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Draft Technical Report

Puget Sound Naval Shipyard (PSNS): Sediment Toxicity Monitoring Report May 2011

Prepared by

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For

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Data Quality Assurance:

- SSC Pacific Bioassay Laboratory is a certified Laboratory under the State of California Department of Health Services, Environmental Laboratory Accreditation Program (ELAP), Certificate No. 2601; State of Washington Department of Ecology, Laboratory ID. No. F893.
- All data have been reviewed and verified.
- Any test data discrepancies or protocol deviations have been noted in the summary report pages.

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EXECUTIVE SUMMARY

Sediment toxicity testing was conducted using standardized protocols with the marine amphipods, *Leptocheirus plumulosus* and *Ampelisca abdita*, the polychaete worm, *Neanthes arenaceodentata*, (USEPA 1994 and Farrar and Bridges 2011, respectively), and Mediterranean mussel (*Mytilus galloprovincialis*) embryos (Anderson et al. 1996) to evaluate the environmental risk of sediment samples collected from Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS&IIMF). The results reported are from a single collection event (April 2011) and included a total of 6 test endpoints for samples from two areas of concern: nearshore areas adjacent to a major storm drain (PS03) and an industrial outfall (PS09).

The results from this study showed that Cu and Zn concentrations did not exceed levels associated with toxic effects to the test organisms. No toxicity was observed for either sediment samples, PS03 or PS09, for the whole sediment test with the marine amphipod, *Leptocheirus plumulosus*, or with the marine polychaete, *Neanthes arenaceodentata*. While, the whole sediment test with the marine amphipod, *Ampelisca abdita*, did not meet test acceptability criteria, the results from PS09 showed a slightly significant increase in toxicity from the control, however it is unlikely toxicity was associated with metal exposure because the sediment, overlying water, pore water, and tissue residue metal concentrations were below ecological effect levels. The controls associated with the exposure at the sediment-water interface using embryos from the bivalve, *Mytilus galloprovincialis*, did not meet test acceptability criteria; however, all samples performed better than the control and a comparative analysis revealed that no toxicity was present for either of the sediment samples. The lines of evidence (LOE) for this study indicated non-toxic effects to test organisms with low potential for effects from Cu and Zn exposure.

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AVS	Acid Volatile Sulfide
CBR	Critical Body Residue
CETIS	Comprehensive Environmental Toxicity Information System
Cu	Copper
DO	Dissolved Oxygen
EC_{50}	Median Effective Concentration
ELAP	Environmental Laboratory Accreditation Program
FSW	Filtered Seawater
HDPE	High Density Polyethylene
LC_{50}	Median Lethal Concentration
NBK	Naval Base Kitsap
NOED	No Observed Effect Dose
NOER	No Observed Effect Residues
NPDES	National Pollutant Discharge Elimination System
PSNS&IMF	Puget Sound Naval Shipyard & Intermediate Maintenance Facility
SEM	Simultaneously Extracted MetalSPAWAR Space and Naval Warfare
SQG	Sediment Quality Guidelines
SSC Pac	SPAWAR Systems Center Pacific
TST	Test for Significant Toxicity
TU	Toxic Unit
USEPA	United States Environmental Protection Agency

1. INTRODUCTION

Copper (Cu) and zinc (Zn) are frequently elevated in marine sediments at coastal U.S. Navy facilities. Although these metals are naturally occurring, and essential for life, there are numerous anthropogenic sources of Cu and Zn that frequently result in elevated, potentially harmful, sediment concentrations. For the Navy, one of the largest sources of Cu and Zn in coastal embayments is from antifouling paint systems on ship hulls. Assessment and regulation of adverse effects in these sediments typically occurs via co-occurrence-based sediment quality guidelines (SQG) using total metal concentration (e.g. Long et al. 1995; Ecology 2013). The bioavailability and potential toxicity of Cu and Zn, however, is not necessarily related to total concentrations measured in bulk sediments, complicating appropriate application of SQGs for environmental regulation.

To address this issue, a research project "Compliance Tools Development for Metals in Antifouling Paints Program"[1] was funded by the Navy to address short-term requirements and data gaps identified by the Navy and the program's technical work group (composed of scientific experts in government, industry, and academia). Funding was provided to support development of improved tools for assessing Cu and Zn bioavailability and toxicity in sediments located at selected Navy facilities, which included two sites at PSNS&IMF. The primary focus of the study was to build on the recent results published by others (e.g. Simpson et al. 2008; Strom et al., 2011), which suggest that expressing sediment Cu concentrations in terms of the metal concentration measured in the fraction of sediment equal or smaller to 63 µm (silt-size fraction of the sediment), normalized to the total organic carbon (TOC) content in the silt-size fraction, provides a vast improvement in the predictability of metal toxicity over current methods based on bulk sediment concentration, or TOC normalization of the bulk concentration. Successful demonstration and validation of this tool could vastly simplify and improve the assessment of contaminant bioavailability and toxicity in DoD sediments, potentially reducing costs associated with their future assessment and remediation.

It is widely recognized that the complexity of sediments and the presence of co-occurring contaminants render definitive identification of Cu or Zn as causal agents in contaminated sediments difficult. The intent of this study was to support the development of improved tools for assessment of Cu and Zn bioavailability and toxicity in sediments at Navy sites, and to support future advances on the ability to model metal toxicity in contaminated marine sediments. The bioavailability of metals is controlled by sediment geochemistry and metals toxicity can be predicted based on the analysis of Simultaneously Extracted Metals (SEM) and Acid Volatile Sulfides (AVS). Sediment quality benchmarks for the protection of benthic

[1] FY 2010 Department of Defense Appropriations Bill, PE 0603721N – Environmental Protection.

organisms from metal exposure have been developed based on the knowledge of AVS, the sum of the SEM (ΣSEM), and fraction of organic carbon (f_{OC}) in the sediment to determine sediments that would not be toxic to benthic organisms when (ΣSEM - AVS)/f_{OC} ≤ 130 umol/g OC (US EPA 2005). However, under oxic and suboxic conditions, the ΣSEM can be more abundant than AVS, therefore this study was focused on relatively oxidized sediments exposed to four different benthic receptors commonly used in sediment quality assessment in North America. The results reported in this document are part of a larger study conducted to advance the state of the science for assessing metal toxicity in sediments (Colvin et al. in prep).

The NIWC Pacific Environmental Sciences Bioassay Laboratory (formerly SPAWAR Pacific Environmental Sciences Bioassay Laboratory) maintains laboratory certifications for bioassays from the Washington State Department of Ecology and the State of California Laboratory Accreditation Programs, employs qualified toxicologists, conducts external and internal audits, and maintains up-to-date standard operating procedures (SOPs) and good laboratory practices (GLP). Sediment toxicity testing using the marine amphipods *Ampelisca abdita, Leptocheirus plumulosus,* the polychaete *Neanthes arenaceodentata*, and embryos from the bivalve *Mytilus galloprovincialis* were performed to evaluate the environmental quality of sediments collected from Puget Sound Naval Shipyard & Immediate Maintenance Facility (PSNS&IMF). The amphipods and polychaete worms were tested in homogenized sediment samples, whereas bivalve embryos were exposed in sediment-water interface (SWI) toxicity tests described by Anderson et al. (2001). Samples were collected April 27, 2011 and testing was conducted at the SPAWAR Systems Center Pacific (SSC Pac) Bioassay Laboratory in San Diego, CA from May 3 through 31, 2011. Sediment chemistry evaluating the metal content as well as grain size and organic content was performed on the samples and is presented herein. Diffusive gradients in thin films (DGTs) were also concurrently deployed to assess the bioavailability of metals associated with the sediment porewater as an additional line of evidence to assess the environmental quality of the sediments tested.

2. MATERIALS AND METHODS

To meet the defined objectives for the project, this study included a series of tasks to characterize toxicity, physico-chemical parameters on overlying water, porewater, sediment, and labile metal concentrations using DGTs. An overview of the approach is shown in Figure 2-1.

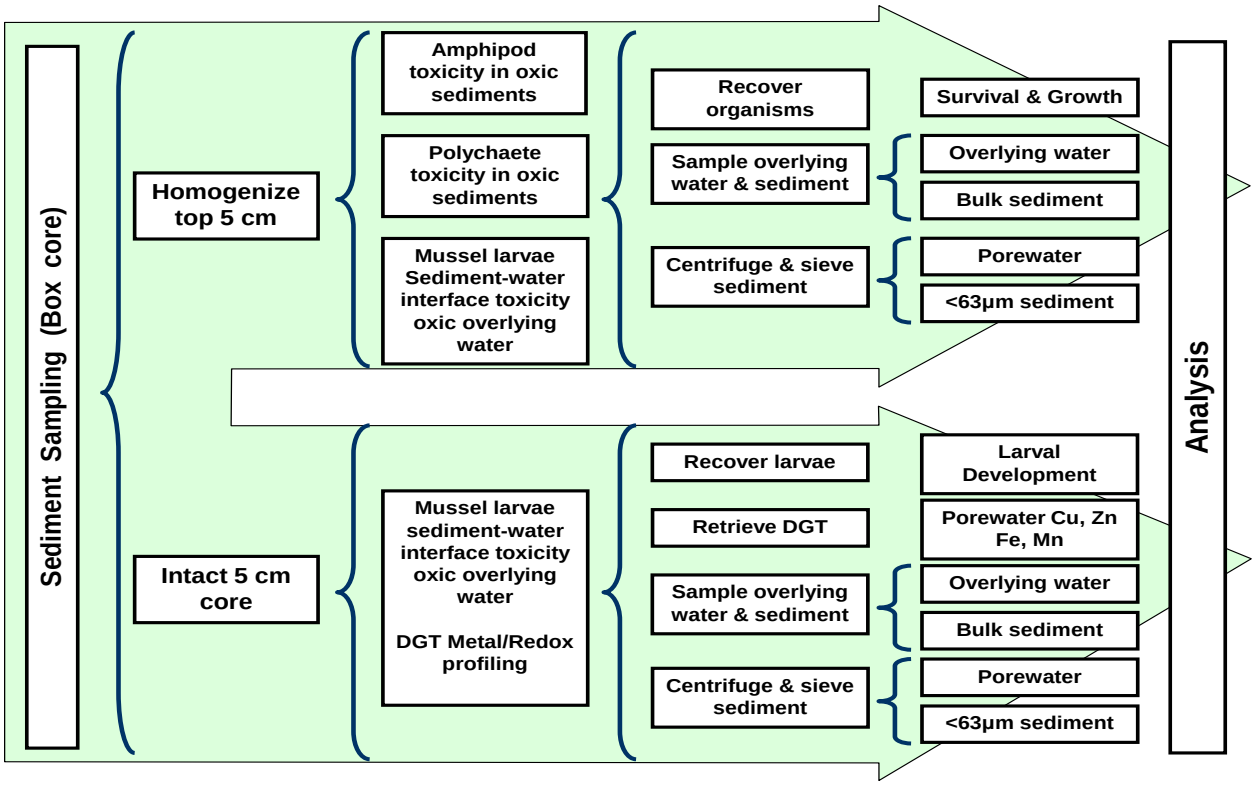

Figure 2-1. Schematic of generalized experimental design.

2.1. *Test Material*

Sediments from PSNS&IMF and Naval Base Kitsap (NBK) located in Bremerton, WA were collected using standard sediment collection, sampling, and storage procedures (ASTM 2008). Sediment samples were collected using a Van Veen sampler to preserve the integrity of *in situ* conditions as best as possible. The sampling equipment was pre-cleaned, and scrubbed and rinsed with site water between grabs, with careful attention not to sample from the sides of the device to avoid cross-contamination. Sampling occurred on the top 5 cm of sediment, focusing on the oxic and suboxic layers. Sediment was composited in pre-cleaned 2 L HDPE wide-mouth bottles for later homogenization and coarse press-sieving (2 mm) at the laboratory to remove native organisms and potential predators. Additionally, intact cores were collected for the SWI toxicity tests using pre-cleaned polycarbonate core tubes following specifications in Anderson et al. (1996). SCUBA divers manually collected the intact core samples from the field by completely filling the tubes and capping the ends of the tube. Caps were taped and shipped to the SSC Pac Bioassay Laboratory in insulated ice chests containing blue ice. Upon receipt in the laboratory, sediments were stored in the dark at 4^{o}C until use, and were used for experimentation as soon as possible. Sediment within the cores was dropped down to a 5 cm mark on the side of each core on the day prior to initiation. Test initiation was targeted for 48 h within collection, with a maximum holding time of two weeks (USEPA, 1994). Sample collection and receipt times are summarized in Table 2-1. Copies of chain of custody forms are provided in Appendix D.

Table 2-1. Sediment Sample Collection and Receipt Times.

Sample/ Station ID	Latitude	Longitude	Type	Sample Collection Date	Sample Receipt Date/Time	Sample Receipt Temperature (°C)
PS03 (NBK)	47.555783	-122.651925	Grab	4/27/2011 11:25	4/29/2011 09:00	6.1
			Intact Core	4/27/2011 10:50	4/29/2011 09:00	6.1
PS09 (PSNS)	47.560127	-122.636493	Grab	4/27/2011 12:35	4/29/2011 09:00	6.1
			Intact Core	4/27/2011 12:20	4/29/2011 09:00	6.1

2.2. *Test Organisms and Acclimation*

Toxicity testing included the following experimental organisms: the two amphipods (*Ampelisca abdita* and *Leptocheirus plumulosus*), the polychaete (*Neanthes arenaceodentata*), and embryos from the bivalve embryos (*Mytilus galloprovincialis*).

Selection of test organisms was based on the desire to assess the responses in benthic invertebrates that differ in sensitivity to Cu and Zn, contaminant exposure route, and geographical location. *A. abdita* (Figure 2-2) is a suspension feeding, sediment ingesting amphipod that builds tubes out of sand grains (Redmond et al., 1994), while *L. plumulosus* is a free burrowing species (USEPA 1994). *N. arenaceodentata* (Figure 2-2) is a surface deposit feeding/predatory omnivorous polychaete, and builds mucoid tubes in surficial sediments (Dillon et al., 1993). All three species occur extensively in North America, are exposed to a combination of overlying water and porewater, in addition to sediment particles, detritus, and prey that might be an exposure source for Cu and Zn, and are frequently employed in testing for regulatory programs.

M. galloprovincialis embryo-larval development tests were incorporated in sediment-water interface (SWI) toxicity exposures (Anderson et al. 1996; Anderson et al. 2001). The relevancy of SWI tests in the assessment of sediment bioavailability and toxicity is high; 1) embryos are negatively buoyant and therefore directly exposed to sediment-associated contaminants during critical phases of cell differentiation; 2) the endpoint plays a major role in the development of saltwater WQC for Cu (USEPA 1995a); 3) the endpoint has served as the primary test for the development of site specific WQC for Cu in water effect ratio (WER) studies (e.g. Rosen et al. 2005, 2009; Earley et al. 2007), and for the development of predictive models of Cu toxicity in surface waters (e.g. Arnold et al. 2006; Chadwick et al. 2008); 4) the SWI toxicity test with *M. galloprovincialis* is a recommended test for the assessment of sediment quality as part of recently derived sediment quality objectives (SQOs) for the state of California (Bay et al. 2007); and 5) the lack of feeding during embryogenesis simplifies the interpretation of data towards the dissolved water concentration only.

Sub-adult *L. plumulosus*, approximately 2-4 mm in length, were obtained from Chesapeake Cultures, Inc. (Hayes, VA). *A. abdita*, approximately 0.71 – 1.18 mm in length, were obtained from Aquatic Research Organisms, Inc. (Hampton, NH). Juvenile *N. arenaceodentata* were obtained from a culture maintained by Aquatic Toxicology Support (Bremerton, WA). Gravid *M. galloprovincialis* were obtained from Carlsbad Aquafarm (Carlsbad, CA).

Amphipods and polychaetes were received at least one day prior to test initiation to allow for acclimation to appropriate test conditions (salinity, temperature, and lighting). Gravid mussels and urchins were received on the morning of the test initiation day. Mussel embryos were obtained from thermal-shock induced spawning from gravid mussels and sea urchins injected with potassium chloride to induce spawning. All organisms were visually inspected to confirm that they were of the proper size, and in good health, prior to use in toxicity testing.

Figure 2-2. Toxicity endpoints for this study included a) polychaete (*Neanthes arenaceodentata*) survival and growth, b) amphipod (*Leptocheirus plumulosus*) survival, c) amphipod (*Ampelisca abdita*) survival, and d) bivalve (*Mytilus galloprovincialis*) embryo-larval development and survival. Photos are not to scale.

2.3. *Toxicity Testing Procedures*

Testing was conducted in accordance with standard methods (USEPA 1994, USEPA 1995, ASTM 1996). The 10-day amphipod survival tests with whole sediment, the 28-day polychaete survival and growth test, and the 2-day sediment-water interface (SWI) bivalve embryo development test were conducted on the samples listed in Table 2-1. Negative controls consisting of sediment from the amphipod collection site was included in the 10-day whole sediment test. For the 2-day SWI test, a chamber control (screen tube) and a seawater negative control were also tested concurrently. Summaries of the test conditions are provided in Table 2-2, Table 2-3, and Table 2-4.

For both the whole sediment and sediment-water interface (SWI) toxicity tests, samples from the overlying water were collected at the beginning and end of the exposures, while porewater, DGT samplers and sediment samples were collected and analyzed at the test termination only. All test chambers were set up with sediment, water and aeration on the day prior to test initiation. Screen tubes for the SWI test were gently introduced to each core tube on the day of test initiation. Water quality parameters including pH, dissolved oxygen (DO), salinity, temperature and ammonia were measured in the overlying water prior to organism addition to ensure that conditions were within those tolerated. Daily observations of

water quality, aeration and sediment condition (e.g. anoxia, microbial growth, etc.) were made. All instruments used for water quality measurements were calibrated daily according to manufacturer specifications.

2.3.1. Sediment-Water Interface Toxicity Tests

The *M. galloprovincialis* embryo-larval development toxicity tests were conducted according to USEPA (1995b) and Anderson et al. (1996). Test conditions and acceptability criteria are summarized in Table 2-2. For the SWI test, early stage (< 4 hour old) embryos were placed at the interface using a screen tube (25 µm mesh) that rests ~1 cm above a 5 cm sediment core (Figure 2-3). Developing larvae were exposed to contaminant flux from the sediment in both intact core and homogenized core tubes (2.5 inches in diameter), which were filled with 300 mL of overlying uncontaminated FSW. The number of surviving normal D-shaped larvae (% normal alive) was determined on an inverted microscope at the end of the test.

Each sample consisted of six replicates, four for organism exposure, one for destructive sampling of the sediments at the beginning of the test, and one for placement of a diffusive gradient in thin-film (DGT) to measure the profile of metal (Cu, Zn, Fe and Mn) concentrations in the porewater and overlying water. The mussel embryos never came into direct contact with the sediment and do not feed, so are exposed primarily to dissolved substances that partition out of the sediment. This test is required in newly established California SQOs (Bay et al. 2007; SSCWRP 2014), and the embryo-larval development endpoint of this species independently dictates ambient saltwater WQC for Cu (USEPA, 1995a) and was used in marine Cu BLM development (Chadwick et al. 2008), and therefore, provides a nice linkage between water and sediment metal bioavailability assessment.

At the end of the exposure period for the SWI toxicity test, screen tubes were carefully removed from the sediment and the embryos were washed into glass scintillation vials, and preserved in 10% buffered formalin for later microscopic examination.

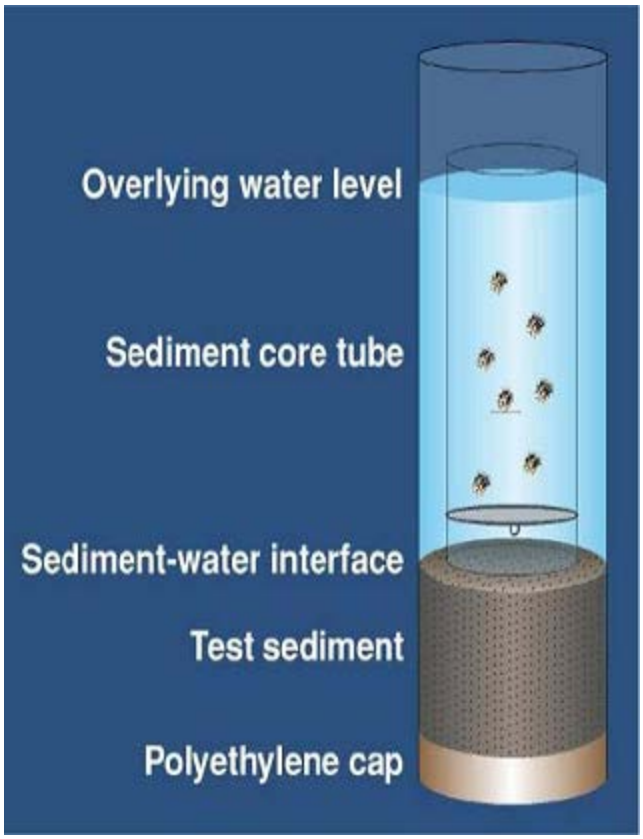

Figure 2-3. Diagram of the sediment-water interface toxicity test.

Table 2-2. Specifications for 2-day Chronic Exposure Using the Mediterranean Mussel Embryo-Larvae at the Sediment-Water Interface.

Test Periods	5/3-5/5/2011
Test organism	Mediterranean mussel – *Mytilus galloprovincialis*
Test organism source	Carlsbad Aquafarm, Carlsbad, CA
Test duration; endpoints	48 hr; embryo-larval survival and development success (proportion normal-alive)
Test solution renewal	None
Feeding	None
Test Chamber size/type	1L glass mason jar w/ polycarbonate screen tubes with 25 µm mesh
Test sediment depth	5 cm
Test sediment manipulation	Undiluted sediment exposed as intact cores
Overlying water volume	300 ml
Test temperature	15 ± 1 °C
Test salinity	30 ± 2 ppt
Light quality	10-20 µE/m^2/s (Ambient laboratory levels)
Photoperiod	16 hr light/ 8 hr dark
Aeration	Laboratory filtered air, continuous (1-2 bubbles per second delivered through a Pasteur pipette) , maintain >90% saturation
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
Overlying water source	Filtered (0.45 µm) natural seawater collected from near the mouth of San Diego Bay at SSC PAC Laboratory
Test acceptability criteria	≥ 80% mean normal-alive in control
Reference toxicant	Copper sulfate, standard EPA laboratory method only; 48 hr water only exposure; five concentrations (5 replicates each)
Test protocol	EPA 600/R-95/136 (USEPA 1995)

2.3.2. Whole Sediment Toxicity Tests

The *A. abdita* and *L. plumulosus* exposures were conducted using minor modifications of standard methods (USEPA, 1994). Recently, a new protocol was published for *N. arenaceodentata* (Farrar and Bridges, 2011) that employs an earlier life stage (≤ 7 day old emergent juveniles) than other standard methods with this species (e.g. ASTM 2000). This method was demonstrated to be considerably more sensitive than methods employing 2-3 week old organisms in comparative round robin testing. In addition, the growth endpoint using the new procedure described by Farrar and Bridges (2011) was among the most sensitive in a multi-species comparison of acute and chronic toxicity in marine sediments (Greenstein et al., 2008).

Summaries of the test conditions and test acceptability criteria for the whole sediment toxicity tests are shown in Table 2-3 and Table 2-4. Briefly, the amphipod tests included approximately 150g of homogenized wet sediment in 1 L glass jars, with 700 mL of overlying uncontaminated 0.45 µm FSW. The polychaete tests contained 75 g of wet sediment and 175 mL of FSW (Farrar and Bridges, 2011). Overlying water in all tests was continuously aerated with filtered laboratory air at a rate of approximately 100 bubbles per minute. A 24-h equilibration period with the overlying water was allowed prior to addition of test organisms (Day 0). Exposures were static for *A. abdita* and *L. plumulosus* for 10 days (acute exposure), while weekly renewals of the overlying water were made in the 28 day exposures with *N. arenaceodentata* (chronic exposure). The organisms were recovered on 0.5 mm sieves at the end of the test and enumerated for survival. For *N. arenaceodentata,* recovered organisms were purged overnight in FSW prior to drying for growth assessment, and then transferred into microcentrifuge vials for acid digestion (nitric acid under heat) and measurement of Cu and Zn in the tissues (Rosen et al., 2008).

Table 2-3. Specifications for 10-day Whole Sediment Acute Exposure Using the Marine Amphipods *Ampelisca abdita* and *Leptocheirus plumulosus*.

Test Periods	5/3-13/2011
Test organism	Marine amphipods – *Ampelisca abdita* and *Leptocheirus plumulosus*
Test organism size at initiation	Adult 3-5 mm
Test organism source	Aquatic Research Organisms, Inc. and Chesapeake Cultures, Inc.
Test duration; endpoint	10-day; survival
Test solution renewal	None
Feeding	None
Test Chamber size/type	1L glass mason jar
Test sediment depth	5 cm (approximately 150 g)
Test sediment manipulation	Homogenized and sieved to <2.0 mm
Overlying water volume	700 ml
Control sediment source	Sediment from amphipod collection site, Yaquina Bay, OR
Test temperature	15 ± 1 °C
Test salinity	30 ± 2 ppt
Light quality	Ambient laboratory illumination
Photoperiod	Continuous light (24 hr), ambient laboratory lighting
Aeration	Laboratory filtered air, continuous (1-2 bubbles per second delivered through a Pasteur pipette), maintain >90% saturation
No. of organisms per chamber	20
No. of replicates	*Leptocheirus* – 3; *Ampelisca* - 4
Overlying water source	Filtered (0.45 µm) natural seawater collected from near the mouth of San Diego Bay at SSC PAC Laboratory
Test acceptability criteria	≥ 90% mean survival in control sediment
Reference toxicant	Copper sulfate, standard EPA laboratory method only; 96-h water only exposure; five concentrations (4 replicates each)
Test protocol	EPA 600/R-94/025 (USEPA 1994)

Table 2-4. Specifications for 28-day Whole Sediment Chronic Exposure Using the Marine Polychaete *Neanthes arenaceodentata* (adapted from Farrar and Bridges 2011).

Test Periods	5/3-31/2011
Test organism	Marine polychaete – *Neanthes arenaceodentata*
Test organism size at initiation	Juvenile ≤ 7 d post-emergent
Test organism source	Aquatic Toxicology Support
Test duration; endpoint	28-day; survival and growth
Test solution renewal	50% once weekly
Feeding	Twice weekly, 2 mg of ground Tetramin © per organism
Test Chamber size/type	400 mL glass beaker
Test sediment depth	2 cm (approximately 75 g)
Test sediment manipulation	Homogenized and sieved to <2.0 mm
Overlying water volume	175 ml
Control sediment source	Sediment from amphipod collection site, Yaquina Bay, OR
Test temperature	15 ± 1 °C
Test salinity	30 ± 2 ppt
Light quality	Ambient laboratory illumination
Photoperiod	Continuous light (24 hr), ambient laboratory lighting
Aeration	Laboratory filtered air, continuous (1-2 bubbles per second delivered through a Pasteur pipette), maintain >90% saturation
No. of organisms per chamber	20
No. of replicates	10
Overlying water source	Filtered (0.45 µm) natural seawater collected from near the mouth of San Diego Bay at SSC PAC Laboratory
Test acceptability criteria	≥ 80% mean survival in control sediment and positive growth in control organisms
Reference toxicant	Copper sulfate, standard EPA laboratory method only; 96-h water only exposure; five concentrations (4 replicates each)
Test protocol	Farrar and Bridges (2011)

2.4. *General Chemistry*

All glassware, plasticware and associated equipment were cleaned thoroughly prior to use by soaking in 10% nitric acid (HNO_3) for 24 h, followed by rinsing in de-ionized water. Glassware used as test chambers also underwent a 24 h soak in 30 ppt 0.45 µm FSW.

2.5. *Total and Dissolved Metal Measurements*

Assessment of metal concentrations was made following methodology recommended by USEPA, including use of trace metal clean sampling techniques in the collection, handling and analysis (USEPA, 1996). Water and porewater samples were collected in 30-mL acid-cleaned low-density polyethylene bottles. Samples were acidified to pH ≤2 with quartz still-grade nitric acid (Q-HNO_3) in a High Efficiency Particle Air (HEPA) class-100 all polypropylene working area.

2.5.1. Metal Concentrations in Water

Overlying Water Sample Collection

Overlying water samples were taken from test chambers at the beginning and end of exposure periods (i.e. time zero and time final). The water samples were decanted from the test chamber using a peristaltic pump, without disturbing the sediment, into acid-cleaned 30 mL high density polyethylene (HDPE) bottles. Samples were collected in duplicate from each test chamber; one replicate was acidified to measure total metals, while the other replicate was filtered at a clean bench with a 0.45 µm filter attached to the pump tubing, and then acidified for quantification of dissolved metals. Each water sample was acidified to a pH of ≤ 2 with 50 µL of $QHNO_3$.

Pore Water Sample Collection

Pore water samples were collected from the test sediments at test termination only. After the overlying water was sampled and/or discarded, replicates of each sediment treatment were combined into a centrifuge tube in an anaerobic chamber. The combined replicate samples were ultra-centrifuged at 9000 rpm for 15 minutes leaving the pore water as a supernatant. The pore water was sampled from the centrifuge tube using a peristaltic pump with a 0.45 µm filter into acid cleaned 15 mL HDPE bottles. Each water sample was acidified to a pH ≤ 2 with 50 µL of $QHNO_3$.

Overlying Water and Porewater Metal Analysis

Metal concentrations in overlying and pore water samples were measured using in-line pre-concentration Flow Injection Analysis and a Perkin-Elmer SCIEX ELAN DRC II inductively coupled plasma with detection by mass spectrometry (ICP-MS; USEPA, 1994b). Each sample first ran through a Flow Injection Analysis System (FIAS) to pre-concentrate the metals, and to reduce the salt-content of the sample. The sample was then directly transferred into the Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for quantification. Blanks were analyzed every 5 samples to make sure the system was clean and to give a

reference point for the background level. A Standard Reference Material (SRM) was analyzed after each blank to ensure that the instrument was measuring accurately and precisely. The blank was NASS 2 (open ocean sea water) and the SRM was CASS 4 (coastal seawater) both from the National Research Council of Canada.

When deemed necessary for samples with high metal concentrations, samples were diluted with 0.1 N Q-HNO_3 made up in high-purity (18 MΩ cm^{-1}) water in order to minimize matrix related interferences. The diluted samples were injected directly into the ICP-MS via a Perkin-Elmer Autosampler 100. Analytical standards were made in CASS4 Nearshore Seawater Reference Material for Trace Metals, National Research Council Canada, with Perkin-Elmer multi-element standard solution (PEMES-3) diluted in 1N Q-HNO_3, and were analyzed at the beginning and end of the run. The analysis also included measurement of the Standard Reference Material (SRM) 1643e from the National Institute of Standards & Technology (NIST), and analytical blanks made up of 1N Q-HNO_3 after every five samples. A coefficient of variation (CV) of ≤5% for replicate measurements was observed, as well as a recovery within 15% for direct injection of SRM 1643e. The method limit of detection is defined as three times the standard deviation of the analytical blanks made of 1N Q-HNO_3.

2.5.2. Metal Concentration in Sediment

Sediment Sample Collection

Sediment samples were collected at the test termination from exposure beakers for both sediment samples. After overlying and pore waters were removed from multi-replicate composites, approximately 120 mL sediment was transferred to a HDPE bottle for bulk metal analysis. The remaining sediment was wet-sieved through a 63 µm sieve, and transferred to a separate 120 mL HDPE bottle for metal and TOC analysis of the <63 µm size fraction (Spadaro et al. 2008).

Sediment Metal analysis – ICP-MS and SEM-AVS

Bulk sediment samples were analyzed using both ICP-MS and SEM-AVS. The <63 µm fraction was only analyzed by ICP-MS. ICP-MS analyses were conducted at SSC Pacific. SEM-AVS analyses were conducted by the Engineer Research and Development Center Laboratory (ERDC).

For ICP-MS analyses, empty 30 mL HDPE bottles were labeled and dried at 60°C in a drying oven for at least 24 hours. The dried bottles were then weighed and the tare mass (g) recorded. Enough wet sediment to get a dry mass of approximately 0.25 g was transferred to each 30 mL bottle. The bottles were placed in the oven with no caps at 60°C for at least 24 hours, followed by verification of complete dryness. The bottles with dry sediment were weighed again and the mass (g) was recorded. One mL of concentrated trace metal grade (TMG) Hydrochloric Acid (HCL) and 0.5 mL of concentrated TMG HNO_3 were added to each sediment sample. The samples were allowed to digest for 24 hours at room temperature capped loosely and put on a warm heating plate (≈60°C) for at least 1 hr. Subsequently, about 30 mL of 1 N TMG HNO_3 was added to each sample and the final mass (g) recorded. After particles were allowed to settle,

sample dilutions of the overlying digestate were made. A 5-fold dilution of each sample was made before metal concentration analysis by transferring 2 mL of sample digestate solution (no particles) to a 15 mL centrifuge tube and adding 8 mL of 1N TMG HNO_3 for a total volume of 10 mL.

Metal concentrations were measured using an ICP-Optical Emissions Spectrometer (ICP-OES). Three duplicate samples were chosen at random for each run. For every 5 samples, a blank was run to make sure the system was clean and to give a reference point for the background level of metals. A SRM was run after each blank to ensure that the instrument was measuring accurately and precisely. The blank was either 1N TMG HNO_3 or 18 MΩ cm^{-1} water and the SRM was 1643e (trace metals in water) from the National Bureau of Standards. In addition, six blanks were prepared from empty 30 mL HDPE bottles which were treated in the same manner as the sediment digestions. All acid additions and dilutions were carried out identically.

2.5.3. Metal Concentration in Tissues

Tissue Collection and Analysis

Tissue from the *Neanthes* whole sediment test were analyzed for metal tissue concentrations following the exposure period of 28 d. At the termination of the bioassay, organisms were allowed to depurate for a minimum of 24 hr. Organisms were examined following the depuration period for debris in their gut and were gently palpated to further remove debris. Organisms were gently rinsed with Milli-Q DI water to remove salts, blotted dry, and then placed into pre-cleaned, dried and pre-weighed polypropylene microcentrifuge tubes (1.5 mL). Wet tissue was then dried at 60°C. Once the tissues were dry, the vials were weighed again. Concentrated Q-HNO3 (50 µL) was added to each vial making sure to cover the tissue as much as possible. The vials were allowed to digest for at least three days at room temperature at a clean bench. Finally, 1500 µL 1N Q-HNO3 was added to each vial and the vial weighed again. One mL of acid was taken from each digestion and analyzed by ICP-MS.

2.5.4. Metal Concentration in DGTs

DGTs were positioned in surrogate test vessels for each of the sediment types, allowing for both DGT determination of overlying water and pore water Cu, Zn, Fe, and Mn measurements in the oxic and suboxic zones. Suboxic zones were defined as those layers of sediment where either or both Fe or Mn was present in the (0.45 µm) porewater.

DGT Collection and Analysis

At test termination, DGTs were recovered and rinsed with DI water. The DGT gel was extracted from the plastic housing and the DGT gel was set at the bottom of pre-cleaned, dried and weighed centrifuge tubes. The gel was then allowed to dry in a class-100 clean bench for several days at room temperature. Once dry, the vials were weighed again. Concentrated Q-HNO3 (50 µL) was added to each vial making sure to cover the DGT gel film as much as possible. The vials were allowed to digest for at least three days at room

temperature in the clean bench. Finally, 1500 µL 1N Q-HNO3 was added to each vial and the vial weighed again. One mL of acid was taken from each digestion and analyzed by ICP-MS.

3. RESULTS

Summaries of statistical, toxicity, and raw test results for the bioassay tests are provided in Tables 3-1 through Table 3-3. Analytical chemistry results are provided for bulk sediment (Table 3-4), sediment <63 um (Table 3-5), overlying and pore water chemistry (Tables 3-7 through 3-10), overlying water ammonia (Table 3-11) and tissue residue results (Table 3-12). Complete statistical summaries and bench water quality sheets are provided in Appendix A.

3.1. *Sediment-Water Interface Toxicity Results*

The chamber control associated with the SWI exposures with *M. galloprovincialis* was slightly outside of test acceptability criteria at 75.6% (acceptability criteria: ≥ 80% mean normal-alive). However, the tests were deemed acceptable based on the responses of the site sediments all performing better than the control. All water quality parameters were within the recommended range for the duration of the test. Table 3-2 summarizes the results of the SWI tests with *M. galloprovincialis*. Mean normal ranged from 80 to 95 percent and mean normal-alive ranged from 79 to 95 percent (Table 3-2). For statistical analyses, each sample was compared to the chamber control using the statistical software Comprehensive Environmental Toxicity Information System (CETIS) v1.8.7.16 (Tidepool 2012). No significant differences were observed for intact cores or homogenized field samples relative to the chamber control tested (all p-values >0.05).

Table 3-1. Summary of Statistical Results for the Sediment-Water Interface Test.

Station ID	Mean % Normal (SD)	% Difference from Control	p-value	Mean % Normal-Alive (SD)	% Difference from Control	p-value
Negative Control – Screen Tube	80.4 (7.3)	-	-	75.6 (13.8)	-	-
PS-03 Intact Core	89.4 (8.1)	11.2	0.9909	85.8 (11.1)	13.5	0.9642
PS-03 Homogenized	95.8 (2.0)	19.2	0.9996	97.4 (3.4)	28.8	0.9994
PS-09 Intact Core	92.0 (4.6)	14.4	0.9986	79.1 (11.9)	4.6	0.8005
PS-09 Homogenized	92.5 (3.3)	15.1	0.9988	83.4 (11.4)	10.3	0.9225

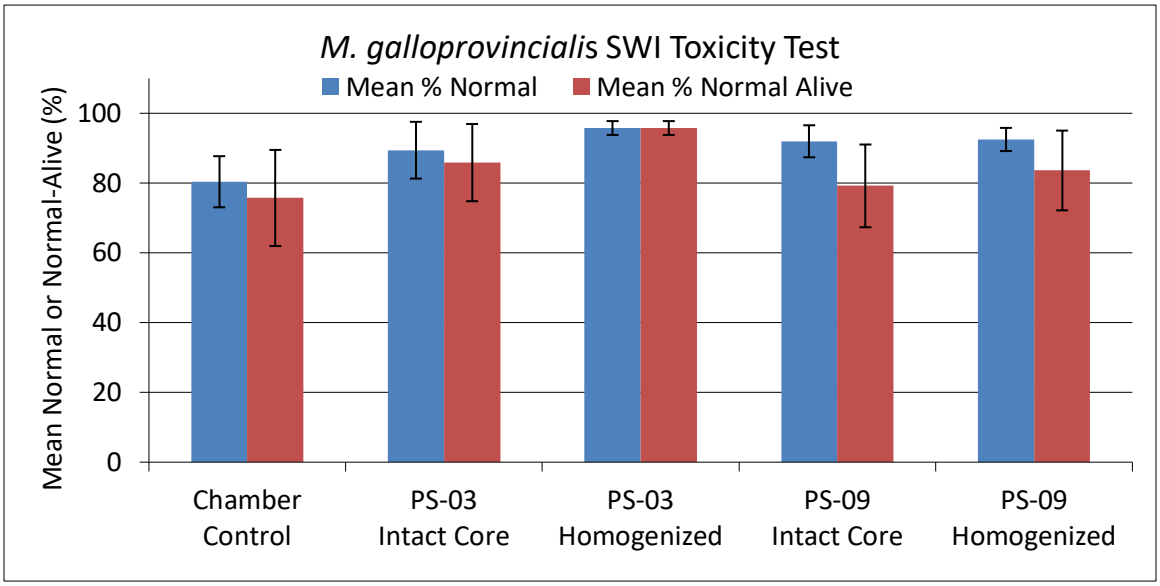

Figure 3-1. Mean percent normal and mean percent normal-alive *M. galloprovincialis* larvae for the sediment-water interface test.

3.2. *Whole Sediment Toxicity Results*

3.2.1. Leptocheirus plumulosus

The control associated with the 10-day whole sediment test with *L. plumulosus* met test acceptability criteria of 90 % survival. All water quality parameters measured were within the recommended range for the duration of the test. Survival was 86 and 93 % for samples PS-03 and PS-09, respectively. Each sample was compared statistically against the laboratory control sediment that was tested concurrently using a CETIS. No significant differences were observed in any of the sediment samples tested compared to the control (all p-values >0.05, Table 3-3, Figure 3-2. Mean percent normal and mean percent normal-alive *M. galloprovincialis* larvae for the sediment-water interface test.).

3.2.2. Ampelisca abdita

For the 10-day whole sediment test with *A. abdita*, dramatic mortality was observed two days into the exposure period. It was decided that ten additional organisms would be added to two of the four replicates (replicates A & B) and based on the average number of mortalities observed across all replicates a normalization of the initial number of organisms in each replicate would be made; 26 for the A & B replicates and 16 organisms for the C & D replicates. The *A. abdita* toxicity test as a whole did not meet test acceptability criteria for the mean survival (90% survival) in the controls. However, samples were compared against the control for interest's sake. Sample PS-09 was significantly decreased from the control sediment (p = 0.0493, Table 3-3, Figure 3-2. Mean percent normal and mean percent normal-alive *M. galloprovincialis* larvae for the sediment-water interface test.) and while PS-03 did not show significance relative to the control, the trend for toxicity is similar to PS-09.

Table 3-1. Summary of Statistical Results for the Whole Sediment Tests with *L. plumulosus* and *A. abdita*.

Station ID	*Leptocheirus plumulosus*			*Ampelisca abdita*		
	Mean % Survival (SD)	**% Difference from Control**	**p-value from Student's t-test**	**Mean % Survival (SD)**	**% Difference from Control**	**p-value from Student's t-test**
Laboratory Control[A]	90.0 (8.7)	-	-	78.5 (17.9)	-	-
PS-03 Homogenized	86.7 (2.9)	-3.7	0.2317	59.3 (12.1)	-24.5	0.0686
PS-09 Homogenized	93.3 (5.8)	3.7	0.6788	58.9 (4.4)	-25.0	**0.0493**

[A] – Control sediment was sediment from Sequim Bay.
Values in **bold** indicate a statistically significant decrease compared to the control.

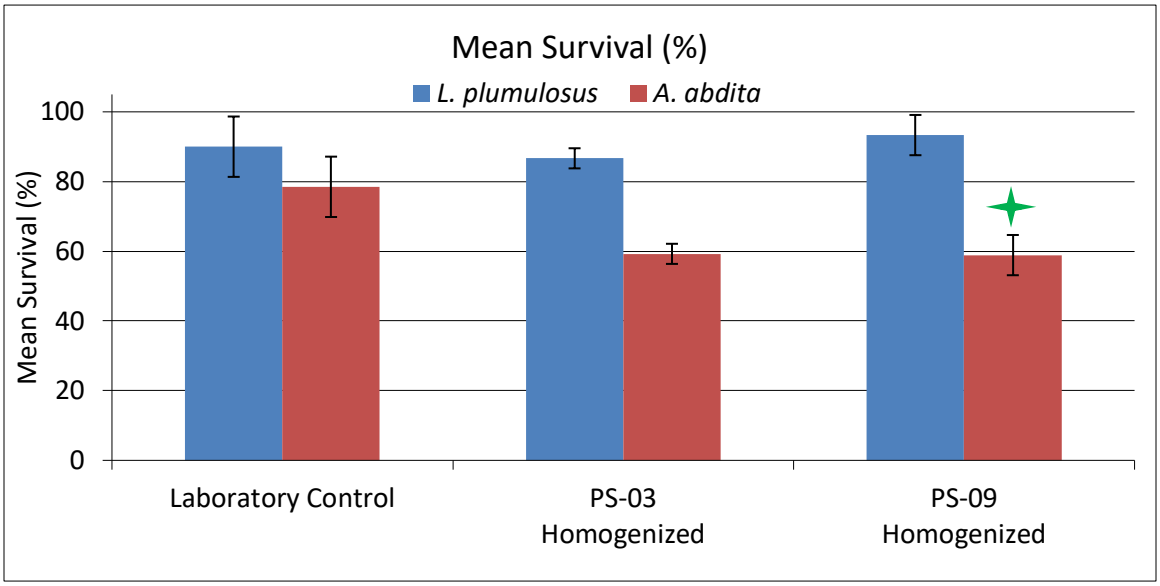

Figure 3-2. Mean percent survival of *L. plumulosus* and *A. abdita.* The star indicates statistical decrease from the respective laboratory control.

3.2.3. Neanthes arenaceodentata

The control associated with the 28-day whole sediment test with *N. arenaceodentata* met test acceptability criteria of 90 % survival and positive growth. All water quality parameters measured were within the recommended range for the duration of the test. Survival was 100 % for both samples (Table 3-3). Growth was 5.1 and 4.7 mg for PS-03 and PS-09, respectively. Each sample was compared statistically against the laboratory control sediment that was tested concurrently using CETIS. No significant differences were observed in any of the sediment samples tested compared to the control for either survival or growth (all p-values >0.05, Table 3-3, Figure 3-3, Figure 3-4).

Table 3-2. Summary of Statistical Results for 28-day Whole Sediment Test with *N. arenaceodentata.*

Station ID	Survival			Growth		
	Mean % Survival (SD)	% Difference from Control	p-value from Student's t-test	Mean Growth (mg) (SD)	% Difference from Control	p-value from Student's t-test
Laboratory Control[A]	90.0 (31.6)	-	-	4.928	-	-
PS-03 Homogenized	100.0 (0.0)	11.1	1.000	5.106	3.6	0.5905
PS-09 Homogenized	100.0 (0.0)	11.1	1.000	4.682	-4.99	0.3840

[A] – Control sediment was sediment from Sequim Bay.

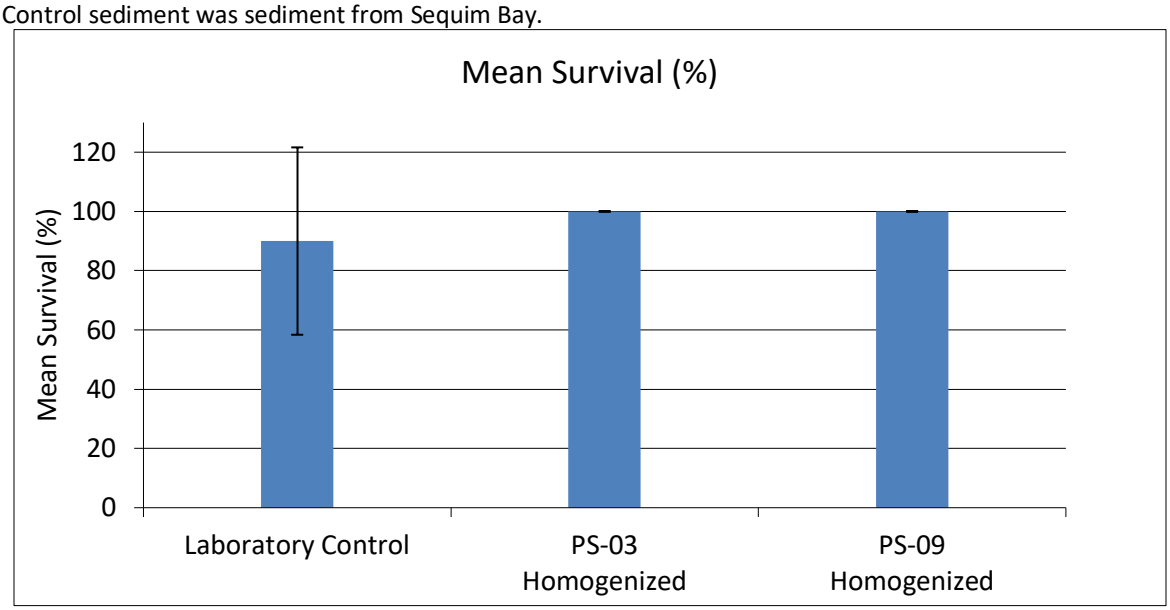

Figure 3-3. Mean percent survival of *N. arenaceodentata*.

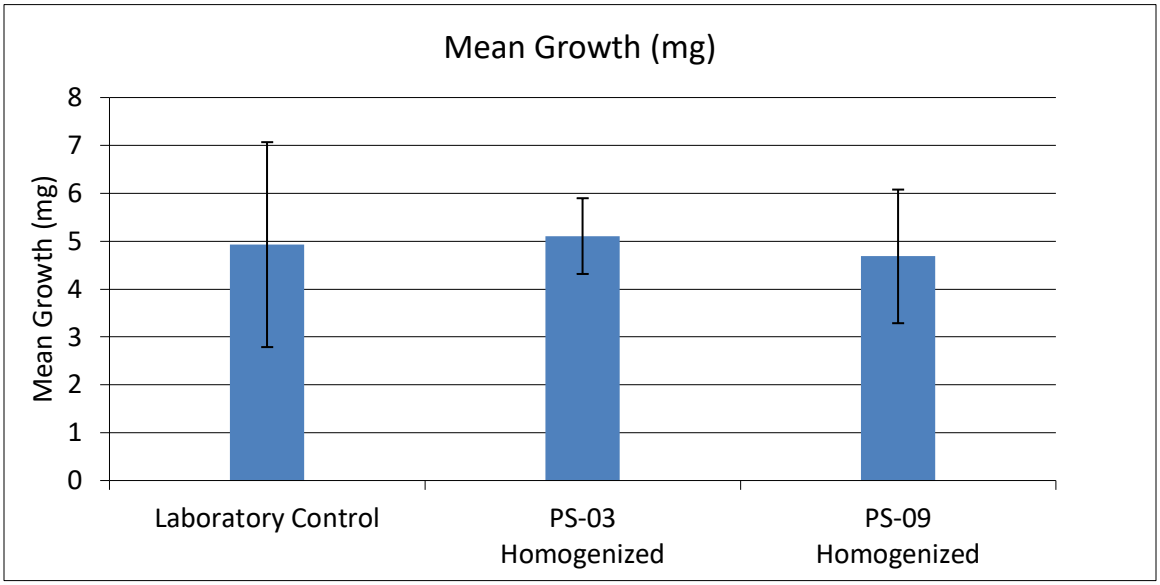

Figure 3-4. Mean growth of *N. arenaceodentata*.

3.3. *Sediment, Overlying Water, Pore Water and Tissue Residue Chemistry*

Results for sediment chemistry, for dissolved and particulate phases of metals in overlying water and pore water, and tissue residues are summarized below. Bulk Sediment Chemistry

As stated previously, bulk sediment samples were collected following decanting of the overlying waters. Bulk samples were analyzed for Cu and Zn and total organic carbon (TOC). Remaining sediment was then processed through a 63 µm sieve for additional metal and TOC analysis. Bulk sediment samples were also evaluated using SEM-AVS by the Army's research lab ERDC and results were normalized to the amount of organic carbon.

The results showed that sediment concentrations of Cu and Zn did not exceed WA SQGs (Ecology 2013), organic carbon normalized SQGs for bulk and <63 um size fractions (Simpson et al. 2008), or threshold of effects for $(\sum SEM\text{-}AVS)/f_{oc}$ (US EPA 2005).

Table 3-3. Sediment Chemistry Results – Bulk Sample.

Sample ID	Silt & Clay <63µm (%)	pH	TOC (%)	Cu (mg/kg)	Zn (mg/kg)	Bulk Sed/TOC Cu (mg/g OC)	Bulk Sed/TOC Zn (mg/g OC)
PS03	71.3	7.26	2.9	199.6	232.0	6.9	8.0
PS09	81.0	7.42	2.6	213.9	258.7	8.2	10.0

Table 3-4. Sediment Chemistry Results – < 63µm Fraction and SEM-AVS.

Sample ID	TOC (%)	Cu (mg/kg)	Zn (mg/kg)	<63µm/TOC Cu (mg/g OC)	<63µm/TOC Zn (mg/g OC)	∑SEM-AVS (µmol/g)	$(\sum SEM\text{-}AVS)/f_{oc}$
PS03	3.5	213.1	229.5	6.1	6.6	-8.96	-309
PS09	4.4	223.7	260.5	5.1	5.9	-12.1	-466

3.3.1. Overlying and Porewater Chemistry

The results for chemical analysis of overlying water (OW) and pore water (PW) showed that Cu and Zn did not exceed chronic water quality standards (Ecology 2016)

Table 3-6. Overlying and Porewater Chemistry Results – *L. plumulosus*.

	Overlying Water – Time Final				Pore Water	
Sample ID	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	Dissolved Cu (µg/L)	Dissolved Zn (µg/L)
PS03	1.2	0.8	3.0	7.2	0.8	10.4
PS09	1.7	0.8	9.4	6.1	0.4	1.0

Table 3-7. Overlying and Porewater Chemistry Results – *A. abdita*.

	Overlying Water – Time Final				Pore Water	
Sample ID	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	Dissolved Cu (µg/L)	Dissolved Zn (µg/L)
PS03	1.1	0.8	17.2	20.8	0.8	10.4
PS09	1.6	1.0	17.6	13.4	0.4	1.0

Table 3-8. Overlying and Porewater Chemistry Results – *N. arenaceodentata*.

	Overlying Water – Time Final				Pore Water		
Sample ID	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	Dissolved Cu (µg/L)	Dissolved Zn (µg/L)	DOC (mg/L)
PS03	1.2	0.8	1.8	0.5	0.4	ND	<5.0
PS09	0.8	0.8	3.1	3.1	0.4	16.5	<5.0

Table 3-9. Overlying Water Chemistry Results – *M. galloprovincialis*. (C indicates intact core, H indicates homogenated core)

	Overlying Water – Time 0					Overlying Water – Time Final				
Sample ID	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	DOC (mg/L)	TOC (mg/L)
PS03 - C	1.4	0.7	4.5	4.3	1.7	0.6	2.4	0.4	<0.5	<0.5
PS03 - H	1.3	0.6	1.7	1.9	2.6	0.6	3.1	ND	<0.5	<0.5
PS09 - C	0.9	0.6	2.4	2.2	1.3	0.6	2.2	ND	<0.5	<0.5
PS09 - H	1.1	0.6	1.6	2.5	4.7	0.6	5.3	ND	<0.5	<0.5

Table 3-10. Porewater Chemistry Results – *M. galloprovincialis*.

Sample ID	Dissolved Cu (µg/L)	Dissolved Zn (µg/L)	DOC (mg/L)
PS03 - C	0.3	153.6	168.0
PS03 - H	0.3	68.6	<0.5
PS09 - C	0.2	49.4	2600
PS09 - H	0.3	32.7	0.6

Table 3-5. Overlying Water Chemistry Results – Ammonia (mg/L).

Sample ID	Initiation			Termination		
	SWI test	10-d whole sediment test	28-d whole sediment test	SWI test	10-d whole sediment test*	28-d whole sediment test
PS03 - C	2.2	-	-	1.0	-	-
PS03 - H	0.2	0.7	1.1	ND	1.6/ND	ND
PS09 - C	0.5	-	-	0.4	-	-
PS09 - H	1.3	ND	0.4	1.1	1.6/ND	0.12

* - first and second values are for *L. plumulosus test* and *A. abdita* tests, respectively
ND – Non-detect

Tissue residue concentrations in Neanthes exposed to NBK and PSNS sediments showed that the worms exposed to sediment from PS03 accumulated about twice as much Cu and Zn than the worms exposured to sediments from PS09. However, the Cu results were below the critical body residues (CBR) or whole body no-observed-effect-residues (NOER) of 21 and 23 mg/kg dw, determined for M. galloprovincialis and S. purpuratus, respectively (Rosen et. 2008). The tissue residue concentrations for Zn were also lower than tissue concentrations equal to the No Observed Effect Dose (NOED) of 200.0 mg/kg wet weight (1333 mg/kg dry weight) reported for Zn in mussels (Applied Biomonitoring 2009), assuming a dry:wet ratio of 15%.

Table 3-6. Site Sediments – *Neanthes* Tissue Chemistry Results (dry weight)

Sample ID	Cu (mg/kg)	Zn (mg/kg)
PS03	20.8	99.9
PS09	8.7	50.9

4. QA/QC**QA/QC**

A thorough QA/QC review of the data and test procedures did not identify any likely impacts on test results; therefore, all presented data were deemed acceptable. Additionally, all test acceptability criteria were met.

All tests were conducted within the recommended 1-month holding time (initiated within three days of receipt). While the temperatures of the samples upon receipt were slightly outside the EPA recommended range of 0-6 °C, the samples were in a state of cooling and this exceedence was not deemed an issue.

Control test acceptability criteria were met for the *Leptocheirus* amphipod and the *Neanthes* polychaete toxicity tests. Control test acceptability criteria for the SWI exposure with embryos of *M. galloprovincialis* was just under the 80% mean normal-alive. However, the tests were deemed acceptable based on the responses of the site sediments all performing better than the control. For the *Ampelisca* amphipod toxicity tests, acceptability criteria were not met (mean survival of controls ≥ 90% survival). However, samples were compared against the control for interest's sake.

The Total ammonia concentrations were below those that would be anticipated to be toxic to the test endpoints. A glossary of the qualifier codes used on the test datasheets is provided in Appendix E.

4.1. *Reference Toxicant Testing*

A 2-day copper sulfate ($CuSO_4$) reference toxicant test was conducted concurrently for the bivalve embryo-larval development test. The lab controls associated with this test did not met test acceptability criteria (TAC) and therefore is not deemed official. However, since the dose response observed was typical and the 2.9 µg/L concentration was above the TAC, the reference toxicant test is shown below and reported for comparative and informational purposes.

The median effective concentration (EC_{50}) was 10.2 and 9.9 µg/L for the proportion normal and proportion normal-alive endpoints, respectively. Each of these endpoints fell within two standard deviations of the laboratory's historical means (Table 4-1); indicating sensitivity to copper was consistent with that historically observed for this species.

Table 4-1. Results Summary for the Copper Reference Toxicant Tests Concurrently Conducted with the NBPL RWM Samples Collected on May 11, 2016.

Species & Endpoint	NOEC (µg/L copper)	LC_{50} or EC_{50} (µg/L copper)	Historical mean ± 2 SD (µg/L copper)
Mediterranean Mussel Embryo-Larval Development:			
Proportion Normal	8.4	9.9	7.1 ± 3.7
Proportion Normal-Survival	8.4	10.2	7.0 ± 4.3

5. SUMMARY

The results from this study showed that Cu and Zn concentrations did not exceed levels associated with toxic effects to the test organisms. No toxicity was observed for either sediment samples, PS03 or PS09, for the whole sediment test with the marine amphipod, *Leptocheirus plumulosus*, or with the marine polychaete, *Neanthes arenaceodentata*. While, the whole sediment test with the marine amphipod, *Ampelisca abdita*, did not meet test acceptability criteria, the results from PS09 showed a slightly significant increase in toxicity from the control, however it is unlikely toxicity was associated with metal exposure because the sediment, overlying water, pore water, and tissue residue metal concentrations were below ecological effect levels. The controls associated with the exposure at the sediment-water interface using embryos from the bivalve, *Mytilus galloprovincialis*, did not meet test acceptability criteria; however, all samples performed better than the control and a comparative analysis revealed that no toxicity was present for either of the sediment samples. The lines of evidence (LOE, Simpson et al. 2013) for this study indicated non-toxic effects to test organisms with low potential for effects from Cu and Zn exposure (Table 5-1).

Table 5-1. Lines of Evidence for Toxicity and Chemistry based on bioassay results from whole sediment (A) and sediment water interface toxicity (B); and overlying water (OW), pore water (PW) chemistry (C), sediment chemistry (D), and tissue residue analysis (E).

	A. Whole Sediment Toxicity			
	L.	*A. adbita*	*N. arenaceodentata*	
Station	Survival	Survival	Survival	Growth (mg)
PS03	87%	59%	100%	5.10
PS09	93%	**59%**	100%	4.68

Key for Toxicity Results	
$p \geq 0.05$	**Non Toxic**
$0.05 > p < 0.01$	**Slightly Toxic**
$p \leq 0.01$	**Toxic**

	B. Sediment Water Interface Toxicity			
	M. galloprovincialis			
	Normal		Normal Alive	
Station	Core	Homogenized	Core	Homogenized
PS03	89%	96%	86%	97%
PS09	92%	93%	79%	83%

		C. Water Chemistry			
		L. plumulosus	*A. adbita*	*N. arenaceodentata*	*M. galloprovincialis*
PS03	OW	Low	Low	Low	Low
	PW	Low	Low	Low	Low
PS09	OW	Low	Low	Low	Low
	PW	Low	Low	Low	Low

	D. Sediment Chemistry		
	Bulk Sed	<63 um	(SEM-AVS)/foc
PS03	Low	Low	Low
PS09	Low	Low	Low

	E. Tissue Residue Chemistry in *Neanthes arenaceodentata*	
	Cu	Zn
PS03	Low	Low
PS09	Low	Low

Key for Chemistry Results	
Low Risk of Effects	**Low**
Medium Risk of Effects	**Mediuim**
High Risk of Effects	**High**

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Appendix A

Test Data and Statistical Summaries

Mediterranean Mussel

Mytilus galloprovincialis

CETIS Summary Report

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Batch ID:	11-0713-8919	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	03 May-11 20:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	05 May-11 18:00	**Species:**	Mytilis galloprovincialis	**Brine:**	Not Applicable
Duration:	46h	**Source:**	Carlsbad Aquafarm	**Age:**	na

Sample ID:	11-3926-0850	**Code:**	43E7BDB2	**Client:**	SPAWAR
Sample Date:	28 Apr-11	**Material:**	Ambient Sediment Sample	**Project:**	Sediment Copper Tools - Round 1
Receive Date:	29 Apr-11 09:00	**Source:**	Sediment Copper Tools		
Sample Age:	5d 20h	**Station:**	PSNS PS03		

Batch Note: SWI - Sediment Water Interface Test. Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Sample Note: Both bulk sediment and core sediment collected. Bulk sediment sieved to 2mm upon receipt in lab. Homogenized sediment distributed to test chambers on 5/2/2011. (alternate ID: B-1 or PSNS-1); Sample Conc 100 = Intact Core; 101 = Homogenized

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-6941-8339	Combined Proportion Norm	101	>101	N/A	27.4%		Dunnett Multiple Comparison Test
15-6702-5543	Proportion Normal	101	>101	N/A	15.6%		Dunnett Multiple Comparison Test

Combined Proportion Normal Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.7559	0.7044	0.8074	0.5607	0.8598	0.06894	0.1379	18.24%	0.0%
100		4	0.8577	0.816	0.8994	0.729	0.9735	0.0558	0.1116	13.01%	-13.46%
101		4	0.9736	0.9609	0.9863	0.9286	1	0.01703	0.03407	3.5%	-28.79%

Proportion Normal Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.8038	0.7764	0.8311	0.6983	0.8598	0.03665	0.07329	9.12%	0.0%
100		4	0.8941	0.8638	0.9245	0.7818	0.9735	0.04066	0.08133	9.1%	-11.24%
101		4	0.9578	0.9504	0.9652	0.9286	0.9727	0.009913	0.01983	2.07%	-19.16%

Combined Proportion Normal Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.757	0.8598	0.8462	0.5607
100		0.9735	0.9247	0.8037	0.729
101		0.9286	0.9658	1	1

Proportion Normal Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.6983	0.8598	0.8462	0.8108
100		0.9735	0.9247	0.7818	0.8966
101		0.9286	0.9658	0.964	0.9727

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID: 15-6702-5543 **Endpoint:** Proportion Normal **CETIS Version:** CETISv1.8.1
Analyzed: 28 Jul-11 17:44 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	0	C > T	Not Run	101	>101	N/A		15.6%

Dunnett Multiple Comparison Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100	-2.063	2.18	6	0.1488	0.9909	Non-Significant Effect
		101	-3.681	2.18	6	0.1488	0.9996	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.126847	0.06342348	2	6.809	0.0158	Significant Effect
Error	0.08383599	0.00931511	9			
Total	0.210683	0.0727386	11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.577	9.21	0.2757	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9434	0.8025	0.5427	Normal Distribution

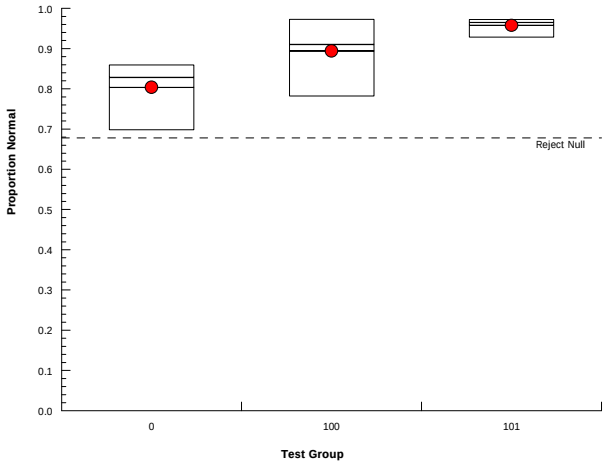
Proportion Normal Summary

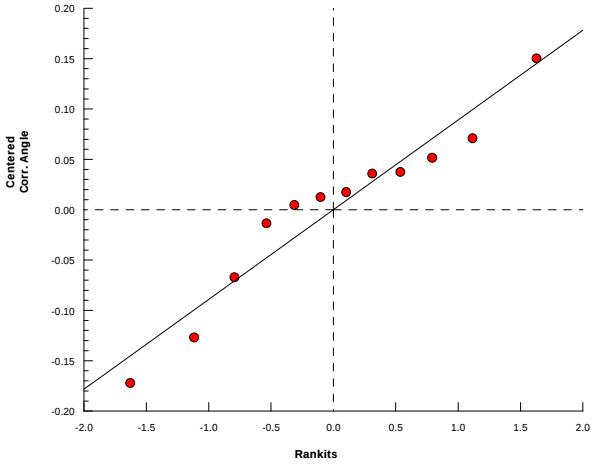
Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.8038	0.7759	0.8316	0.6983	0.8598	0.03665	0.07329	9.12%	0.0%
100		4	0.8941	0.8632	0.9251	0.7818	0.9735	0.04066	0.08133	9.1%	-11.24%
101		4	0.9578	0.9502	0.9653	0.9286	0.9727	0.009914	0.01983	2.07%	-19.16%

Angular (Corrected) Transformed Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1.116	1.082	1.15	0.9893	1.187	0.04454	0.08908	7.98%	0.0%
100		4	1.257	1.206	1.308	1.085	1.407	0.06687	0.1337	10.64%	-12.61%
101		4	1.367	1.35	1.385	1.3	1.405	0.02304	0.04609	3.37%	-22.51%

Graphics





Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID: 15-6941-8339 **Endpoint:** Combined Proportion Normal **CETIS Version:** CETISv1.8.1
Analyzed: 28 Jul-11 17:44 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	0	C > T	Not Run	101	>101	N/A		27.4%

Dunnett Multiple Comparison Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100	-1.374	2.18	6	0.2295	0.9642	Non-Significant Effect
		101	-3.499	2.18	6	0.2295	0.9994	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2755946	0.1377973	2	6.216	0.0202	Significant Effect
Error	0.1995258	0.02216953	9			
Total	0.4751204	0.1599668	11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.5549	9.21	0.7577	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9308	0.8025	0.3890	Normal Distribution

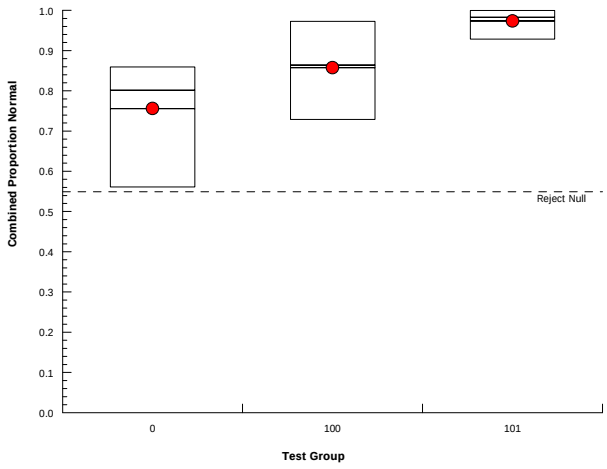
Combined Proportion Normal Summary

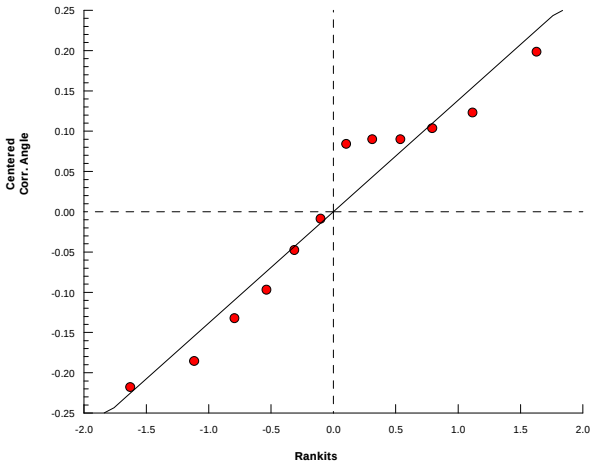
Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.7559	0.7035	0.8084	0.5607	0.8598	0.06894	0.1379	18.24%	0.0%
100		4	0.8577	0.8153	0.9002	0.729	0.9735	0.0558	0.1116	13.01%	-13.46%
101		4	0.9736	0.9606	0.9866	0.9286	1	0.01703	0.03407	3.5%	-28.79%

Angular (Corrected) Transformed Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1.064	1.005	1.124	0.8463	1.187	0.07819	0.1564	14.7%	0.0%
100		4	1.209	1.143	1.275	1.023	1.407	0.08671	0.1734	14.35%	-13.59%
101		4	1.432	1.391	1.474	1.3	1.522	0.05473	0.1095	7.64%	-34.62%

Graphics





CETIS Summary Report

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Batch ID:	11-0713-8919	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	03 May-11 20:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	05 May-11 18:00	**Species:**	Mytilis galloprovincialis	**Brine:**	Not Applicable
Duration:	46h	**Source:**	Carlsbad Aquafarm	**Age:**	na

Sample ID:	04-9494-1312	**Code:**	1D803480	**Client:**	SPAWAR
Sample Date:	28 Apr-11	**Material:**	Ambient Sediment Sample	**Project:**	Sediment Copper Tools - Round 1
Receive Date:	29 Apr-11 09:00	**Source:**	Sediment Copper Tools		
Sample Age:	5d 20h	**Station:**	PSNS PS09		

Batch Note: SWI - Sediment Water Interface Test. Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Sample Note: Both bulk sediment and core sediment collected. Bulk sediment sieved to 2mm upon receipt in lab. Homogenized sediment distributed to test chambers on 5/2/2011. (alternate ID: B-2 or PSNS-2); Sample Conc 100 = Intact Core; 101 = Homogenized

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-1236-3644	Combined Proportion Norm	101	>101	N/A	29.3%		Dunnett Multiple Comparison Test
05-7543-3244	Proportion Normal	101	>101	N/A	13.2%		Dunnett Multiple Comparison Test

Combined Proportion Normal Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.7559	0.7044	0.8074	0.5607	0.8598	0.06894	0.1379	18.24%	0.0%
100		4	0.7906	0.7461	0.8351	0.6355	0.9194	0.05954	0.1191	15.06%	-4.58%
101		4	0.8341	0.7915	0.8767	0.729	0.9626	0.05702	0.114	13.67%	-10.34%

Proportion Normal Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.8038	0.7764	0.8311	0.6983	0.8598	0.03665	0.07329	9.12%	0.0%
100		4	0.9197	0.9026	0.9368	0.8641	0.9765	0.02294	0.04589	4.99%	-14.42%
101		4	0.9249	0.9125	0.9373	0.8889	0.9626	0.0166	0.03319	3.59%	-15.07%

Combined Proportion Normal Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.757	0.8598	0.8462	0.5607
100		0.7757	0.8318	0.6355	0.9194
101		0.9626	0.729	0.8972	0.7477

Proportion Normal Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.6983	0.8598	0.8462	0.8108
100		0.9765	0.8641	0.9189	0.9194
101		0.9626	0.907	0.9412	0.8889

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID: 05-7543-3244 **Endpoint:** Proportion Normal **CETIS Version:** CETISv1.8.1
Analyzed: 28 Jul-11 17:46 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	0	C > T	Not Run	101	>101	N/A		13.2%

Dunnett Multiple Comparison Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100	-3.023	2.18	6	0.128	0.9986	Non-Significant Effect
		101	-3.104	2.18	6	0.128	0.9988	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.08633389	0.04316695	2	6.259	0.0198	Significant Effect
Error	0.06207319	0.006897021	9			
Total	0.1484071	0.05006397	11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.3493	9.21	0.8398	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9815	0.8025	0.9889	Normal Distribution

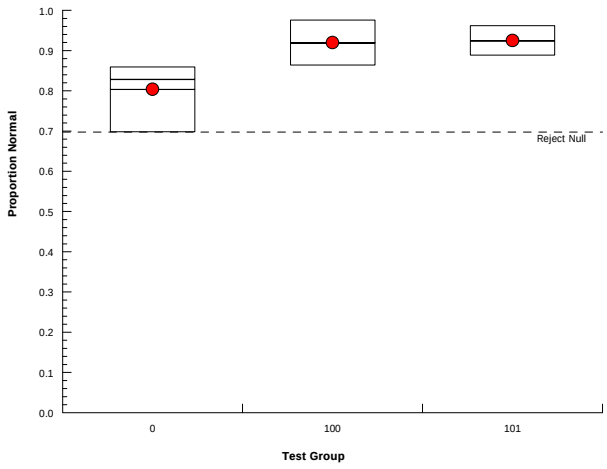
Proportion Normal Summary

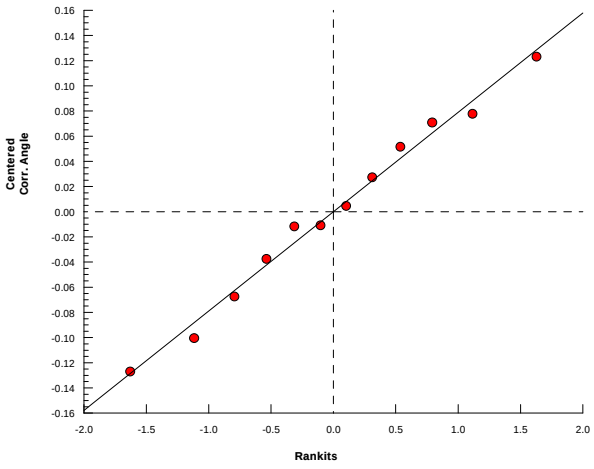
Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.8038	0.7759	0.8316	0.6983	0.8598	0.03665	0.07329	9.12%	0.0%
100		4	0.9197	0.9023	0.9372	0.8641	0.9765	0.02294	0.04589	4.99%	-14.42%
101		4	0.9249	0.9123	0.9375	0.8889	0.9626	0.0166	0.03319	3.59%	-15.07%

Angular (Corrected) Transformed Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1.116	1.082	1.15	0.9893	1.187	0.04454	0.08908	7.98%	0.0%
100		4	1.294	1.259	1.329	1.193	1.417	0.0461	0.0922	7.13%	-15.9%
101		4	1.298	1.274	1.323	1.231	1.376	0.03262	0.06523	5.02%	-16.33%

Graphics





Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID: 15-1236-3644 **Endpoint:** Combined Proportion Normal **CETIS Version:** CETISv1.8.1
Analyzed: 28 Jul-11 17:45 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	0	C > T	Not Run	101	>101	N/A		29.3%

Dunnett Multiple Comparison Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100	-0.3896	2.18	6	0.2444	0.8005	Non-Significant Effect
		101	-0.9634	2.18	6	0.2444	0.9225	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02360935	0.01180467	2	0.4697	0.6397	Non-Significant Effect
Error	0.2261879	0.02513199	9			
Total	0.2497972	0.03693666	11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.03732	9.21	0.9815	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9446	0.8025	0.5605	Normal Distribution

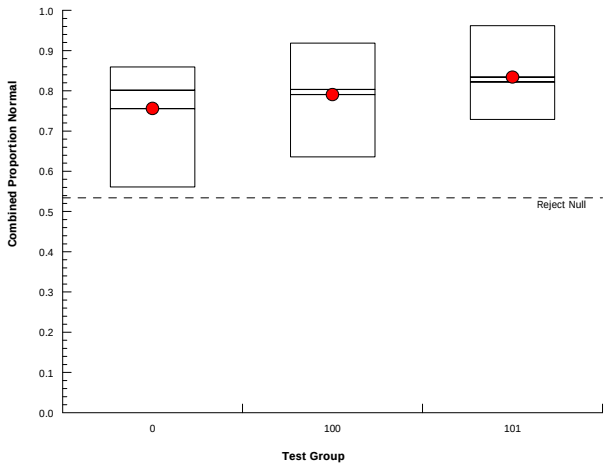
Combined Proportion Normal Summary

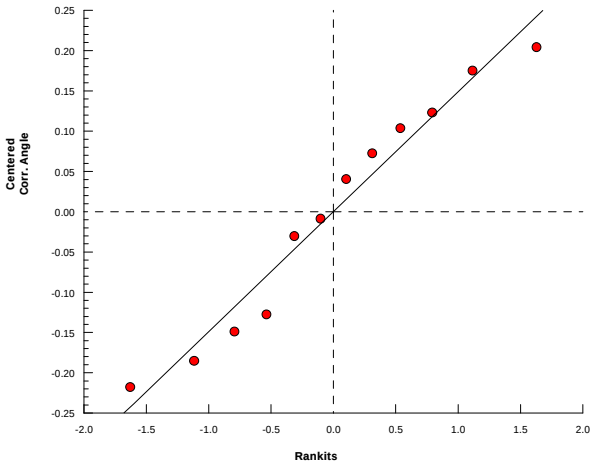
Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.7559	0.7035	0.8084	0.5607	0.8598	0.06894	0.1379	18.24%	0.0%
100		4	0.7906	0.7453	0.8359	0.6355	0.9194	0.05954	0.1191	15.06%	-4.58%
101		4	0.8341	0.7907	0.8775	0.729	0.9626	0.05702	0.114	13.67%	-10.34%

Angular (Corrected) Transformed Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1.064	1.005	1.124	0.8463	1.187	0.07819	0.1564	14.7%	0.0%
100		4	1.108	1.051	1.165	0.9226	1.283	0.07499	0.15	13.54%	-4.1%
101		4	1.172	1.108	1.236	1.023	1.376	0.08433	0.1687	14.39%	-10.15%

Graphics





Embryo Larval Bioassay

48-hour Development

Project: Internal - Cu Availability Study

Sample ID: Ambient Sediment Samples

Test Species: *M.galloprovincialis*

Start Date: 5/3/2011

End Date: 5/5/2011

Sample ID	Replicate	Number Normal	Number Abnormal	Technician Initials	
Water Only Control CORE BLANK	A	81	35	GR	
	B	92	15	GR	
	C	110	20	MC	
	D	60	14	GR	
Shelter Island - Intact Core	A	92	16	GR	
	B	97	11	GR	
	C	98	10	GR	
	D	102	2	MC	
Shelter Island - Homogenized	A	101	5	GR	
	B	85	31	GR	
	C	127	6	GR	
	D	117	0	GR	
Naval Base SD - Intact Core	A	71	7	MC	
	B	83	17	GR	
	C	80	9	MC	
	D	41	26	MC	* term. early
Naval Base SD - Homogenized	A	100	11	GR	
	B	108	8	MC	
	C	102	8	GR	
	D	91	5	MC	
Bremerton PS03 (B1) - Intact Core	A	110	3	GR	
	B	135	11	GR	
	C	86	24	GR	
	D	~~78~~ 78	9	MC	
Bremerton PS03 (B1) - Homogenized	A	117	9	GR	
	B	113	4	GR	
	C	107	4	GR	
	D	107	3	MC	
Bremerton PS09 (B2) - Intact Core	A	83	2	MC	
	B	89	14	GR	
	C	46 (48)	5 (6)	MC (MC-filtered)	* LOTS OF DEBRIS
	D	114	10	GR	
Bremerton PS09 (B2) - Homogenized	A	103	9	MC	filtered
	B	78	8	MC	
	C	96	6	MC	filtered
	D	80	10	MC	filtered

QC Check: _____ **Final Review:** _____

fine zero counts - A: 194
B: 198
C: 216
~~D~~ E: 209
D ~~E~~: 187

Marine Chronic Bioassay

Water Quality Measurements

Project: Internal - Cu Availability Study

Sample ID: Ambient Sediment Samples

Test Species: *M.galloprovincialis*

Start Date/Time: 5/3/2011 2000

End Date/Time: 5/5/2011 1800

Sample ID	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Water Only Control	30.1	30.0	29.6	14.4	15.8	15.8	7.9	6.8	6.6	8.03	8.08	8.09
Shelter Island - Core	30.2	30.3	30.6	15.1	15.8	15.9	7.7	7.4	7.2	8.07	8.15	8.21
Shelter Island - Hom.	30.8	30.9	30.8	15.1	15.5	16.0	7.6	7.1	7.0	8.10	8.14	8.21
Naval Base SD - Core	30.4	30.9	30.4	14.8	15.9	15.8	7.1	7.4	7.3	8.05	8.15	8.23
Naval Base SD - Hom.	30.6	30.8	30.8	15.2	15.9	15.9	7.9	7.2	7.2	8.10	8.16	8.21
B1 Bivalve - Bremerton PS03 - Core	30.0	30.3	30.3	14.1	15.8	15.7	7.5	7.3	7.2	8.05	8.13	8.21
B1 Bivalve - Bremerton PS03 - Hom.	30.1	30.2	30.2	14.8	15.7	15.8	7.3	7.0	7.4	8.09	8.16	8.21
B2 Bivalve - Bremerton PS09 - Core	30.1	30.2	30.1	15.0	15.6	15.1	7.8	7.5	7.1	8.05	8.11	8.16
B2 Bivalve - Bremerton PS09 - Hom.	30.3	30.3	30.3	14.5	15.4	15.7	7.9	7.1	7.0	8.08	8.15	8.20

	0	24	48
WQ Readings:	ML	MC	MC
Dilutions made by:	MC		

Technician Initials:

Animal Source/Date Received: Carlsbad Aquafarms 5/3/2011

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check:

Final Review:

CuSO4 Reference Toxicant Concentration Calculations
Mar-11

Bivalve: 1ppm

Volume / concentration needed= 100 mL

C1	V1	C2	V2
(below)	100	1000	(below)

Conc's:	Volume (mL) of 1000 µg/L stock	Volume (mL) of SW
0	0.000	100.0
2.9	0.290	99.7
4.1	0.410	99.6
5.8	0.580	99.4
8.4	0.840	99.2
12	1.200	98.8
17.2	1.720	98.3
~~24~~	~~2.400~~	97.6
~~35~~	~~3.500~~	~~96.5~~
~~50~~	~~5.000~~	~~95.0~~

15.94 mL of stock needed

SUBSTOCK CALCULATIONS

Stock	1 g/L		
substock	1 mg/L	1000 µg/L	100

1PPM **1000 µg/L:** **0.1 mL of 1 g/L stock into 100 mL**

Cu Stock Date

Cu Stock Date
QC:

Embryo-Larval Development Test – SPAWNING CHECKLIST & CALCULATIONS

Batch ID: 050311Mg Spawn/Test Date: 05/03/11 Test Species M. galloprovincialis
Analyst: GR

Task	Time
Spawning Inducement Initiated	1400 HR
Spawning Begins	1500 HR 5 ♂, 0 ♀
Females/Males Isolated in Incubator	1820 HR
Fertilization Initiated	1825 HR
Fertilzation Terminated/eggs rinsed	1835 HR
Embryo Counts	1900 HR
Embryo addition to vials	2000 HR

1815 HR : 1 ♀

Embryo Counts:
Embryo Stock #1: 59, 52, 59 Mean = 56.7/20 uL * 1000 uL/mL = 2,833 cells/mL
Embryo Stock #2: ___, ___, ___ Mean = ___/___ uL * 1000 uL/mL = _____ cells/mL
Embryo Stock #3: ___, ___, ___ Mean = ___/___ uL * 1000 uL/mL = _____ cells/mL

Adjust selected embryo stock to 2000 embryos/ml. Confirm density:

Selected Stock : 50, 40, 36 Mean = ~~120~~ 42/20 uL * 1000 uL/mL = 2,100 cells/mL
GR

Add 100 µl of 2000 embryo/ml stock to obtain 20 embryos/ml in test vials.

Notes: Only 1 ♀ spawner out of ~100 mussels.
Sperm solution was composite of 4 viable males.
Add 100 µl suspension for ~200 embryos/core.
This was a SWI exposure for sediment Copper Tools project (Phase I).

Marine Amphipod

Leptocheirus plumulosus

CETIS Summary Report

Leptocheirus 10-d Survival and Reburial Sediment Test — **SPAWAR Systems Center**

Batch ID:	14-5299-7720	**Test Type:**	Survival-Reburial	**Analyst:**	
Start Date:	03 May-11 10:30	**Protocol:**	EPA/600/R-94/025 (1994)	**Diluent:**	Laboratory Seawater
Ending Date:	13 May-11 09:00	**Species:**	Leptocheirus plumulosus	**Brine:**	Not Applicable
Duration:	9d 22h	**Source:**	Chesapeake Cultures	**Age:**	

Sample ID:	11-3926-0850	**Code:**	43E7BDB2	**Client:**	SPAWAR
Sample Date:	28 Apr-11	**Material:**	Ambient Sediment Sample	**Project:**	Sediment Copper Tools - Round 1
Receive Date:	29 Apr-11 09:00	**Source:**	Sediment Copper Tools		
Sample Age:	5d 10h	**Station:**	PSNS PS03		

Batch Note: Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Sample Note: Both bulk sediment and core sediment collected. Bulk sediment sieved to 2mm upon receipt in lab. Homogenized sediment distributed to test chambers on 5/2/2011. (alternate ID: B-1 or PSNS-1)

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
04-3540-0521	Survival Rate	100	>100	N/A	12.8%		Equal Variance t Two-Sample Test

Survival Rate Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	3	0.9	0.8677	0.9323	0.8	0.95	0.05	0.0866	9.62%	0.0%
100		3	0.8667	0.8559	0.8774	0.85	0.9	0.01667	0.02887	3.33%	3.7%

Survival Rate Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3
0	Lab Control	0.95	0.8	0.95
100		0.85	0.85	0.9

CETIS Analytical Report

Leptocheirus 10-d Survival and Reburial Sediment Test **SPAWAR Systems Center**

Analysis ID: 04-3540-0521 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.1
Analyzed: 28 Jul-11 18:48 **Analysis:** Parametric-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Angular (Corrected)	0	C > T	Not Run	Sample passes survival rate endpoint	12.8%

Equal Variance t Two-Sample Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100	0.8101	2.132	4	0.1776	0.2317	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.006832685	0.006832685	1	0.6562	0.4633	Non-Significant Effect
Error	0.04165075	0.01041269	4			
Total	0.04848344	0.01724537	5			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	9.831	199	0.1847	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8595	0.43	0.1874	Normal Distribution

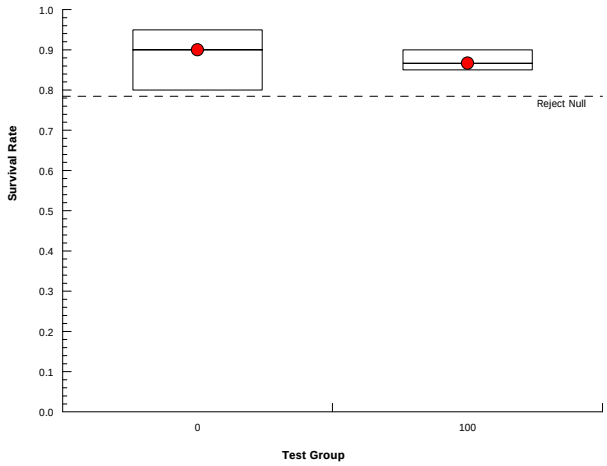
Survival Rate Summary

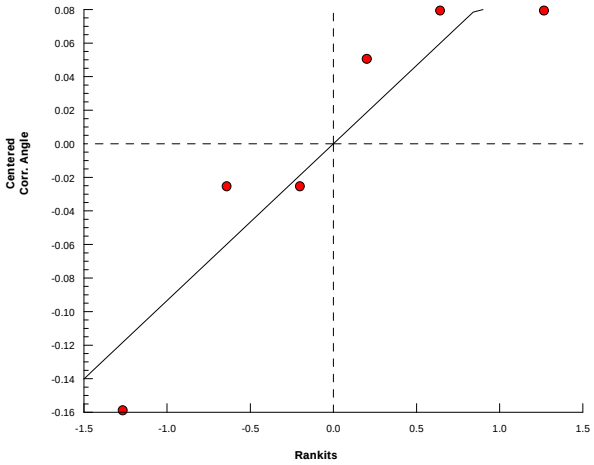
Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	3	0.9	0.8671	0.9329	0.8	0.95	0.05	0.0866	9.62%	0.0%
100		3	0.8667	0.8557	0.8776	0.85	0.9	0.01667	0.02887	3.33%	3.7%

Angular (Corrected) Transformed Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	3	1.266	1.214	1.318	1.107	1.345	0.07938	0.1375	10.86%	0.0%
100		3	1.198	1.182	1.215	1.173	1.249	0.02532	0.04385	3.66%	5.33%

Graphics





CETIS Summary Report

Leptocheirus 10-d Survival and Reburial Sediment Test — **SPAWAR Systems Center**

Batch ID:	14-5299-7720	**Test Type:**	Survival-Reburial	**Analyst:**	
Start Date:	03 May-11 10:30	**Protocol:**	EPA/600/R-94/025 (1994)	**Diluent:**	Laboratory Seawater
Ending Date:	13 May-11 09:00	**Species:**	Leptocheirus plumulosus	**Brine:**	Not Applicable
Duration:	9d 22h	**Source:**	Chesapeake Cultures	**Age:**	

Sample ID:	04-9494-1312	**Code:**	1D803480	**Client:**	SPAWAR
Sample Date:	28 Apr-11	**Material:**	Ambient Sediment Sample	**Project:**	Sediment Copper Tools - Round 1
Receive Date:	29 Apr-11 09:00	**Source:**	Sediment Copper Tools		
Sample Age:	5d 10h	**Station:**	PSNS PS09		

Batch Note: Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Sample Note: Both bulk sediment and core sediment collected. Bulk sediment sieved to 2mm upon receipt in lab. Homogenized sediment distributed to test chambers on 5/2/2011. (alternate ID: B-2 or PSNS-2)

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-3721-3818	Survival Rate	100	>100	N/A	17.3%		Equal Variance t Two-Sample Test

Survival Rate Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	3	0.9	0.8677	0.9323	0.8	0.95	0.05	0.0866	9.62%	0.0%
100		3	0.9333	0.9118	0.9549	0.9	1	0.03333	0.05774	6.19%	-3.7%

Survival Rate Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3
0	Lab Control	0.95	0.8	0.95
100		0.9	0.9	1

CETIS Analytical Report

Report Date: 28 Jul-11 18:51 (p 1 of 1)
Test Code: 75B9DE53 | 19-7511-5347

Leptocheirus 10-d Survival and Reburial Sediment Test — **SPAWAR Systems Center**

Analysis ID: 00-3721-3818 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.1
Analyzed: 28 Jul-11 18:51 **Analysis:** Parametric-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Angular (Corrected)	0	C > T	Not Run	Sample passes survival rate endpoint	17.3%

Equal Variance t Two-Sample Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100	-0.5015	2.132	4	0.2255	0.6788	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.004220693	0.004220693	1	0.2515	0.6424	Non-Significant Effect
Error	0.06712493	0.01678123	4			
Total	0.07134563	0.02100193	5			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.289	199	0.8736	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9092	0.43	0.4314	Normal Distribution

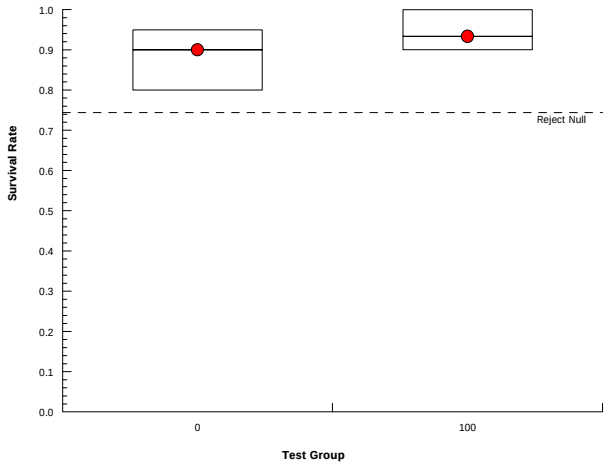
Survival Rate Summary

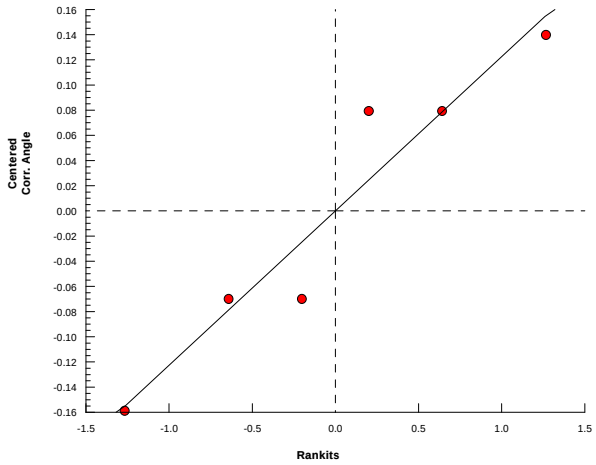
Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	3	0.9	0.8671	0.9329	0.8	0.95	0.05	0.0866	9.62%	0.0%
100		3	0.9333	0.9114	0.9553	0.9	1	0.03333	0.05773	6.19%	-3.7%

Angular (Corrected) Transformed Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	3	1.266	1.214	1.318	1.107	1.345	0.07938	0.1375	10.86%	0.0%
100		3	1.319	1.273	1.365	1.249	1.459	0.0699	0.1211	9.18%	-4.19%

Graphics





Marine Sediment Bioassay **Organism Survival**

Client: Internal - Cu Availability Study

Ambient Sediment Samples

Test Species: *Leptocheirus plumulosus*

Start Date/Time: 5/13/2011 1030

End Date/Time: 1:15pm 5/13/2011

Sample ID	Replicate	Initial No.	No. Recovered	Technician Initials	
Lab Control	A	20			
	B	20			
	C	20			
	D	20			
Shelter Island	A	20	9	7 bodies BLS	
	B	20	9	4 bodies BLS	
	C	20	11	6 bodies BLS	
	D	20			
Naval Base SD	A	20	15	5 bodies BLS	generally in poor condition
	B	20	13	2 bodies BLS	
	C	20	12	5 bodies BLS	
	D	20			
PSNS - 1	A	20	17	N/S	looked strong
	B	20	17	1 body BLS	
	C	20	18	0 BLS	
	D	20			
PSNS - 2	A	20	18	6R, 1 polychaete, looked strong.	
	B	20	18	6R, looks strong.	
	C	20	20	6R, " "	
	D	20			
	A	20			
	B	20			
	C	20			
	D	20			

QC Check: LLL 5/25/2011 **Final Review:** _____

Naval Base - only one rep used for sediment (Lepto) collection; too few.

BR1 - only one rep for sediment.

10-Day Marine Sediment Bioassay
Static Conditions

Water Quality Measurements

Client: Internal - Cu Availability Study

Test Species: *Leptocheirus plumulosus*

Sample ID: Bremerton PS09- B2

Start Date/Time: 5/3/2011 1030

End Date/Time: 5/13/2011 1315

Test Day	Salinity (ppt)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH (units)	Technician Initials	Comments
0	30.1	19.7	6.1	7.93	MC	
1	30.2	20.1	6.4	8.09	MC	
2	30.4	20.1	6.7	8.12	MC	
3		20.0		8.07	CC	
4		19.9		8.09	CC	
5		20.1		8.10	CC	
6	30.5	19.7	6.1	8.08	MC	
7	30.4	19.9	6.2	8.12	MC	
8	30.6	19.7	6.9	8.08	MC	
9		20.1		8.01	CC	
10	30.7	19.9	6.5	8.09	MC	

QC Check: MC 5/25/2011

Final Review:

10-Day ..arine Sediment Bioassay
Static Conditions

Water Quality Meas..ments

Client: Internal - Cu Availability Study

Test Species: *Leptocheirus plumulosus*

Sample ID: Bremerton PSO3 - B1

Start Date/Time: 5/3/2011 1030

End Date/Time: 5/13/2011 1315

Test Day	Salinity (ppt)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH (units)	Technician Initials	Comments
0	30.1	19.5	6.4	7.94	MC	
1	30.3	19.9	6.7	8.05	MC	
2	30.5	20.2	6.8	8.09	MC	
3		20.3		8.17	CC	
4		20.2		8.34	CC	
5		20.3		8.45	CC	
6	30.4	20.0	6.0	8.59	MC	
7	30.4	19.9	6.1	8.61	MC	
8	30.7	20.0	6.7	8.69	MC	
9		20.3		8.66	CC	
10	30.7	19.8	6.4	8.76	MC	

QC Check: MC 5/25/2011

Final Review:

Marine Amphipod

Ampelisca abdita

CETIS Summary Report

Ampelisca 10-d Survival Sediment Test — **SPAWAR Systems Center**

Batch ID:	04-6863-0121	**Test Type:**	Survival	**Analyst:**	
Start Date:	03 May-11 19:00	**Protocol:**	EPA/600/R-94/025 (1994)	**Diluent:**	Laboratory Seawater
Ending Date:	13 May-11 13:00	**Species:**	Ampelisca abdita	**Brine:**	Not Applicable
Duration:	9d 18h	**Source:**	Aquatic Research Organisms, NH	**Age:**	

Sample ID:	11-3926-0850	**Code:**	43E7BDB2	**Client:**	SPAWAR
Sample Date:	28 Apr-11	**Material:**	Ambient Sediment Sample	**Project:**	Sediment Copper Tools - Round 1
Receive Date:	29 Apr-11 09:00	**Source:**	Sediment Copper Tools		
Sample Age:	5d 19h	**Station:**	PSNS PS03		

Batch Note: Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Sample Note: Both bulk sediment and core sediment collected. Bulk sediment sieved to 2mm upon receipt in lab. Homogenized sediment distributed to test chambers on 5/2/2011. (alternate ID: B-1 or PSNS-1)

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-7267-7946	Survival Rate	100	>100	N/A	28.1%		Equal Variance t Two-Sample Test

Survival Rate Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.7849	0.7179	0.8518	0.5625	1	0.08967	0.1793	22.85%	0.0%
100		4	0.5925	0.5475	0.6376	0.5	0.7692	0.06028	0.1206	20.35%	24.5%

Survival Rate Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.8077	0.7692	0.5625	1
100		0.7692	0.5385	0.5	0.5625

CETIS Analytical Report

Ampelisca 10-d Survival Sediment Test **SPAWAR Systems Center**

Analysis ID: 08-7267-7946 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.1
Analyzed: 28 Jul-11 18:24 **Analysis:** Parametric-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Angular (Corrected)	0	C > T	Not Run	Sample passes survival rate endpoint	28.1%

Equal Variance t Two-Sample Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100	1.715	1.943	6	0.2699	0.0686	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.113528	0.113528	1	2.942	0.1371	Non-Significant Effect
Error	0.2315116	0.03858527	6			
Total	0.3450396	0.1521132	7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	3.716	47.47	0.3096	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9216	0.6451	0.4429	Normal Distribution

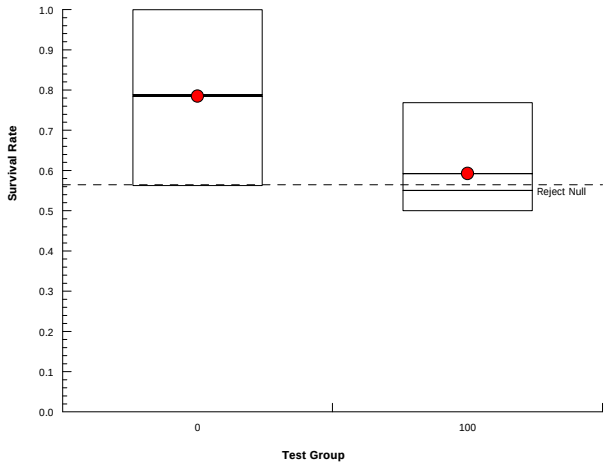
Survival Rate Summary

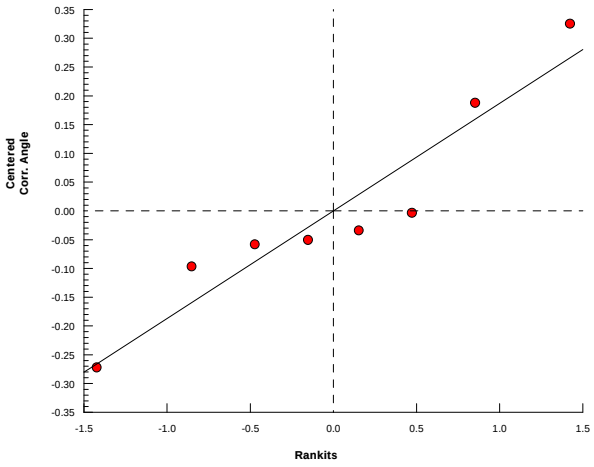
Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.7849	0.7166	0.8531	0.5625	1	0.08967	0.1793	22.85%	0.0%
100		4	0.5925	0.5467	0.6384	0.5	0.7692	0.06028	0.1206	20.35%	24.5%

Angular (Corrected) Transformed Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1.12	1.026	1.214	0.8481	1.445	0.1233	0.2466	22.02%	0.0%
100		4	0.8818	0.8331	0.9304	0.7854	1.07	0.06396	0.1279	14.51%	21.27%

Graphics





CETIS Summary Report

Ampelisca 10-d Survival Sediment Test — **SPAWAR Systems Center**

Batch ID:	04-6863-0121	**Test Type:**	Survival	**Analyst:**	
Start Date:	03 May-11 19:00	**Protocol:**	EPA/600/R-94/025 (1994)	**Diluent:**	Laboratory Seawater
Ending Date:	13 May-11 13:00	**Species:**	Ampelisca abdita	**Brine:**	Not Applicable
Duration:	9d 18h	**Source:**	Aquatic Research Organisms, NH	**Age:**	

Sample ID:	04-9494-1312	**Code:**	1D803480	**Client:**	SPAWAR
Sample Date:	28 Apr-11	**Material:**	Ambient Sediment Sample	**Project:**	Sediment Copper Tools - Round 1
Receive Date:	29 Apr-11 09:00	**Source:**	Sediment Copper Tools		
Sample Age:	5d 19h	**Station:**	PSNS PS09		

Batch Note: Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Sample Note: Both bulk sediment and core sediment collected. Bulk sediment sieved to 2mm upon receipt in lab. Homogenized sediment distributed to test chambers on 5/2/2011. (alternate ID: B-2 or PSNS-2)

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-2926-6458	Survival Rate	<100	100	N/A	24.8%		Equal Variance t Two-Sample Test

Point Estimate Summary

Analysis ID	Endpoint	Level		95% LCL	95% UCL	TU	Method
17-7521-7542	Survival Rate	LC50	>100	N/A	N/A		Linear Interpolation (ICPIN)

Survival Rate Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.7849	0.7179	0.8518	0.5625	1	0.08967	0.1793	22.85%	0.0%
100		4	0.5889	0.5726	0.6053	0.5625	0.6538	0.0219	0.0438	7.44%	24.96%

Survival Rate Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.8077	0.7692	0.5625	1
100		0.5769	0.6538	0.5625	0.5625

Ampelisca 10-d Survival Sediment Test **SPAWAR Systems Center**

Analysis ID: 10-2926-6458 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.1
Analyzed: 28 Jul-11 18:26 **Analysis:** Parametric-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Angular (Corrected)	0	C > T	Not Run	Sample fails survival rate endpoint	24.8%

Equal Variance t Two-Sample Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100*	1.954	1.943	6	0.2435	0.0493	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1199362	0.1199362	1	3.818	0.0985	Non-Significant Effect
Error	0.1884838	0.03141397	6			
Total	0.30842	0.1513502	7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	30.08	47.47	0.0194	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8637	0.6451	0.1306	Normal Distribution

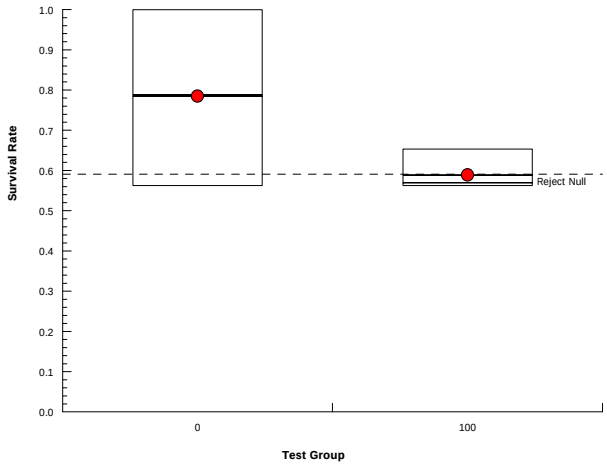
Survival Rate Summary

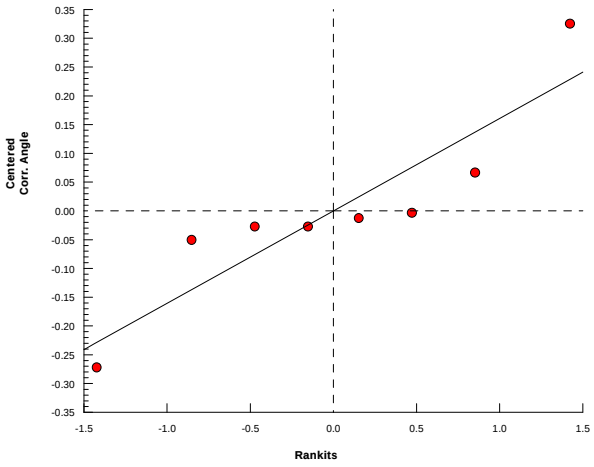
Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.7849	0.7166	0.8531	0.5625	1	0.08967	0.1793	22.85%	0.0%
100		4	0.5889	0.5723	0.6056	0.5625	0.6538	0.0219	0.0438	7.44%	24.96%

Angular (Corrected) Transformed Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1.12	1.026	1.214	0.8481	1.445	0.1233	0.2466	22.02%	0.0%
100		4	0.8751	0.858	0.8922	0.8481	0.9418	0.02248	0.04496	5.14%	21.86%

Graphics





CETIS Analytical Report

Ampelisca 10-d Survival Sediment Test **SPAWAR Systems Center**

Analysis ID: 17-7521-7542 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.1
Analyzed: 28 Jul-11 18:26 **Analysis:** Linear Interpolation (ICPIN) **Official Results:** Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	41733800	200	Yes	Two-Point Interpolation

Point Estimates

Level		95% LCL	95% UCL
LC50	>100	N/A	N/A

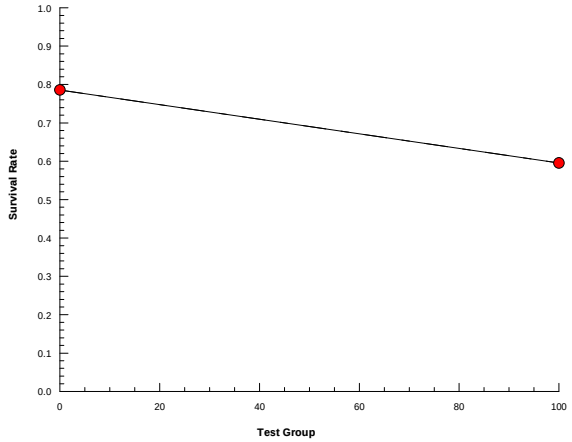
Survival Rate Summary

			Calculated Variate(A/B)								
Test Group	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	0.7849	0.5625	1	0.08967	0.1793	22.85%	0.0%	66	84
100		4	0.5889	0.5625	0.6538	0.0219	0.0438	7.44%	24.96%	50	84

Survival Rate Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	0.8077	0.7692	0.5625	1
100		0.5769	0.6538	0.5625	0.5625

Graphics



Marine Sediment Bioassay

Organism Survival

Client: Internal - Cu Availability Study

Ambient Sediment Samples

Test Species: *Ampelisca abdita*

Start Date/Time: 5/3/2011

End Date/Time: 5/13/2011 1300

Sample ID	Replicate	Initial No.	No. Recovered	Technician Initials # on Surface Sediment (Dead)
Lab Control Ampelisca Home Sed.	A	~~20~~ 30	21	Ø look good.
	B	~~20~~ 30	20	3
	C	20	9	3
	D	20	18	1
Shelter Island	A	~~20~~ 30	~~X~~ (PD) 17	7
	B	~~20~~ 30	13	16 - Black Spots → dead amphipods on sediment surface
	C	20	11	6
	D	20	9	9 - Black Spots
Naval Base SD	A	~~20~~ 30	9	8 - Black Spots (BS)
	B	~~20~~ 30	12	13 - Black Spots
	C	20	6	12 Black Spots
	D	20	7	8 - BS
PSNS - 1 (B1) PS03	A	~~20~~ 30	20	2 (PD)
	B	~~20~~ 30	14	10
	C	20	8	6
	D	20	9	
PSNS - 2 (B2) PS09	A	~~20~~ 30	15	2
	B	~~20~~ 30	17	4
	C	20	9	5
	D	20	9	3
	A	20	~~21~~	
	B	20		
	C	20		
	D	20		

QC Check: 5/25/2011 **Final Review:** _____

10-Day . .arine Sediment Bioassay
Static Conditions

Water Quality Meas. ments

Client: Internal - Cu Availability Study

Sample ID: Amp. Control

Test Species: *Ampelisca abdita*

Start Date/Time: 5/3/2011 1900

End Date/Time: 5/13/2011 1300

Test Day	Salinity (ppt)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH (units)	Technician Initials	Comments
0	30.2	15.7	6.9	8.00	MC	
1	29.3	14.7	6.3	7.65	MC	
2	29.1	15.3	5.8	7.72	MC	5 A, 3 B, 1 C, 2 D) # dead/still @ sediment surface; 10 added to ~~control~~ A+B
3		12.7		7.73	CC	
4		13.7		7.82	CC	
5		13.7		7.96	CC	
6	29.3	13.2	6.8	7.92	MC	
7	29.5	15.0	7.1	7.98	MC	
8	29.5	14.0	7.1	8.20	MC	
9		15.3		8.14	CC	
10	29.5	15.3	6.5	8.20	MC	

QC Check: ____________

Final Review: ____________

10-Day Marine Sediment Bioassay
Static Conditions

Water Quality Measurements

Client: Internal - Cu Availability Study

Test Species: *Ampelisca abdita*

Sample ID: Bremerton PS03 - B1

Start Date/Time: 5/3/2011 1900

End Date/Time: 5/13/11 1300

Test Day	Salinity (ppt)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH (units)	Technician Initials	Comments
0	30.0	14.9	7.3	8.03	MC	
1	30.0	14.3	7.5	8.00	MC	
2	30.2	14.2	7.3	8.00	MC	A-1 B-10 C-9 D-4 # dead/still @ sediment surface; ID Added to A+B
3		12.9		8.03	QC	
4		13.7		8.10	QC	
5		13.7		8.13	QC	
6	30.5	13.7	7.6	8.17	MC	
7	30.4	14.7	7.8	8.12	MC	
8	30.5	14.1	7.5	8.35	MC	
9		15.0		8.39	QC	
10	~~30.8~~ 30.8 MC	15.2	7.0	8.48	MC	

QC Check: _____

Final Review: _____

10-Day . arine Sediment Bioassay
Static Conditions

Water Quality Meas ments

Client: Internal - Cu Availability Study

Test Species: *Ampelisca abdita*

Sample ID: Bremerton PS09. B2

Start Date/Time: 5/3/2011 1900

End Date/Time: 5/13/2011 1500

Test Day	Salinity (ppt)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH (units)	Technician Initials	Comments
0	30.2	14.3	7.8	8.01	MC	
1	30.3	14.4	7.9	8.01	MC	
2	30.3	14.3	8.0	8.02	MC	1-A, 2-B, 3-C, 4-D > # dead/stike sediment surface; 10 Added to A+B
3		12.5		8.04	CC	
4		13.4		8.08	CC	
5		13.6		8.08	CC	
6	30.4	13.2	7.7	8.09	MC	
7	30.3	14.6	7.9	8.10	MC	
8	30.4	14.3	8.0	8.11	MC	
9		15.3		8.11	CC	
10	30.6	14.6	7.7	8.19	MC	

QC Check:

Final Review:

Marine Ploychaete

Neanthes arenaceodentata

CETIS Summary Report

Neanthes Survival and Growth Test — **SPAWAR Systems Center**

Batch ID:	19-1064-5197	**Test Type:**	Survival-Growth	**Analyst:**	
Start Date:	03 May-11 14:30	**Protocol:**	Farrar and Bridges 2011	**Diluent:**	Seawater
Ending Date:	31 May-11 09:00	**Species:**	Neanthes arenaceodentata	**Brine:**	Not Applicable
Duration:	27d 19h	**Source:**	Aquatic Toxicology Support	**Age:**	6d

Sample ID:	11-3926-0850	**Code:**	43E7BDB2	**Client:**	SPAWAR
Sample Date:	28 Apr-11	**Material:**	Ambient Sediment Sample	**Project:**	Sediment Copper Tools - Round 1
Receive Date:	29 Apr-11 09:00	**Source:**	Sediment Copper Tools		
Sample Age:	5d 14h	**Station:**	PSNS PS03		

Sample Note: Both bulk sediment and core sediment collected. Bulk sediment sieved to 2mm upon receipt in lab. Homogenized sediment distributed to test chambers on 5/2/2011. (alternate ID: B-1 or PSNS-1)

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-2197-3227	Mean Dry Weight-mg	100	>100	N/A	28.1%		Unequal Variance t Two-Sample Test
13-0542-9912	Survival Rate	100	>100	N/A	N/A		Fisher Exact Test

Mean Dry Weight-mg Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	9	0.004928	0.004129	0.005727	0.00076	0.00747	0.0007134	0.00214	43.43%	0.0%
100		10	0.005106	0.004811	0.005401	0.00394	0.00611	0.0002502	0.0007912	15.5%	-3.62%

Survival Rate Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	10	0.9	0.7819	1	0	1	0.1	0.3162	35.14%	0.0%
100		10	1	1	1	1	1	0	0	0.0%	-11.11%

Mean Dry Weight-mg Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	0.00247	0.00545	0.00519	0.00419	0.00647	0.00573	0.00747	0.00662	0.00076	
100		0.00445	0.00502	0.00577	0.00396	0.00518	0.00611	0.00394	0.0056	0.00601	0.00502

Survival Rate Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	1	1	0	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1

CETIS Analytical Report

Test Code: 58FE136 | 00-9331-5382

Neanthes Survival and Growth Test **SPAWAR Systems Center**

Analysis ID: 13-0542-9912 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.1
Analyzed: 27 Jul-11 14:37 **Analysis:** Single 2x2 Contingency Table **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result
Untransformed		C > T	Not Run	Sample passes survival rate endpoint

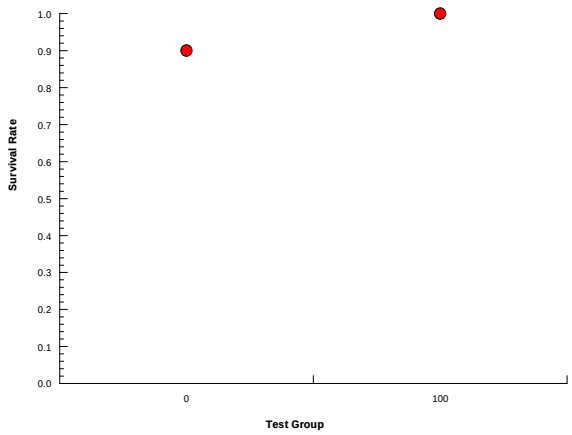
Fisher Exact Test

Control	vs	Test Group	Test Stat	P-Value	Decision(0.05)
Lab Control		100	1	1.0000	Non-Significant Effect

Data Summary

Test Group	Control Type	No-Resp	Resp	Total
0	Lab Control	9	1	10
100		10	0	10

Graphics



CETIS Analytical Report

Neanthes Survival and Growth Test **SPAWAR Systems Center**

Analysis ID: 05-2197-3227 **Endpoint:** Mean Dry Weight-mg **CETIS Version:** CETISv1.8.1
Analyzed: 27 Jul-11 14:38 **Analysis:** Parametric-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Untransformed	0	C > T	Not Run	Sample passes mean dry weight-mg endpoint	28.1%

Unequal Variance t Two-Sample Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100	-0.2357	1.833	9	0.001386	0.5905	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.504654E-07	1.504654E-07	1	0.0605	0.8087	Non-Significant Effect
Error	4.227953E-05	2.487031E-06	17			
Total	0.00004243	2.637497E-06	18			

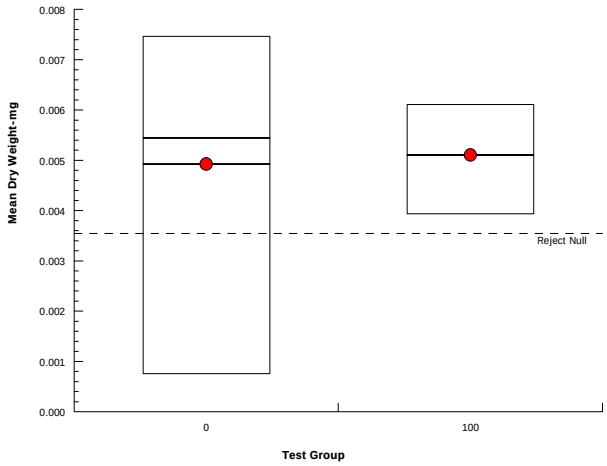
Distributional Tests

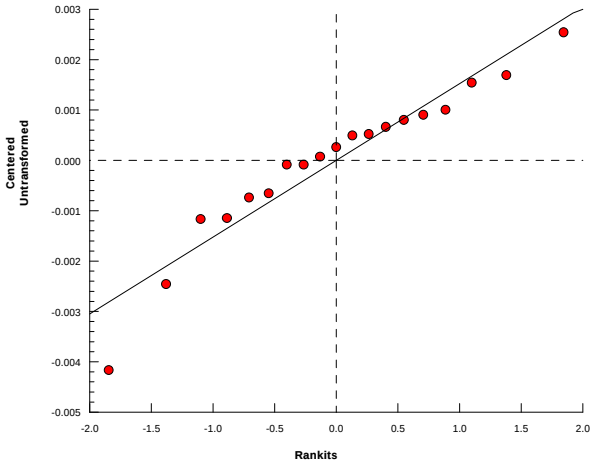
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	7.318	6.693	0.0073	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9323	0.8605	0.1913	Normal Distribution

Mean Dry Weight-mg Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	9	0.004928	0.004114	0.005742	0.00076	0.00747	0.0007134	0.00214	43.43%	0.0%
100		10	0.005106	0.004805	0.005407	0.00394	0.00611	0.0002502	0.0007912	15.5%	-3.62%

Graphics





CETIS Summary Report

Neanthes Survival and Growth Test — **SPAWAR Systems Center**

Batch ID:	19-1064-5197	**Test Type:**	Survival-Growth	**Analyst:**	
Start Date:	03 May-11 14:30	**Protocol:**	Farrar and Bridges 2011	**Diluent:**	Seawater
Ending Date:	31 May-11 09:00	**Species:**	Neanthes arenaceodentata	**Brine:**	Not Applicable
Duration:	27d 19h	**Source:**	Aquatic Toxicology Support	**Age:**	6d

Sample ID:	04-9494-1312	**Code:**	1D803480	**Client:**	SPAWAR
Sample Date:	28 Apr-11	**Material:**	Ambient Sediment Sample	**Project:**	Sediment Copper Tools - Round 1
Receive Date:	29 Apr-11 09:00	**Source:**	Sediment Copper Tools		
Sample Age:	5d 14h	**Station:**	PSNS PS09		

Sample Note: Both bulk sediment and core sediment collected. Bulk sediment sieved to 2mm upon receipt in lab. Homogenized sediment distributed to test chambers on 5/2/2011. (alternate ID: B-2 or PSNS-2)

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-3531-0838	Mean Dry Weight-mg	100	>100	N/A	29.0%		Equal Variance t Two-Sample Test
15-6033-2933	Survival Rate	100	>100	N/A	N/A		Fisher Exact Test

Mean Dry Weight-mg Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	9	0.004928	0.004129	0.005727	0.00076	0.00747	0.0007134	0.00214	43.43%	0.0%
100		10	0.004682	0.004161	0.005203	0.00105	0.00604	0.0004413	0.001395	29.81%	4.99%

Survival Rate Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	10	0.9	0.7819	1	0	1	0.1	0.3162	35.14%	0.0%
100		10	1	1	1	1	1	0	0	0.0%	-11.11%

Mean Dry Weight-mg Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	0.00247	0.00545	0.00519	0.00419	0.00647	0.00573	0.00747	0.00662	0.00076	
100		0.00604	0.00563	0.00444	0.00437	0.00545	0.00545	0.00494	0.00442	0.00503	0.00105

Survival Rate Detail

Test Group	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Lab Control	1	1	0	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1

CETIS Analytical Report

Neanthes Survival and Growth Test **SPAWAR Systems Center**

Analysis ID: 15-6033-2933 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.1
Analyzed: 27 Jul-11 15:36 **Analysis:** Single 2x2 Contingency Table **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result
Untransformed		C > T	Not Run	Sample passes survival rate endpoint

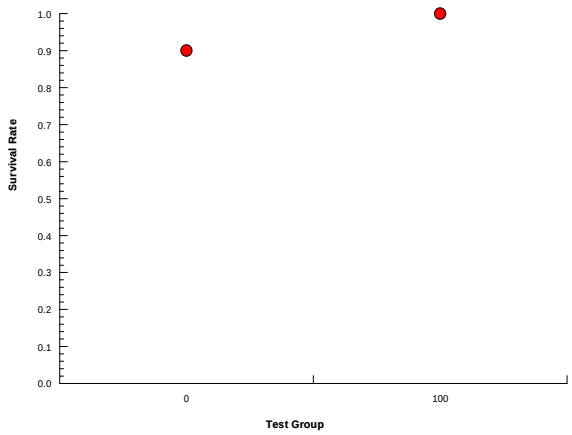
Fisher Exact Test

Control	vs	Test Group	Test Stat	P-Value	Decision(0.05)
Lab Control		100	1	1.0000	Non-Significant Effect

Data Summary

Test Group	Control Type	No-Resp	Resp	Total
0	Lab Control	9	1	10
100		10	0	10

Graphics



CETIS Analytical Report

Neanthes Survival and Growth Test **SPAWAR Systems Center**

Analysis ID: 13-3531-0838 **Endpoint:** Mean Dry Weight-mg **CETIS Version:** CETISv1.8.1
Analyzed: 27 Jul-11 15:36 **Analysis:** Parametric-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Untransformed	0	C > T	Not Run	Sample passes mean dry weight-mg endpoint	29.0%

Equal Variance t Two-Sample Test

Control	vs	Test Group	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Lab Control		100	0.2997	1.74	17	0.001427	0.3840	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.861617E-07	2.861617E-07	1	0.0898	0.7681	Non-Significant Effect
Error	5.417202E-05	3.18659E-06	17			
Total	5.445819E-05	3.472751E-06	18			

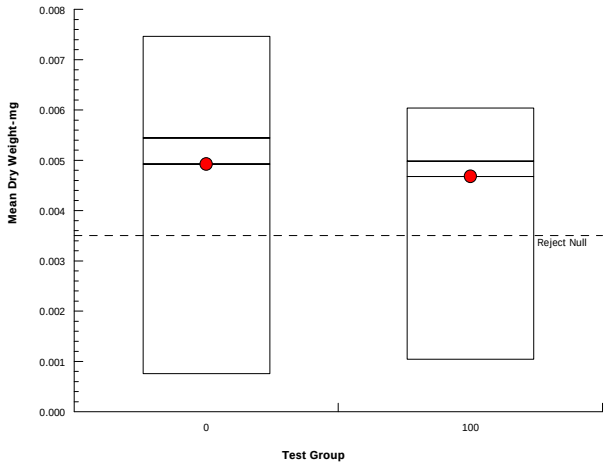
Distributional Tests

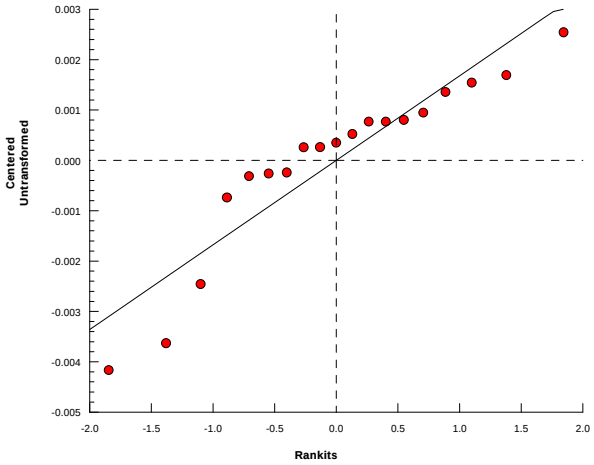
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.352	6.693	0.2244	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8732	0.8605	0.0164	Normal Distribution

Mean Dry Weight-mg Summary

Test Group	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	9	0.004928	0.004114	0.005742	0.00076	0.00747	0.0007134	0.00214	43.43%	0.0%
100		10	0.004682	0.004151	0.005213	0.00105	0.00604	0.0004413	0.001395	29.81%	4.99%

Graphics





Marine Sediment Bioassay

Organism Survival

Client: Internal - Cu Availability Study

Ambient Sediment Samples

Test Species: *N. arenaceodentata*

Start Date/Time: _____

End Date/Time: _____

Sample ID	Replicate	Initial No.	No. Recovered	Pan Weight ~~(mg)~~ g	Pan + Org. Weight (mg) WET	Pan + Org. Weight ~~(mg)~~ DRY g	Technician Initials
Shelter Island (SI)	A	1	1	1.20179		1.20542	
	B	1	1	1.05324		1.05683	
	C	1	1	1.15351		1.15545	
	D	1	1	1.19365		1.19414	
	E	1	1	1.09436		1.09898	
	F	1	1	1.13763		1.14173	
	G	1	1	0.99341		0.99725	
	H	1	1	1.23698		1.23922	
	I	1	1	1.24023		1.24339	
	J	1	1	1.16114		1.16530	
Naval Base SD (NB)	A	1	1	1.20162		1.20498	
	B	1	1	1.15929		1.16368	
	C	1	1	1.13636		1.13928	
	D	1	1	1.11471		1.11644	
	E	1	1	1.13801		1.14160	
	F	1	1	1.17650		1.18066	
	G	1	1	1.18646		1.19144	
	H	1	1	1.13461		1.13897	
	I	1	1	1.16520		1.16813	
	J	1	1	1.16015		1.16416	
PSNS - 1 (B1)	A	1	1	1.17409	1.17854	~~1.19686~~	
	B	1	1	1.15708	1.16210	~~1.20029~~	
	C	1	1	1.20240	1.20817	~~1.14042~~	
	D	1	1	1.05658	1.06054		
	E	1	1	1.13384	1.13902		
	F	1	1	1.11611	1.12222		
	G	1	1	1.17535	1.17929		
	H	1	1	1.25224	1.25784		
	I	1	1	1.11449	1.12050		
	J	1	1	1.11407	1.11909		
PSNS - 2 (B2)	A	1	1	1.19082		1.19686	
	B	1	1	1.19466		1.20029	
	C	1	1	1.13608		1.14042	
	D	1	1	1.13247		1.13684	
	E	1	1	1.17211		1.17756	
	F	1	1	1.12455		1.13000	
	G	1	1	1.18611		1.19105	
	H	1	1	1.12150		1.12592	
	I	1	1	1.12573		1.13076	
	J	1	1	1.08371		1.08482	

very small

QC Check: _____

Final Review: _____

P id 1 Neanthes Metals Analysis - 6/2/11

ombined 3 reps (each w/ 1 worm) into 2mL acid-cleaned microcentr Tubes (2mL)

Treatment	Empty Tube (g)	Tube + Dry Worms (g)	Comment
LCB/1 (4)	1.00390	1.01847	clean guts
LCB/2 (4)	1.00298	1.01399	clean guts
LCB/3 (4)	1.00227	1.01336	a couple of sediment particle.
100/1 (100)	1.00043	1.01013	clean
100/2	1.00251	1.01430	clean
100/3	1.00333	1.01898	4 worms, possible 1-2 sed particle
300/1	0.99953	1.01055	clear
300/2	1.00186	1.01317	clear
300/3	1.00043	1.01206	clear
500/1	1.00196	1.01041	possible minor sed.
500/2	0.99845	1.00482	possible minor sed.
500/3	1.00008	1.00602	possible minor sed.
SI/1	0.99994	1.00749	2 worms, clean
SI/2	1.00023	1.01023	minor sed.
SI/3	0.99812	1.00699	minor sed.
NB/1	1.00191	1.01187	clean
NB2	1.00034	1.00684	clean (small)
NB3	1.00165	1.01683	4 worms, minor sed.
B1/1	1.00034	1.01467	minor sed
B1/2	1.00028	1.01000	minor sed
B1/3	1.00091	1.01338	medium sed
B2/1	1.00153	1.01508	clean
B2/2	1.00028	1.01884	clean
B2/3	1.00024	1.00843	minor sed

Continued on Page

Read and Understood By

B1 = PSNS1 (PSO3), B2 = PSNS2 (PSO9)

Signed _____ Date _____ Signed _____ Date _____

28-Day Marine Sediment Bioassay
Static-Renewal Conditions

Water Quality Measurements

Client: Internal - Cu Availability Study

Test Species: *N. arenaceodentata*

mple ID: Bremerton DS03 - B1

Start Date/Time: 5/3/2011 1000

End Date/Time:

Test Day	Salinity (ppt)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH (units)	Fed	Water Change	NH_3 Analysis	Technician Initials	Comments
0	30.1	19.8	5.8	7.84	Y	N	✓	MC	
1	30.4	19.9	6.0	7.93				MC	
2	30.5	19.9	6.4	8.16				MC	
3		19.9		8.15	Y	N		CC	
4		20.0		8.24	N	N		CC	
5		19.9		8.59	N	N		CC	
6	30.8	19.8	5.6	8.86	N	N		MC	
7	30.7	19.9	5.8	8.89	Y	Y		MC	
8	30.5	19.8	6.5	9.00	N	N		MC	
9	30.7	19.9	6.72	9.05	N	N		CC	
10	30.4	19.8	6.4	9.18	Y	N		MC	
11	30.5	19.3	6.7	8.83	N	Y		MC	
12		19.9		8.91	N	N		CC	
13	30.9	19.9	6.8	8.94	N	N		MC	
14	27.8	20.0	5.59	8.82	Y	Y		CC	
15			~~7.68~~						
16	28.0	19.7	7.6	8.68	N	N		CC	
17		20.1		8.38				BL	
18	28.7	19.9	6.7	8.38	Y	MC Y N		MC	
19	28.5	19.8	6.9	8.36				MC	
20	28.6	19.9	7.2	8.41				MC	
21	29.1	20.0	6.3	8.23	Y	Y		MC	
22	30.1	19.9	6.8	8.10				MC	
23	29.5	20.1	6.9	8.36	N	N		CC	
24	29.8	20.0	6.9	8.24	Y	N		MC	
25									
26	30.1	20.1	7.2	8.32				MC	
27									
28	30.0	19.9	6.5	8.36			Y	MC	

QC Check:

Final Review:

28-Day Marine Sediment Bioassay
Static-Renewal Conditions

Water Quality Measurements

Client: Internal - Cu Availability Study

Test Species: *N. arenaceodentata*

ıple ID: Bremerton BS09 - B2

Start Date/Time: 3/3/2011 1600

End Date/Time:

Test Day	Salinity (ppt)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH (units)	Fed	Water Change	NH_3 Analysis	Technician Initials	Comments
0	30.2	19.7	5.4	7.83	Y	N	✓	MC	
1	30.8	19.9	6.2	8.08				MC	
2	30.5	20.1	6.4	8.03				MC	
3		19.7		7.91	Y	N		CC	
4		19.9		7.86	N	N		CC	
5		19.8		8.03	N	N		CC	
6	30.8	19.7	6.1	8.17	N	N		MC	
7	30.7	19.9	6.3	8.21	Y	Y		MC	
8	29.9	19.7	6.7	8.21	N	N		MC	
9	30.7	19.7	6.62	8.21	N	N		CC	
10	30.8	19.7	6.5	8.31	Y	N		MC	
11	30.9	19.5	6.7	8.20	N	N		MC	
12		19.8		8.32	N	N		CC	
13	31.2	19.8	7.1	8.27	N	N		MC	
14	28.4	19.8	5.01	8.29	Y	Y		CC	
15	—	—	—	—	—	—			
16	28.6	19.5	6.5	8.16	N	N		CC	
17		20.0		8.08				CC	
18	29.3	19.9	6.6	8.13	Y	MC Y N		MC	
19	29.1	19.9	6.8	8.14				MC	
20	29.1	20.0	6.9	8.17				MC	
21	29.0	19.8	6.5	8.26	Y	Y		MC	
22	29.9	20.1	6.7	8.14				MC	
23	~~29.0~~ 30.0	19.9	6.5	8.22	N	N		CC	
24	30.1	20.1	6.8	8.18	Y	N		MC	
25									
26	30.3	20.0	6.9	8.21				MC	
27									
28	31.1	19.8	6.4	8.28			Y	MC	

QC Check:

Final Review:

Appendix B

Reference Toxicant Test Data and Statistical Summaries

Bivalve Larval Survival and Development Test **SPAWAR Systems Center**

Batch ID:	11-0713-8919	**Test Type:**	Development-Survival	**Analyst:**	Marienne A Colvin
Start Date:	03 May-11 20:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	05 May-11 18:00	**Species:**	Mytilis galloprovincialis	**Brine:**	Not Applicable
Duration:	46h	**Source:**	Carlsbad Aquafarm	**Age:**	na

Sample ID:	21-1579-3046	**Code:**	7E1C7096	**Client:**	NAVSEA
Sample Date:	03 May-11	**Material:**	Copper sulfate	**Project:**	Sediment Copper Tools - Round 1
Receive Date:		**Source:**	Reference Toxicant		
Sample Age:	20h	**Station:**	Reference Toxicant		

Batch Note: SWI - Sediment Water Interface Test. Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-4993-3967	Combined Proportion Norm	8.4	12	10.04	20.6%		Bonferroni Adj t Test
02-9314-7267	Proportion Normal	8.4	12	10.04	13.5%		Wilcoxon/Bonferroni Adj Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
01-3687-9098	Combined Proportion Norm	EC50	10.22	8.939	10.79		Linear Regression (MLE)
16-9997-0667	Proportion Normal	EC50	9.86	9.484	10.23		Linear Regression (MLE)

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-9314-7267	Proportion Normal	Control Resp	0.8321	0.9 - NL	Yes	Below Acceptability Criteria
16-9997-0667	Proportion Normal	Control Resp	0.8321	0.9 - NL	Yes	Below Acceptability Criteria
15-4993-3967	Combined Proportion Norm	PMSD	0.2056	NL - 0.25	No	Passes Acceptability Criteria

Combined Proportion Normal Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.5087	0.4375	0.5799	0.4627	0.5622	0.02238	0.04475	8.8%	0.0%
2.9		5	0.5522	0.4945	0.61	0.4826	0.602	0.02081	0.04654	8.43%	-8.56%
4.1		5	0.5821	0.5342	0.6299	0.5373	0.6269	0.01723	0.03854	6.62%	-14.43%
5.8		5	0.6189	0.5403	0.6975	0.5124	0.6716	0.0283	0.06328	10.23%	-21.66%
8.4		5	0.5493	0.4565	0.642	0.4925	0.6766	0.0334	0.07468	13.6%	-7.97%
12		5	0.03881	0	0.08147	0.01493	0.08955	0.01537	0.03436	88.55%	92.37%
17.2		4	0	0	0	0	0	0	0		100.0%

Proportion Normal Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.8321	0.7681	0.8962	0.7737	0.8661	0.02014	0.04027	4.84%	0.0%
2.9		5	0.9388	0.9142	0.9633	0.9151	0.9646	0.00883	0.01974	2.1%	-12.81%
4.1		5	0.9551	0.9161	0.9942	0.9153	0.9919	0.01406	0.03144	3.29%	-14.78%
5.8		5	0.9351	0.9283	0.9419	0.9279	0.9403	0.002448	0.005474	0.59%	-12.38%
8.4		5	0.8055	0.6837	0.9273	0.6897	0.9	0.04387	0.09809	12.18%	3.2%
12		5	0.07037	0	0.1431	0.0283	0.1607	0.02621	0.05861	83.29%	91.54%
17.2		4	0	0	0	0	0	0	0		100.0%

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Combined Proportion Normal Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.5622	0.4826	0.5274	0.4627	
2.9		0.5423	0.5871	0.602	0.5473	0.4826
4.1		0.5473	0.6269	0.607	0.592	0.5373
5.8		0.6716	0.6617	0.6219	0.5124	0.6269
8.4		0.4975	0.6766	0.4925	0.5373	0.5423
12		0.01493	0.01493	0.0597	0.01493	0.08955
17.2		0	0	0	0	

Proportion Normal Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8433	0.8661	0.7737	0.8455	
2.9		0.9646	0.9291	0.9528	0.9322	0.9151
4.1		0.9322	0.9767	0.9919	0.9597	0.9153
5.8		0.931	0.9366	0.9398	0.9279	0.9403
8.4		0.6897	0.8947	0.7174	0.9	0.8258
12		0.0303	0.0283	0.09917	0.03333	0.1607
17.2		0	0	0	0	

Combined Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	113/201	97/201	106/201	93/201	
2.9		109/201	118/201	121/201	110/201	97/201
4.1		110/201	126/201	122/201	119/201	108/201
5.8		135/201	133/201	125/201	103/201	126/201
8.4		100/201	136/201	99/201	108/201	109/201
12		3/201	3/201	12/201	3/201	18/201
17.2		0/201	0/201	0/201	0/201	

Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	113/134	97/112	106/137	93/110	
2.9		109/113	118/127	121/127	110/118	97/106
4.1		110/118	126/129	122/123	119/124	108/118
5.8		135/145	133/142	125/133	103/111	126/134
8.4		100/145	136/152	99/138	108/120	109/132
12		3/99	3/106	12/121	3/90	18/112
17.2		0/74	0/82	0/90	0/79	

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID:	02-9314-7267	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Oct-16 15:28	**Analysis:**	Nonparametric-Multiple Comparison	**Official Results:**	Yes
Batch ID:	11-0713-8919	**Test Type:**	Development-Survival	**Analyst:**	Marienne A Colvin
Start Date:	03 May-11 20:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	05 May-11 18:00	**Species:**	Mytilis galloprovincialis	**Brine:**	Not Applicable
Duration:	46h	**Source:**	Carlsbad Aquafarm	**Age:**	na
Sample ID:	21-1579-3046	**Code:**	7E1C7096	**Client:**	NAVSEA
Sample Date:	03 May-11	**Material:**	Copper sulfate	**Project:**	Sediment Copper Tools - Round 1
Receive Date:		**Source:**	Reference Toxicant		
Sample Age:	20h	**Station:**	Reference Toxicant		

Batch Note: SWI - Sediment Water Interface Test. Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	13.5%	8.4	12	10.04	

Wilcoxon/Bonferroni Adj Test

Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.9	35	NA	0	7	1.0000	Exact	Non-Significant Effect
		4.1	35	NA	0	7	1.0000	Exact	Non-Significant Effect
		5.8	35	NA	0	7	1.0000	Exact	Non-Significant Effect
		8.4	24	NA	0	7	1.0000	Exact	Non-Significant Effect
		12*	15	NA	0	7	0.0397	Exact	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.8321	0.9 - NL	Yes	Below Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.444386	0.8888772	5	132.4	<0.0001	Significant Effect
Error	0.154369	0.006711697	23			
Total	4.598755		28			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	16.44	15.09	0.0057	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.98	0.9004	0.8390	Normal Distribution

Proportion Normal Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.8321	0.7681	0.8962	0.8444	0.7737	0.8661	0.02014	4.84%	0.0%
2.9		5	0.9388	0.9142	0.9633	0.9322	0.9151	0.9646	0.00883	2.1%	-12.81%
4.1		5	0.9551	0.9161	0.9942	0.9597	0.9153	0.9919	0.01406	3.29%	-14.78%
5.8		5	0.9351	0.9284	0.9419	0.9366	0.9279	0.9403	0.002448	0.59%	-12.38%
8.4		5	0.8055	0.6837	0.9273	0.8258	0.6897	0.9	0.04387	12.18%	3.2%
12		5	0.07037	0	0.1431	0.03333	0.0283	0.1607	0.02621	83.29%	91.54%
17.2		4	0	0	0	0	0	0	0		100.0%

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID: 02-9314-7267 **Endpoint:** Proportion Normal **CETIS Version:** CETISv1.8.7
Analyzed: 27 Oct-16 15:28 **Analysis:** Nonparametric-Multiple Comparison **Official Results:** Yes

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.15	1.067	1.234	1.165	1.075	1.196	0.02617	4.55%	0.0%
2.9		5	1.323	1.271	1.376	1.307	1.275	1.382	0.01904	3.22%	-15.04%
4.1		5	1.37	1.267	1.473	1.369	1.275	1.481	0.03698	6.04%	-19.08%
5.8		5	1.313	1.3	1.327	1.316	1.299	1.324	0.004946	0.84%	-14.17%
8.4		5	1.124	0.968	1.28	1.14	0.9799	1.249	0.05618	11.18%	2.3%
12		5	0.2521	0.1162	0.388	0.1836	0.169	0.4125	0.04895	43.42%	78.09%
17.2		4	0.0556	0.052	0.05921	0.05576	0.05273	0.05816	0.001132	4.07%	95.17%

Proportion Normal Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8433	0.8661	0.7737	0.8455	
2.9		0.9646	0.9291	0.9528	0.9322	0.9151
4.1		0.9322	0.9767	0.9919	0.9597	0.9153
5.8		0.931	0.9366	0.9398	0.9279	0.9403
8.4		0.6897	0.8947	0.7174	0.9	0.8258
12		0.0303	0.0283	0.09917	0.03333	0.1607
17.2		0	0	0	0	

Angular (Corrected) Transformed Detail

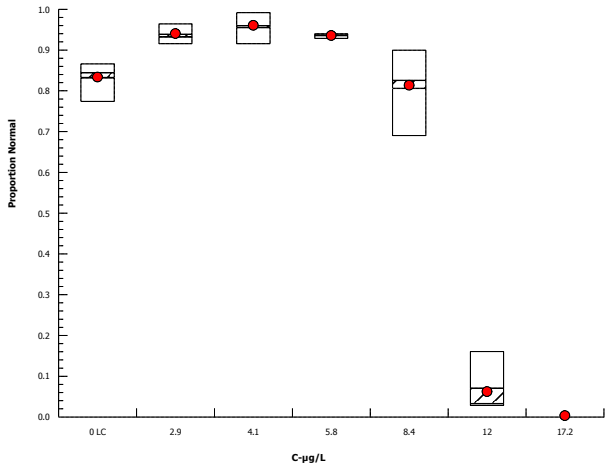
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	1.164	1.196	1.075	1.167	
2.9		1.382	1.301	1.352	1.307	1.275
4.1		1.307	1.418	1.481	1.369	1.275
5.8		1.305	1.316	1.323	1.299	1.324
8.4		0.9799	1.24	1.01	1.249	1.14
12		0.175	0.169	0.3204	0.1836	0.4125
17.2		0.05816	0.05524	0.05273	0.05628	

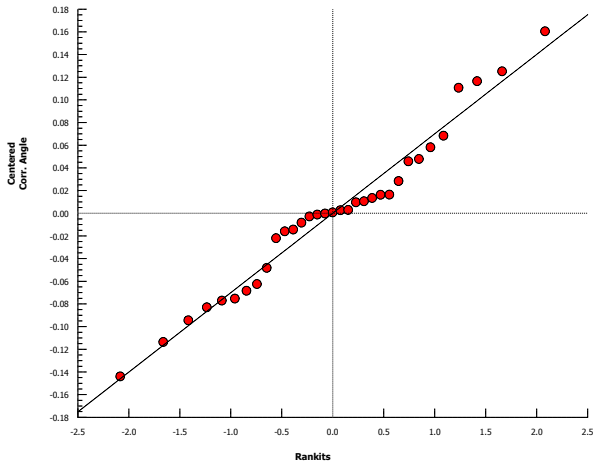
Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	113/134	97/112	106/137	93/110	
2.9		109/113	118/127	121/127	110/118	97/106
4.1		110/118	126/129	122/123	119/124	108/118
5.8		135/145	133/142	125/133	103/111	126/134
8.4		100/145	136/152	99/138	108/120	109/132
12		3/99	3/106	12/121	3/90	18/112
17.2		0/74	0/82	0/90	0/79	

Bivalve Larval Survival and Development Test					**SPAWAR Systems Center**
Analysis ID:	02-9314-7267	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Oct-16 15:28	**Analysis:**	Nonparametric-Multiple Comparison	**Official Results:**	Yes

Graphics





Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID:	15-4993-3967	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Oct-16 15:28	**Analysis:**	Parametric-Multiple Comparison	**Official Results:**	Yes
Batch ID:	11-0713-8919	**Test Type:**	Development-Survival	**Analyst:**	Marienne A Colvin
Start Date:	03 May-11 20:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	05 May-11 18:00	**Species:**	Mytilis galloprovincialis	**Brine:**	Not Applicable
Duration:	46h	**Source:**	Carlsbad Aquafarm	**Age:**	na
Sample ID:	21-1579-3046	**Code:**	7E1C7096	**Client:**	NAVSEA
Sample Date:	03 May-11	**Material:**	Copper sulfate	**Project:**	Sediment Copper Tools - Round 1
Receive Date:		**Source:**	Reference Toxicant		
Sample Age:	20h	**Station:**	Reference Toxicant		

Batch Note: SWI - Sediment Water Interface Test. Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	20.6%	8.4	12	10.04	

Bonferroni Adj t Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.9	-1.039	2.5	0.105	7	1.0000	CDF	Non-Significant Effect
		4.1	-1.756	2.5	0.105	7	1.0000	CDF	Non-Significant Effect
		5.8	-2.66	2.5	0.105	7	1.0000	CDF	Non-Significant Effect
		8.4	-0.9813	2.5	0.105	7	1.0000	CDF	Non-Significant Effect
		12*	14.5	2.5	0.105	7	<0.0001	CDF	Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
PMSD	0.2056	NL - 0.25	No	Passes Acceptability Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.872349	0.3744698	5	95.08	<0.0001	Significant Effect
Error	0.09058353	0.003938414	23			
Total	1.962932		28			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.464	15.09	0.6288	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9672	0.9004	0.4857	Normal Distribution

Combined Proportion Normal Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.5087	0.4375	0.5799	0.505	0.4627	0.5622	0.02238	8.8%	0.0%
2.9		5	0.5522	0.4945	0.61	0.5473	0.4826	0.602	0.02081	8.43%	-8.56%
4.1		5	0.5821	0.5342	0.6299	0.592	0.5373	0.6269	0.01723	6.62%	-14.43%
5.8		5	0.6189	0.5403	0.6975	0.6269	0.5124	0.6716	0.0283	10.23%	-21.66%
8.4		5	0.5493	0.4565	0.642	0.5373	0.4925	0.6766	0.0334	13.6%	-7.97%
12		5	0.03881	0	0.08147	0.01493	0.01493	0.08955	0.01537	88.55%	92.37%
17.2		4	0	0	0	0	0	0	0		100.0%

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID: 15-4993-3967 **Endpoint:** Combined Proportion Normal **CETIS Version:** CETISv1.8.7
Analyzed: 27 Oct-16 15:28 **Analysis:** Parametric-Multiple Comparison **Official Results:** Yes

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.7941	0.7228	0.8655	0.7904	0.7481	0.8477	0.02242	5.65%	0.0%
2.9		5	0.8379	0.7798	0.896	0.8327	0.768	0.8881	0.02093	5.59%	-5.51%
4.1		5	0.8681	0.8195	0.9166	0.878	0.8227	0.9137	0.01748	4.5%	-9.31%
5.8		5	0.9061	0.8259	0.9863	0.9137	0.7978	0.9606	0.02888	7.13%	-14.1%
8.4		5	0.8354	0.7407	0.9302	0.8227	0.7779	0.9659	0.03414	9.14%	-5.2%
12		5	0.1836	0.07667	0.2906	0.1225	0.1225	0.3039	0.03852	46.91%	76.88%
17.2		4	0.03527	0.03526	0.03529	0.03527	0.03527	0.03527	0	0.0%	95.56%

Combined Proportion Normal Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.5622	0.4826	0.5274	0.4627	
2.9		0.5423	0.5871	0.602	0.5473	0.4826
4.1		0.5473	0.6269	0.607	0.592	0.5373
5.8		0.6716	0.6617	0.6219	0.5124	0.6269
8.4		0.4975	0.6766	0.4925	0.5373	0.5423
12		0.01493	0.01493	0.0597	0.01493	0.08955
17.2		0	0	0	0	

Angular (Corrected) Transformed Detail

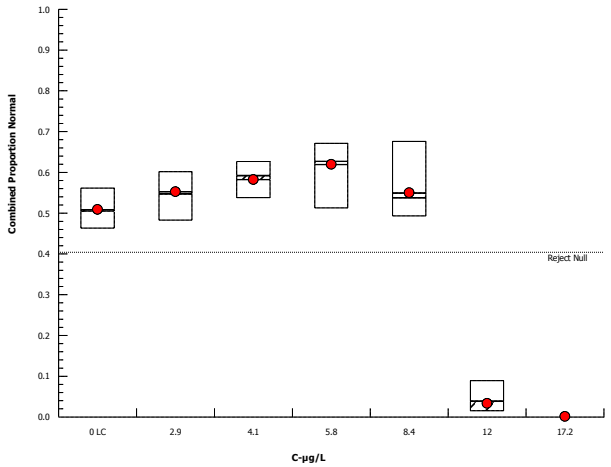
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8477	0.768	0.8128	0.7481	
2.9		0.8277	0.8729	0.8881	0.8327	0.768
4.1		0.8327	0.9137	0.8932	0.878	0.8227
5.8		0.9606	0.95	0.9085	0.7978	0.9137
8.4		0.7829	0.9659	0.7779	0.8227	0.8277
12		0.1225	0.1225	0.2468	0.1225	0.3039
17.2		0.03527	0.03527	0.03527	0.03527	

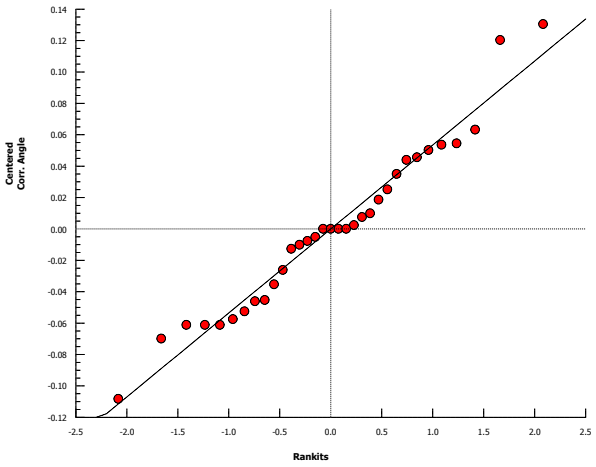
Combined Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	113/201	97/201	106/201	93/201	
2.9		109/201	118/201	121/201	110/201	97/201
4.1		110/201	126/201	122/201	119/201	108/201
5.8		135/201	133/201	125/201	103/201	126/201
8.4		100/201	136/201	99/201	108/201	109/201
12		3/201	3/201	12/201	3/201	18/201
17.2		0/201	0/201	0/201	0/201	

Bivalve Larval Survival and Development Test					**SPAWAR Systems Center**
Analysis ID:	15-4993-3967	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Oct-16 15:28	**Analysis:**	Parametric-Multiple Comparison	**Official Results:**	Yes

Graphics





Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID:	16-9997-0667	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Oct-16 15:28	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes
Batch ID:	11-0713-8919	**Test Type:**	Development-Survival	**Analyst:**	Marienne A Colvin
Start Date:	03 May-11 20:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	05 May-11 18:00	**Species:**	Mytilis galloprovincialis	**Brine:**	Not Applicable
Duration:	46h	**Source:**	Carlsbad Aquafarm	**Age:**	na
Sample ID:	21-1579-3046	**Code:**	7E1C7096	**Client:**	NAVSEA
Sample Date:	03 May-11	**Material:**	Copper sulfate	**Project:**	Sediment Copper Tools - Round 1
Receive Date:		**Source:**	Reference Toxicant		
Sample Age:	20h	**Station:**	Reference Toxicant		

Batch Note: SWI - Sediment Water Interface Test. Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	0.170385	Yes	No	Yes	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
5	-1140	2288	2291	0.9939	0.06077	0.9369	5.976	2.743	0.0015	Significant Lack of Fit

Point Estimates

Level	µg/L	95% LCL	95% UCL
EC50	9.86	9.484	10.23

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	0.8321	0.9 - NL	Yes	Below Acceptability Criteria

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.08047	0.01224	0.05547	0.1055	6.574	<0.0001	Significant Parameter
Slope	16.46	1.649	13.09	19.83	9.978	<0.0001	Significant Parameter
Intercept	-16.36	1.652	-19.73	-12.98	-9.9	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	2277.623	2277.623	1	477.1	<0.0001	Significant
Lack of Fit	68.60464	17.15116	4	5.976	0.0015	Significant
Pure Error	74.62022	2.870008	26			
Residual	143.2249	4.774162	30			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	143.2	43.77	<0.0001	Significant Heterogenity
	Likelihood Ratio GOF	135.9	43.77	<0.0001	Significant Heterogenity
Variances	Bartlett Equality of Variance	51.52	12.59	<0.0001	Unequal Variances
	Mod Levene Equality of Variance	3.313	2.573	0.0187	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9443	0.9354	0.0908	Normal Distribution
	Anderson-Darling A2 Normality	0.7333	2.492	0.0557	Normal Distribution

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID: 16-9997-0667 **Endpoint:** Proportion Normal **CETIS Version:** CETISv1.8.7
Analyzed: 27 Oct-16 15:28 **Analysis:** Linear Regression (MLE) **Official Results:** Yes

Proportion Normal Summary

			Calculated Variate(A/B)								
C-µg/L	**Control Type**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**	**A**	**B**
0	Lab Control	4	0.8321	0.7737	0.8661	0.02014	0.04027	4.84%	0.0%	409	493
2.9		5	0.9388	0.9151	0.9646	0.00883	0.01974	2.1%	-12.81%	555	591
4.1		5	0.9551	0.9153	0.9919	0.01406	0.03144	3.29%	-14.78%	585	612
5.8		5	0.9351	0.9279	0.9403	0.002448	0.005473	0.59%	-12.38%	622	665
8.4		5	0.8055	0.6897	0.9	0.04387	0.09809	12.18%	3.2%	552	687
12		5	0.07037	0.0283	0.1607	0.02621	0.05861	83.29%	91.54%	39	528
17.2		4	0	0	0	0	0		100.0%	0	325

Proportion Normal Detail

C-µg/L	**Control Type**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**
0	Lab Control	0.8433	0.8661	0.7737	0.8455	
2.9		0.9646	0.9291	0.9528	0.9322	0.9151
4.1		0.9322	0.9767	0.9919	0.9597	0.9153
5.8		0.931	0.9366	0.9398	0.9279	0.9403
8.4		0.6897	0.8947	0.7174	0.9	0.8258
12		0.0303	0.0283	0.09917	0.03333	0.1607
17.2		0	0	0	0	

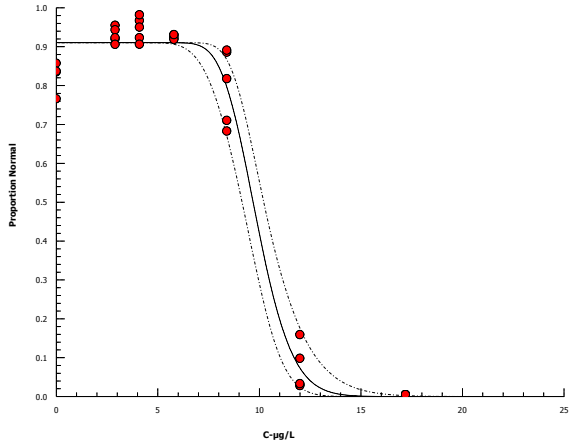
Proportion Normal Binomials

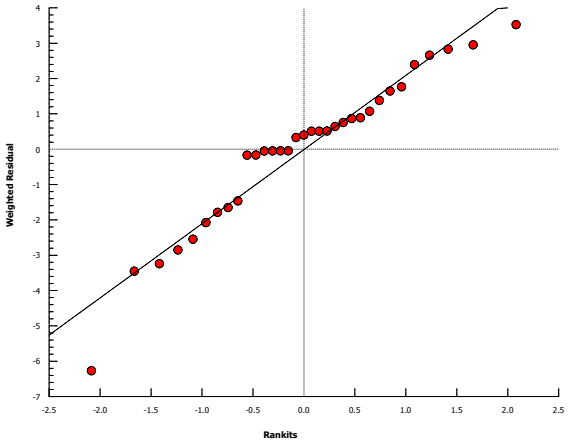
C-µg/L	**Control Type**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**
0	Lab Control	113/201	97/201	106/201	93/201	
2.9		109/201	118/201	121/201	110/201	97/201
4.1		110/201	126/201	122/201	119/201	108/201
5.8		135/201	133/201	125/201	103/201	126/201
8.4		100/201	136/201	99/201	108/201	109/201
12		3/201	3/201	12/201	3/201	18/201
17.2		0/201	0/201	0/201	0/201	

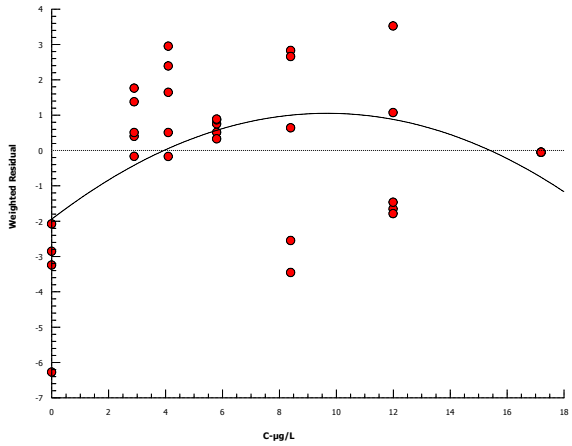
Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

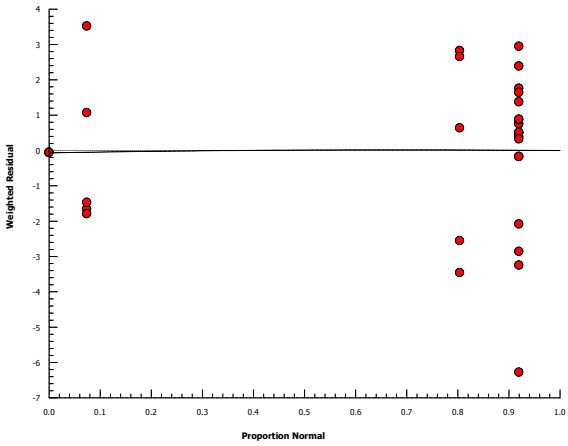
Analysis ID:	16-9997-0667	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Oct-16 15:28	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

Graphics — Log-Normal [NED=A+B*log(X)]









Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID:	01-3687-9098	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Oct-16 15:28	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes
Batch ID:	11-0713-8919	**Test Type:**	Development-Survival	**Analyst:**	Marienne A Colvin
Start Date:	03 May-11 20:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	05 May-11 18:00	**Species:**	Mytilis galloprovincialis	**Brine:**	Not Applicable
Duration:	46h	**Source:**	Carlsbad Aquafarm	**Age:**	na
Sample ID:	21-1579-3046	**Code:**	7E1C7096	**Client:**	NAVSEA
Sample Date:	03 May-11	**Material:**	Copper sulfate	**Project:**	Sediment Copper Tools - Round 1
Receive Date:		**Source:**	Reference Toxicant		
Sample Age:	20h	**Station:**	Reference Toxicant		

Batch Note: SWI - Sediment Water Interface Test. Overlying Water consisted of 0.45µm filter laboratory seawater diluted to 30ppt with Nano-pure DI water.

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	0.491294	Yes	No	Yes	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
7	-3468	6943	6946	1.01	0.0467	0.9379	2.069	2.743	0.1140	Non-Significant Lack of Fit

Point Estimates

Level	µg/L	95% LCL	95% UCL
EC50	10.22	8.939	10.79

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.4315	0.01445	0.402	0.461	29.86	<0.0001	Significant Parameter
Slope	21.41	4.828	11.55	31.27	4.435	0.0001	Significant Parameter
Intercept	-21.62	5.177	-32.19	-11.04	-4.176	0.0002	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	1579.036	1579.036	1	485.6	<0.0001	Significant
Lack of Fit	23.54807	5.887017	4	2.069	0.1140	Non-Significant
Pure Error	73.99361	2.845908	26			
Residual	97.54169	3.25139	30			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	97.54	43.77	<0.0001	Significant Heterogenity
	Likelihood Ratio GOF	96.95	43.77	<0.0001	Significant Heterogenity
Variances	Mod Levene Equality of Variance	0.9946	2.573	0.4544	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9642	0.9354	0.3378	Normal Distribution
	Anderson-Darling A2 Normality	0.4114	2.492	0.3454	Normal Distribution

Combined Proportion Normal Summary — **Calculated Variate(A/B)**

C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	0.5087	0.4627	0.5622	0.02238	0.04475	8.8%	0.0%	409	804
2.9		5	0.5522	0.4826	0.602	0.02081	0.04654	8.43%	-8.56%	555	1005
4.1		5	0.5821	0.5373	0.6269	0.01723	0.03854	6.62%	-14.43%	585	1005
5.8		5	0.6189	0.5124	0.6716	0.0283	0.06328	10.23%	-21.66%	622	1005
8.4		5	0.5493	0.4925	0.6766	0.0334	0.07468	13.6%	-7.97%	552	1005
12		5	0.03881	0.01493	0.08955	0.01537	0.03436	88.55%	92.37%	39	1005
17.2		4	0	0	0	0	0		100.0%	0	804

Bivalve Larval Survival and Development Test — **SPAWAR Systems Center**

Analysis ID:	01-3687-9098	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Oct-16 15:28	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

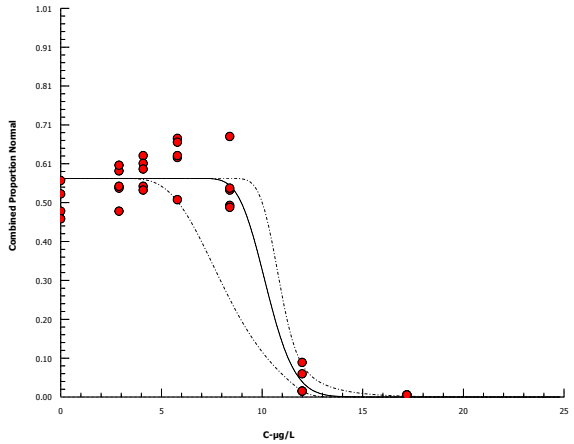
Combined Proportion Normal Detail

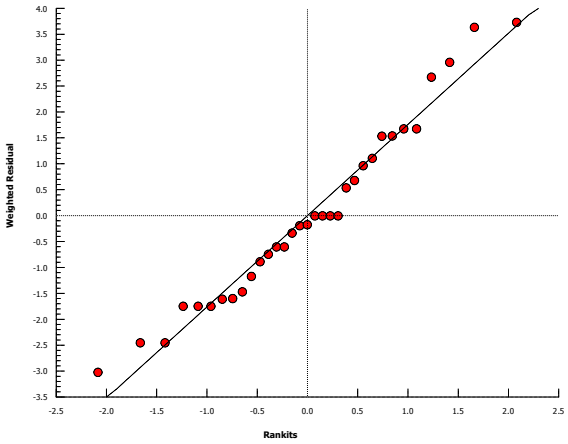
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.5622	0.4826	0.5274	0.4627	
2.9		0.5423	0.5871	0.602	0.5473	0.4826
4.1		0.5473	0.6269	0.607	0.592	0.5373
5.8		0.6716	0.6617	0.6219	0.5124	0.6269
8.4		0.4975	0.6766	0.4925	0.5373	0.5423
12		0.01493	0.01493	0.0597	0.01493	0.08955
17.2		0	0	0	0	

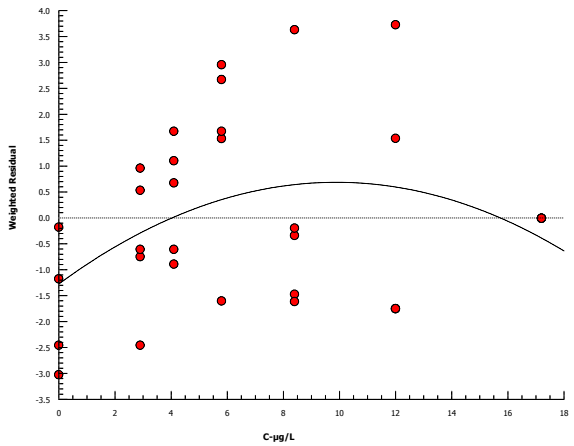
Combined Proportion Normal Binomials

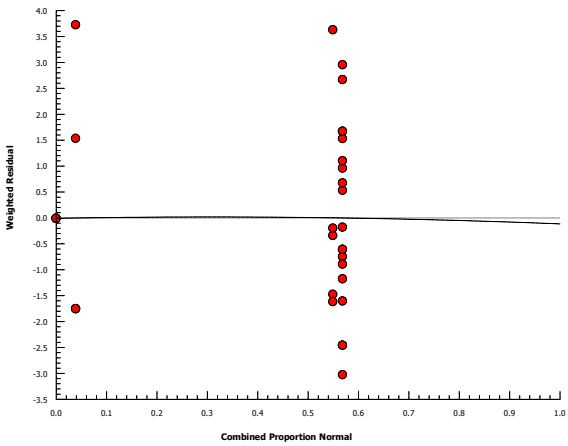
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	113/201	97/201	106/201	93/201	
2.9		109/201	118/201	121/201	110/201	97/201
4.1		110/201	126/201	122/201	119/201	108/201
5.8		135/201	133/201	125/201	103/201	126/201
8.4		100/201	136/201	99/201	108/201	109/201
12		3/201	3/201	12/201	3/201	18/201
17.2		0/201	0/201	0/201	0/201	

Graphics Log-Normal [NED=A+B*log(X)]









Embryo Larval Bioassay

48-hour Development

Project: SPAWAR

Sample ID: Reference Toxicant CuSO4

Test Species: *M.galloprovincialis*

Start Date: 5/3/2011

End Date: 5/5/2011

Random #	Number Normal	Number Abnormal	Technician Initials	
1	103	8	GR	
2	106	31	GR	
3				term. early
4	133	9	MC	
5	125	8	GR	
6	97	15	MC	
7	18	94	MC	
8	Ø	74	MC	
9	3	96	GR	
10	97	9	MC	
11	122	1	MC	
12	108	10	MC	
13	136	16	MC	
14	Ø	79	MC	
15	110	8	MC	
16	135	10	MC	
17	109	4	MC	
18	118	9	MC	
19	Ø	90	MC	
20	99	39	GR	
21	~~Ø~~ Ø mc	79	MC	
22	120	8	MC	
23	Ø	82	MC	
24	12	109	MC	
25	119	5	MC	
26	110	8	MC	
27	109	23	MC	
28	100	45	MC	
29	121	6	MC	
30	120	3	MC	
31	3	103	MC	
32	mc ~~Ø~~ 93	mc ~~110~~ 17	MC	
33	108	12	MC	
34	113	21	MC	
35	3	87	MC	

QC Check: _____

Final Review: _____

Marine Chronic Bioassay

Water Quality Measurements

Project: **SPAWAR**

Sample ID: Copper Sulfate Reftox

Test Species: *M.galloprovincialis*

Start Date/Time: 5/3/2011 2000

End Date/Time: 5/5/2011 1800

Concentration (%)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	29.9	30.5	31.2	15.0	15.3	14.7	~~15.0~~ 7.4 mc	7.3	7.4	8.04	8.05	8.11
2.9	29.6	30.3	32.0	15.0	15.4	15.4	7.3	7.4	7.5	8.06	8.09	8.13
4.1	30.0	30.5	31.8	15.0	15.6	14.8	7.3	7.4	7.4	8.07	8.09	8.15
5.8	30.5	30.8	31.8	15.0	15.6	14.9	7.4	7.3	7.2	8.07	8.10	8.15
8.4	30.5	30.7	31.6	15.0	15.5	14.9	7.3	7.2	7.1	8.07	8.11	8.15
12	30.6	30.7	31.5	15.0	15.6	15.2	7.4	7.3	7.3	8.07	8.11	8.15
17.2	30.6	30.8	31.8	15.0	15.6	15.5	7.4	7.4	7.2	8.07	8.10	8.15

	0	24	48
WQ Readings:	MC	MC	MC
Dilutions made by:	MC		

Technician Initials:

Animal Source/Date Received: Carlsbad Aquafarms 5/3/2011

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check:

Final Review:

Appendix C

Sample Information

SAMPLE COLLECTION AND ARRIVAL LOG

Project	Sample ID	Sampling			Matrix	Received By	Receipt at Lab		
		Date	Time	Temp. (°C)			Date	Time	Temp. (°C)
PSNS AMB06	M3.1	3/23/11	1434	9.0	SW	SD	3/23/11	1020	0.8
↓	M4	↓	1403	8.9	↓	↓	↓	↓	0.8
↓	TEST	↓	1421	9.0	↓	↓	↓	↓	0.6
PS03 - bulk sed.	Cu Sed. Project	4/27/2011	1125	NR	Sed	MC	4/29/11	0900	6.1
PS09 - bulk sed.	↓	↓	1235	↓	↓	↓	↓	↓	6.1
PS03 - core sed.	↓	↓	1050	↓	↓	↓	↓	↓	6.1
PS08 - core sed.	↓	↓	NR	↓	↓	↓	↓	↓	6.1
PS09 - core sed.	↓	3/31/11	1120	↓	↓	↓	↓	↓	6.1
Cu tools	Seavim Sed	~~4/27/11~~	1005	4	Sed	MC/HM	6/14/11	1005	2.5
PSNS AMB07	NPDES 18	6/21/11	1300	4.0	SW	SD	6/22/11	0950	4.1
↓	NPDES 15	↓	1200	4.0	↓	↓	↓	↓	4.1
↓	NPDES 2	↓	1200	4.0	↓	↓	↓	↓	4.1
↓	PS01	↓	1127	13.8	↓	↓	↓	↓	1.6
↓	PS03	↓	0938	13.9	↓	↓	↓	↓	2.9
↓	PS08	↓	1010	13.2	↓	↓	↓	↓	2.9
↓	~~PS09~~	↓	~~1028~~	~~13.2~~	↓	↓	↓	↓	~~1.6~~ (5)
↓	PS14	↓	0925	13.5	↓	↓	↓	↓	2.9
↓	PS15	↓	1019	13.2	↓	↓	↓	↓	2.9
↓	M3.1	↓	1233	12.6	↓	↓	↓	↓	1.6
↓	M4	↓	1203	15.9	↓	↓	↓	↓	1.6
↓	TEST	↓	1215	15.6	↓	↓	↓	↓	3.9

Comments (5) Sample leaked during shipment

Total Ammonia Analysis
Marine Samples

Client: **Internal - Cu Availability Study**
Initial Measurements of Overlying Water
Test Type: **Sediment Toxicity Exposure**

N x 1.22

Sample ID	Sample Date	Test Day	pH (units)	Salinity (ppt)	Nitrogen (mg/L)	Ammonia (mg/L)	Technician Initials
Blank Spike (10 mg/L NH_3)	NA	NA	NA	NA	9.8	12.0	GR
Neanthes:							
Shelter Island	5/3/2011	0			0.6	0.7	
Naval Base SD	5/3/2011	0			0.4	0.5	
Neanthes - Bremerton PS03 BV	5/3/2011	0			0.9	1.1	
Neanthes - Bremerton PS09 BV	5/3/2011	0			0.3	0.4	
Cu - Lab control	5/3/2011	0			0.9	1.1	
Cu - 100 mg/kg	5/3/2011	0			1.1	1.3	
Cu - 500 mg/kg	5/3/2011	0			0.7	0.9	
Cu - 4500 mg/kg	5/3/2011	0			1.2	1.5	
Ampelisca:					SEE		
Shelter Island	5/3/2011	0			LEPTOCHEIRUS		
Naval Base SD	5/3/2011	0			DATA		
Neanthes - Bremerton PS03	5/3/2011	0					
Neanthes - Bremerton PS09	5/3/2011	0					
Cu - Lab control	5/3/2011	0					
Cu - 100 mg/kg	5/3/2011	0					
Cu - 500 mg/kg	5/3/2011	0					
~~Cu - 4500~~ mg/kg	5/3/2011	0					
Leptocheirus:							
Shelter Island	5/3/2011	0			0.4	0.5	
Naval Base SD	5/3/2011	0			0.3	0.4	
Neanthes - Bremerton PS03	5/3/2011	0			0.6	0.7	
Neanthes - Bremerton PS09	5/3/2011	0			ND	ND	
Cu - Lab control	5/3/2011	0			ND	ND	
Cu - 100 mg/kg	5/3/2011	0			0.5	0.6	
Cu - 500 mg/kg	5/3/2011	0			0.3	0.4	
Cu - 4500 mg/kg	5/3/2011	0			0.1	0.1	

QC Check: WE 6/6/11

Total Ammonia Analysis
Marine Samples

Client: **Internal - Cu Availability Study**

Initial Measurements of Overlying Water
Test Type: **Sediment Toxicity Exposure**

N x 1.22

Sample ID	Sample Date	Test Day	pH (units)	Salinity (ppt)	Nitrogen (mg/L)	Ammonia (mg/L)	Technician Initials
Blank Spike (10 mg/L NH_3)	NA	NA	NA	NA	9.8	12.0	EP
Bivalve:							
Shelter Island - Core	5/3/2011	0			ND	ND	
Shelter Island - Hom.	5/3/2011	0			ND	ND	
Naval Base SD - Core	5/3/2011	0			ND	ND	
Naval Base SD - Hom.	5/3/2011	0		0.7	~~ND~~	0.9	
Bivalve - Bremerton PS03 - Core B1	5/3/2011	0			1.8	2.2	
Bivalve - Bremerton PS03 - Hom. B1	5/3/2011	0			0.2	0.2	
Bivalve - Bremerton PS09 - Core B2	5/3/2011	0			0.4	0.5	
Bivalve - Bremerton PS09 - Hom. B2	5/3/2011	0			1.1	1.3	
Cu - Lab control	5/3/2011	0			0.4	0.5	
Cu - 100 mg/kg	5/3/2011	0			0.9	1.1	
Cu - 500 mg/kg	5/3/2011	0			0.3	0.4	
Cu - 1500 mg/kg	5/3/2011	0			~~0.7~~	0.9	
					0.7		
Ampelisca control sed	5/4/2011	1			1.3	1.6	ME
spike					10.0	12.2	ME

QC Check: ME 6/16/11

Total Copper Analysis
Marine Samples

Client: **Internal - Cu Availability Study**

Pre-Testing Measurements of Overlying Water within Neanthes Test Chambers

Test Type: **Sediment Toxicity Exposure**

Sample ID	Sample Date	Test Day	pH (units)	Total Copper (mg/kg)	Technician Initials
~~Blank Spike (10 mg/L NH_3)~~	NA	NA	NA		
Lab control	5/2/2011		8.14	ND	ML
100 mg/kg	5/2/2011		8.24	ND	
300 mg/kg	5/2/2011		8.28	ND	
500 mg/kg	5/2/2011		8.24	4	
1500 mg/kg	5/2/2011		8.24	34	
4500 mg/kg	5/2/2011		8.20	234	
Lab control	5/3/2011				
100 mg/kg	5/3/2011				
300 mg/kg	5/3/2011				
500 mg/kg	5/3/2011				
1500 mg/kg	5/3/2011				
4500 mg/kg	5/3/2011				
Naval Base SD	5/3/2011	0		5	
Shelter Island SWI				4	
PS03 (B1)				4	
PS09 (B2)				11	MC
↳ Homogenized					

QC Check: _____

5/2/11 SWI 1500 -18 pH 8.21
SWI 4500 -85 pH 8.19

Total Ammonia and Copper Analysis
Marine Samples

Client: **Internal - Cu Availability Study**

Final Measurements of Overlying Water
Test Type: **Sediment Water Interface Toxicity Exposure**

N x 1.22

Sample ID	Sample Date	Test Day	Nitrogen (mg/L)	Ammonia (mg/L)	Copper (µg/L)	Technician Initials
Blank Spike (10 mg/L NH_3)	NA	NA	9.3	11.3	-	uc
Bivalve:						
Water Only Control	5/5/2011	2	0.7	0.9	ND	
Shelter Island - Core	5/5/2011	2	0.4	0.5	ND	
Shelter Island - Hom.	5/5/2011	2	MS 20.5	0.6	ND	
Naval Base SD - Core	5/5/2011	2	0.2	0.2	ND	
Naval Base SD - Hom.	5/5/2011	2	0.0	0.0	ND	
Bivalve - Bremerton PS03 - Core	5/5/2011	2	0.8	1.0	ND	
Bivalve - Bremerton PS03 - Hom.	5/5/2011	2	0.0	0.0	ND	
Bivalve - Bremerton PS09 - Core	5/5/2011	2	0.3	0.4	ND	
Bivalve - Bremerton PS09 - Hom.	5/5/2011	2	0.9	1.1	ND	
Cu - Lab control	5/5/2011	2	1.7	2.1	ND	
Cu - 100 mg/kg	5/5/2011	2	0.5	0.6	ND	
Cu - 300 mg/kg	5/5/2011	2	1.0	MS 1.2	ND	
Cu - 500 mg/kg	5/5/2011	2	2.0	2.4	ND	
Cu - 1500 mg/kg	5/5/2011	2	0.8	1.0	ND	
Cu - 4500 mg/kg	5/5/2011	2	1.0	1.2	113	

ND = Non Detect

QC Check: uc 6/6/11

Total Ammonia and Copper Analysis
Marine Samples

Client: Internal - **Cu Availability Study**

Final Measurements of Overlying Water
Test Type: **Sediment Toxicity Exposure**

N x 1.22

Sample ID	Sample Date	Test Day	pH (units)	Salinity (ppt)	Nitrogen (mg/L)	Ammonia (mg/L)	Technician Initials
Blank Spike (10 mg/L NH_3)	NA	NA	NA	NA	8.0	9.8	MC
Leptocheirus:							
Shelter Island	5/13/2011	10			0.3	0.4	
Naval Base SD	5/13/2011	10			0.2	0.2	
Bremerton 1 PS03	5/13/2011	10			1.3	1.6	
Bremerton 2 PS09	5/13/2011	10			1.3	1.6	
Cu - Lab control	5/13/2011	10			0.6	0.7	
Cu - 100 mg/kg	5/13/2011	10			ND	ND	
Cu - 300 mg/kg	5/13/2011	10			0.5	0.6	
Cu - 500 mg/kg	5/13/2011	10			0.2	0.2	
Cu - 1500 mg/kg	5/13/2011	10			0.4	0.5	
Cu - 4500 mg/kg	5/13/2011	10			0.0	0.0	
Ampelisca:							
Shelter Island	5/13/2011	10			0.9	1.1	
Naval Base SD	5/13/2011	10			0.1	.1	
Bremerton 1 PS03	5/13/2011	10			~~MC~~ ND	ND	
Bremerton 2 PS09	5/13/2011	10			ND	ND	
Cu - Lab control	5/13/2011	10			0.5	0.6	
Cu - 100 mg/kg	5/13/2011	10			ND	ND	
Cu - 300 mg/kg	5/13/2011	10			0.7	0.9	
Cu - 500 mg/kg	5/13/2011	10			0.7	0.9	
Cu - 1500 mg/kg	5/13/2011	10			0.2	0.2	
Cu - 4500 mg/kg	5/13/2011	10			0.7	0.9	
Ampelisca home sediment control	5/13/2011	10			1.5	1.8	
~~Eoh LL Cu~~	Shelter	11			4.4	5.4	~~MC~~
~~Eoh 500 Cu~~					2.9	3.5	
~~Eoh 1500 Cu~~					2.2	2.7	
~~Eoh Spike~~					9.7	11.8	

Note

QC Check: ML 6/16/11

lepto, Amp + Eoh - 10 days
day 10

Total Ammonia Analysis
Marine Samples

Client: **Internal - Cu Availability Study**

Final **Measurements of ~~Pore~~ Overlying Water**

Test Type: **Eohaustorius 10-day Termination Day (16May11)**

N x 1.22

Sample ID	Sample Date	Test Day	Nitrogen (mg/L)	Ammonia (mg/L)	Copper (µg/L)	Technician Initials
Blank Spike (10 mg/L NH_3)	NA	NA	9.6	11.7		GR
Water Only Control	5/16/2011	10	NA	–		
Shelter Island - Core	5/16/2011	10	2.8	3.4		
Shelter Island - Hom.	5/16/2011	10	no sample. –			
Naval Base SI - Core	5/16/2011	10	0.7	0.9		
Naval Base SD - Hom.	5/16/2011	10	4.5	5.5		
Bremerton PS03 - Core PS03	5/16/2011	10	2.4	2.9		
Bremerton PS03 - Hom. 03	5/16/2011	10	4.4	5.4		
Bremerton PS09 - Core 09	5/16/2011	10	no sample –			
Bremerton PS09 - Hom. 09	5/16/2011	10	no sample –			
Cu - Lab control	5/16/2011	10	6.1	7.4		
Cu - 150 mg/kg	5/16/2011	10	3.8	4.6		
Cu - 300 mg/kg	5/16/2011	10	4.8	5.9		
Cu - 500 mg/kg	5/16/2011	10	3.8	4.6		
Cu - 1500 mg/kg	5/16/2011	10	5.8	7.1		
Cu - 3200 mg/kg	5/16/2011	10	5.8	7.1		

QC Check: MC 6/6/2011

Eoh - 10 day - day 10

6/1/2011
ll

Total Amm[illegible] and Copper Analysis
Marine Sa[illegible]s

Client: **Internal - Cu Availability Study**

Final **Measurements of Overlying Water**
Test Type: **Sediment Toxicity Exposure**

N x 1.22

[illegible]ple ID		Sample Date	Test Day	pH (units)	Salinity (ppt)	Nitrogen (mg/L)	Ammonia (mg/L)	Technician Initials
Blank spike [illegible]9 mg/L NH_3)		NA	NA	NA	NA	9.8	12.0	BS/J
Neanthes								
Shelto[illegible]	SJ	5/31/2011	28			0.3	0.37	
Naval [illegible]s	NB	5/31/2011	28			0.7	0.85	
Brem[illegible]n D503	B1	5/31/2011	28			-0.4	ND	
Bremer[illegible] P509	B2	5/31/2011	28			0.1	0.12	
Cu - L[illegible]	LC	5/31/2011	28			-0.1	ND	
Cu - [illegible]	100	5/31/2011	28			-0.2	ND	
Cu - [illegible]	300	5/31/2011	28			-0.2	ND	
Cu - [illegible]	500	5/31/2011	28			-0.1	ND	
Cu - [illegible]	1500	5/31/2011	28			-0.1	ND	
Cu - [illegible]	4500	5/31/2011	28			1.0	1.22	
Blank						-0.1	ND	

ll 6/6/2011

Neanthes - 28day - day28

Appendix D

Chain-of-Custody Forms

SPAWAR
Systems Center San Diego

ENVIRONMENTAL SCIENCES AND
APPLIED SYSTEMS BRANCH, CODE 71750
53605 HULL STREET
SAN DIEGO, CA 92152-5000

Chain of Custody Record

Date: 4/29/2011

Project Title/Project Number:			Project Leader:
Remarks/Air Bill:			Contact:
Sampler(s): (Signature)			Contact Tel:
Tel:	Fax:	Email:	Requested Analyses
Special Instructions:			Biossay

Field Sample Identification	Date	Time	Matrix	Type	Temp (°C) on receipt	
PS03 - core sample (5)	4/27/2011 me	1050	Sediment		6.1	X
PS08 - core sample (5)	4/27/2011 me	NR	↓		↓	↓
PS09 - Core sample (5)	4/27/2011	1220				
PS03 - bulk sample (1)	4/27/2011 me	1125				
~~PS08 - bulk sample (1)~~	~~4/27/2011~~ me		↓		↓	↓
PS09 - bulk sample (1)	4/27/2011	1235	↓		6.1	X

Relinquished by: (Signature)	Received by: (Signature)	Date:	Time:
		4/29/2011	0900
Relinquished by: (Signature)	Received by: (Signature)	Date:	Time:

Appendix E

Glossary of Qualifier Codes

Glossary of Qualifier Codes:

Q1 – pH out of recommended range; refer to CAR

Q2 – Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log

Q3 – Temperatures out of recommended range; no action taken, test terminated same day

Q4 – Sample aerated prior to initiation or renewal

Q5 – Salinity out of recommended range; refer to QA section of report

Q6 – Spilled test chamber/ Lost test animal

Q7– Instrumentation Error/Failure; refer to CAR

Q8 – Inadequate sample volume, 50% renewal performed

Q9 – Inadequate sample volume, no renewal performed

Q10 – Sample out of holding time; refer to QA section of report

Q11 – Refer to QA section of report for explanation

Q12 – Supplemental information is footnoted

Q13 – Test initiated with an incorrect number of test organisms

Q14 – Replicate(s) not initiated; excluded from data analysis

Q15 – Survival counts not recorded due to poor visibility or heavy debris

Q16 – Test aerated due to dissolved oxygen levels dropping below 4.0 mg/L

Q17 – Test initiated with aeration due to an anticipated drop in dissolved oxygen

Q18 – Airline obstructed or fell out of replicate and replaced, drop in dissolved oxygen occurred

Q19 – Animals out of appropriate age range at test initiation

Q20 – Readings not taken, tech error

Q21 – Organisms in replicate not counted, tech error

Q22 – Dissolved oxygen above recommended range, but remained within the 100% ±10% saturation requirement