sample failed the standard compliance test, but a co-collected/tested receiving water sample did not show any negative impact, then added outfall monitoring would include pulsed exposure testing using site and event-specific durations to help understand the magnitude of the exceedance. If the pulsed testing passes, then routine compliance monitoring would resume. If the pulsed exposure exhibited an exceedance, then enhanced monitoring potentially including toxicity identification evaluations (TIEs) would be implemented along. Any toxicity observed in the receiving environment should also be followed up with additional monitoring and investigative studies.

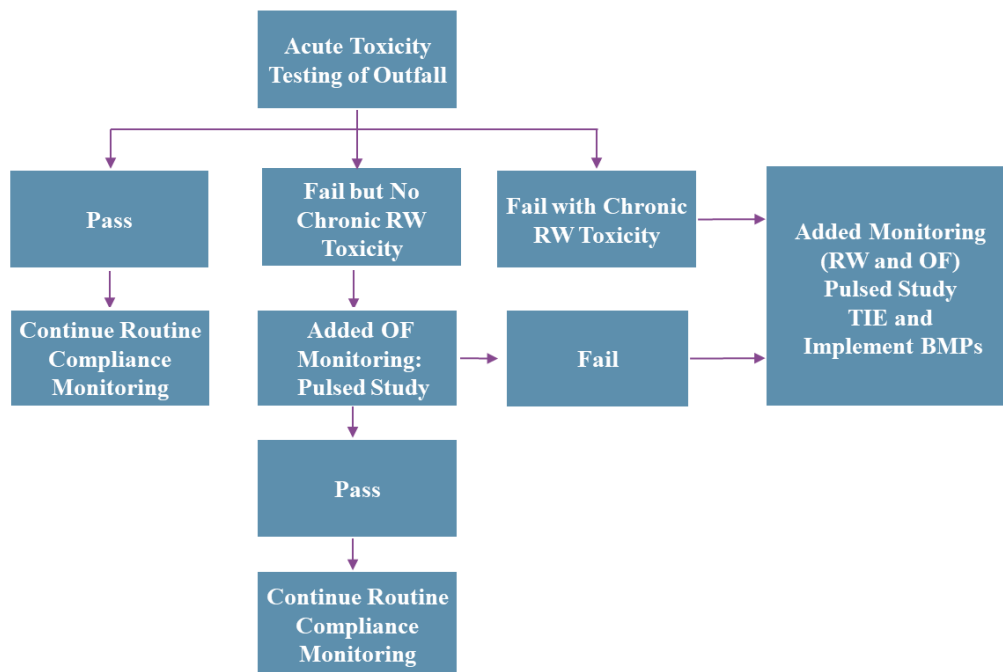


Figure 8-1. Example of monitoring framework for an outfall discharge to a large receiving water.

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APPENDIX B
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This report describes a research effort to modify whole effluent toxicity (WET) testing protocols designed for continuous flow discharges for application to episodic and/or ephemeral discharges such as those associated with storm water runoff. The effort was undertaken in response to a Naval Base San Diego industrial stormwater National Pollutant Discharge Elimination System (NPDES) permit (R9-2013-0064) condition that allows the Navy to assess and propose alternative testing parameters. The research was conducted under the Environmental Security Technology Certification Program (ESTCP) by environmental toxicologists at the Navy's Information Warfare Center Pacific (NIWC Pacific). In order to gain concurrence and support for pulsed exposure methodologies, the following tasks were conducted: (1) establish of a scientific advisory panel for critical review/debate; (2) extensive literature review and analysis of historical rainfall duration and flow nationwide; (3) demonstration of a standardized pulsed exposure toxicity test procedure for assessment of episodic discharges to amend current US EPA WET test methods; (4) validate the pulsed exposure methods with concurrent traditional end-of-pipe collection and testing methods, in addition to <i>in situ</i> testing in the receiving environment to validate protectiveness; (5) conduct an interlaboratory comparison study to assess the ability of contract laboratories to provide consistent and defensible data using the modified methodology; and (6) present the modified methodology to regional regulators for modification of current toxicity testing methodologies that were originally developed for continuous point source discharges.					
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