

# BIOASSAY TESTING RESULTS

## PORT OF FRIDAY HARBOR REMEDIAL INVESTIGATION

### FRIDAY HARBOR, WASHINGTON

**Prepared for**

Leon Environmental  
8047 Burke Ave N  
Seattle, WA 98103

**Prepared by**

EcoAnalysts, Inc.  
Port Gamble Environmental Laboratory  
4770 NE View Drive  
PO Box 216  
Port Gamble, Washington 98364

**EcoAnalysts Report ID**

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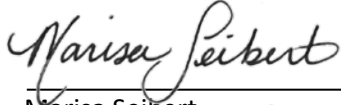
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**PREPARED BY:**



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Marisa Seibert

Project Manager / Laboratory Manager

Julia Levensgood

Project Manager/ Senior Aquatic Toxicologist

QA Review:

Mary Ann Rempel-Hester

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## ACRONYMS AND ABBREVIATIONS

AFDW:	Ash-free dry weight
CSL:	Cleanup Screening Level
°C:	Degrees Celsius
EC <sub>50</sub> :	Effective Concentration that results in a 50% reduction in a sub-lethal endpoint
g:	Grams
LC <sub>50</sub> :	Lethal Concentration that results in a 50% reduction in survival
L:	Liter
µm:	Micrometer
mg:	Milligram
mg/ind/day:	Milligrams per Individual per Day
mg/L:	Milligrams per liter
MIG:	Mean Individual Growth Rate
mL:	Milliliter
mm:	Millimeter
NELAP:	National Environmental Laboratory Accreditation Program
NOEC:	No Observed Effect Concentration
OR:	Oregon
ppt:	parts per thousand
PSEP:	Puget Sound Estuary Protocols (PSEP 1995)
SCO:	Sediment Cleanup Objective
SCU:	Sediment Cleanup Unit
SCUM:	Sediment Cleanup User's Manual
SD:	Standard Deviation
SMS:	Sediment Management Standards
SMARM:	Sediment Management Annual Review Meeting
SOP:	Standard operation procedure
UIA:	Un-ionized ammonia
USACE:	United States Army Corps of Engineers
USEPA:	United States Environmental Protection Agency
WA:	Washington State
WDOE:	Washington (State) Department of Ecology

## 1. INTRODUCTION

EcoAnalysts conducted biological toxicity testing on samples collected by Leon Environmental as part of a remedial sediment investigation at Albert Jensen and Sons Boatyard and Marina located in the Port of Friday Harbor, Washington. Sediments were evaluated for biological effects according to guidance outlined in the Washington State Department of Ecology (WDOE), the Sediment Cleanup User's Manual (SCUM; WDOE 2021), and modifications made through the DMMP annual review process (Sediment Management Annual Review Meetings [SMARM] updates).

All biological testing was performed in strict compliance with Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (PSEP 1995). This report presents the results of the toxicity testing portion of the sediment investigation.

## 2. METHODS

This section summarizes the test methods followed for this biological characterization. Test methods followed guidance provided by the Puget Sound Estuary Program (PSEP 1995), the Sediment Cleanup User's Manual (SCUM; WDOE 2021), and the various updates presented during the Sediment Management Annual Review Meeting (SMARM). Sediment toxicity was evaluated using three standard PSEP bioassays; the 10-day amphipod test, the 20-day juvenile polychaete survival and growth test, and the benthic larval development test.

### 2.1 Sample Collection

Samples were collected by Leon Environmental personnel on March 20<sup>th</sup>, 21<sup>st</sup>, and 23<sup>rd</sup>, 2023 and were received at the EcoAnalysts laboratory on March 24<sup>th</sup>, 2023. Reference sediment CARR18-23-REF, CARR40-23-REF, and CARR62-23-REF were collected from Carr Inlet, Washington on May 10<sup>th</sup>, 2023, and received the same day. Sediment samples were stored in a walk-in cold room at  $4 \pm 2^{\circ}\text{C}$  in the dark. All tests were conducted within the eight-week holding time.

Native *Eohaustorius estuarius* sediment from Yaquina Bay, Oregon was provided by Northwest Amphipod for use as the control sediment treatment for the amphipod and juvenile polychaete tests.

### 2.2 Sample Grain Size and Reference Comparison

Sediment grain size is one of the characteristics used in selecting the appropriate reference sediment(s) to compare the chemical and biological responses of project sediments. The percent fines value is defined as the amount of sediment that passes through a 62.5- $\mu\text{m}$  sieve, expressed as a percentage of the total sample analyzed (PSEP 1986). This value can also be derived from the sum of the silt and clay fractions of the sample when determined by conventional particle-size determination (Plumb 1981). The reference sediment grain size should have a percent fines value within 20% of the corresponding test sediment to which it is being compared to (WDOE 2021).

Wet-sieve grain size results for the reference sediments was conducted in the field (at the time of collection) and confirmed in the laboratory upon receipt. The percent-fines determination of the project sediments is summarized in Table 2-1.

**Table 2-1. Sample and Reference Grain Size Comparison**

Sample ID	Laboratory ID	Compared To	Percent Fines
CARR18-23-REF	P230510.02		18
CARR40-23-REF	P230510.03		40
CARR62-23-REF	P230510.04		62
SED-07G:0-10	P230324.01	CARR18-23-REF	12
SED-11G:0-10	P230324.02		26
SED-01G:0-10	P230324.06	CARR40-23-REF	34
SED-27G:0-10	P230324.08		44
SED-15G:0-10	P230324.04		50
SED-03G:0-10	P230324.05		50
SED-14G:0-10	P230324.03	CARR62-23-REF	58
SED-05G:0-10	P230324.07		64

Test samples were compared to each reference collected from Carr Inlet for the purposes of evaluating the sediment under the sediment management standards. The percent fines of the reference sediment were within the ≤20% objective for the project sample.

Station coordinates for the reference sample are summarized in Table 2-2.

**Table 2-2. Reference Station Coordinates**

Station	Sample ID	Latitude	Longitude
CARR	CARR40-23-REF	47.33300	-122.66409
New Site	CARR18-23-REF	47.33642	-122.66318
New Site 2	CARR62-23-REF	47.33366	-122.66342

## 2.3 Bulk Sample Porewater Ammonia and Water Quality Measurements

Prior to testing, bulk sediment porewater ammonia concentrations and salinity were measured to determine whether any methods modifications or supplemental testing would be required (Table 2-3). Bulk sediments are homogenized test samples that have not been further processed for bioassay testing. The bulk porewater analysis represents a potential worst-case scenario for ammonia and sulfide exposure. In the actual solid phase test exposures, the sediment is established with overlying water (175mL sediment/775mL overlying water) and aeration and allowed to acclimate until test initiation on the subsequent day. A different setup is prepared for the sediment larval test that includes 18g sediment and 900mL of overlying water that is agitated for 10 seconds and allowed to settle for four hours before test initiation. Test mockups were performed for both the solid phase and larval sediment test to get a better idea of ammonia and sulfide levels in the test exposures for those samples with ammonia and/or sulfide exceedances in the bulk sediment. The results of the pretest sediment analyses are summarized in Table 2-3.

Table 2-3: Bulk and Mock Sediment Porewater Measurements

Sample ID	Sample Type	Total Ammonia <sup>1,3</sup> (mg/L)	Unionized Ammonia <sup>1,3</sup> (mg/L)	Total Sulfide (µg/L as S)	Hydrogen Sulfide <sup>2</sup> (mg/L as H <sub>2</sub> S)	Salinity	pH
SED-01G: 0-10	Bulk Sediment	7.72	<b>0.069 - 0.093</b>	ND	ND	31	7.5
SED-03G: 0-10		3.39	<b>0.030 - 0.041</b>	ND	ND	31	7.5
SED-05G: 0-10		5.99	<b>0.043 - 0.058</b>	7	0.0013 – 0.0014	31	7.4
SED-07G: 0-10		29.1	<b>0.517 - 0.692</b>	26	0.0021 – 0.0024	31	7.8
SED-11G: 0-10		23.6	<b>0.334 - 0.448</b>	ND	ND	30	7.7
SED-14G: 0-10		9.94	<b>0.089 - 0.120</b>	ND	ND	31	7.5
SED-15G: 0-10		6.46	<b>0.046 - 0.062</b>	18	<b>0.0032 – 0.0037</b>	31	7.4
SED-27G: 0-10		12.4	<b>0.140 - 0.188</b>	25	<b>0.0030 – 0.0035</b>	31	7.6
SED-07G: 0-10	Mock Solid Phase - Porewater	7.17	0.069			29	7.4
SED-01G: 0-10	Mock Larval	0.00	0.000			28	7.9
SED-03G: 0-10		0.00	0.000			28	7.9
SED-05G: 0-10		0.00	0.000			28	7.8
SED-07G: 0-10		0.108	0.002			28	7.9
SED-11G: 0-10		0.185	0.004			28	7.9
SED-14G: 0-10		0.00	0.000			28	7.9
SED-15G: 0-10		0.00	0.000	13	0.0010	28	7.9
SED-27G: 0-10		0.098	0.002	ND	ND	28	7.8

<sup>1</sup> Unionized Ammonia Purging triggers: >0.04 mg/L for larval, >0.8 mg/L *Eoh* and >0.46 mg/L *Neanthes* (Inouye et al. 2015)

<sup>2</sup> Hydrogen Sulfide Purging triggers: >0.0025 mg/L larval, >0.122 mg/L *Eoh* and >3.4 mg/L *Neanthes* (Inouye et al. 2015)

<sup>3</sup> Estimated values at test temperatures of 16 and 20°C

ND= not detected

**Bold** values exceed purging triggers for larval sediment test

**Bold and Italicized** values exceed purging triggers for larval and *Neanthes* test

Potential effects-based concentrations summarized in Table 2-4 were used to estimate which sample(s) may have sufficiently elevated ammonia and/or sulfides present to produce negative biological effects in the target test organisms. Bulk porewater ammonia exceeded trigger values in all samples for the larval test but only SED-07G for the polychaete test. No amphipod trigger values were exceeded. Two samples, SED-15G and SED-27G, exceeded hydrogen sulfide values for the larval tests in the bulk porewater (Inouye, Hoffman, & Fox, 2015), but there were no exceedances of the polychaete or amphipod trigger values. A test mock-up was performed on the samples with exceedances to determine whether ammonia and sulfide levels in the test exposure would also exceed threshold values. Based on measurements gathered from the mock test chambers, all measurements were below trigger values and no purging was necessary.



**Table 2-4. Threshold Ammonia Concentrations for Benthic Test Species**

Parameter	Amphipod ( <i>Eohaustorius estuarius</i> )	Polychaete	Larval (Bivalve)
Total Ammonia	<60 mg/L <sup>1</sup> 75.1 mg/L <sup>2</sup>	129.4 mg/L <sup>2</sup>	3.7 mg/L <sup>2</sup>
Unionized Ammonia	0.8 mg/L <sup>3</sup> Purging Trigger	0.46 <sup>3</sup> mg/L Purging Trigger	0.04 mg/L <sup>3</sup> Purging Trigger
Hydrogen Sulfide	0.122 <sup>3</sup> mg/L Purging Trigger	3.4 mg/L <sup>3</sup> Purging Trigger	0.0025 mg/L <sup>3</sup> Purging Trigger

<sup>1</sup> EPA 1994

<sup>2</sup> Mean NOEC (Internal Lab Derived)

<sup>3</sup> Inouye et al. 2015

In addition to standard water quality testing, ammonia and total sulfides was measured on surrogate chambers for each test sample, as indicated in Table 2-5.

**Table 2-5. Ammonia and Sulfide Analysis Schedule**

Bioassay	Overlying water	Porewater
Amphipod	Days 0, 10	Days 0 and 10
<i>Neanthes</i>	Days 0, 20	Days 0 and 20
Larval	Days 0, Final	Not Applicable

## 2.4 10-day Amphipod Bioassay

The 10-day amphipod acute toxicity test was conducted with *Eohaustorius estuarius*. The test was conducted with organisms supplied by Northwest Amphipod in Newport, Oregon. The test organisms were held in native sediment from Yaquina Bay, Oregon. They were acclimated to then held at  $15 \pm 2^\circ\text{C}$  prior to test initiation. The native sediment was also used as a control treatment sediment in the test. This matrix has been used successfully in prior tests with this species and is known to support positive organism health and survival.

The amphipod bioassay was conducted as 10-day static exposures with five replicates for each test treatment, reference treatment, and control. Two centimeters of sediment (approximately 175 mL) were placed into each 1-L glass chamber with 775 mL of overlying water. Trickle-flow aeration was provided through glass pipettes, and care was taken to avoid disturbing the sediment surface. Test chambers were placed into randomly assigned positions and allowed to equilibrate to test conditions overnight.

Prior to the test initiation, water quality measurements were taken in a surrogate replicate for each test treatment and included dissolved oxygen, temperature, salinity, and pH. Ammonia and sulfide concentrations were measured in both porewater and overlying water following the schedule in Table 2-5. These measurements were made from a sacrificial surrogate chamber for each test treatment. Sediment porewater was extracted via centrifugation. During the test, water quality was monitored daily in one surrogate replicate per treatment. All water quality instruments were calibrated daily or on their recommended schedule. Records of instrument calibration were retained in the laboratory logs.

To initiate the test, organisms were randomly allocated to each of the test chambers. Initial stocking densities were 20 organisms per test chamber. Amphipods that did not bury within approximately one hour were replaced with healthy amphipods. No food was provided during the 10-day exposure for the amphipod test.

At test termination, sediment from each test chamber was sieved through a 0.5-mm screen to recover all organisms. The number of surviving and dead amphipods was then enumerated.

## 2.5 20-day Juvenile Polychaete Bioassay

The 20-day polychaete survival and growth test was conducted with juvenile polychaete worms (*Neanthes arenaceodentata*). Test organisms were obtained from Aquatic Toxicology Support in Bremerton, Washington and held in seawater at 20°C (*Neanthes* were cultured in water-only and were not held in sediment prior to testing). Native sediment of the amphipod *Eohaustorius estuarius* from Yaquina Bay, Oregon was provided by Northwestern Aquatic Sciences for use as the control treatment sediment for the test. This coarse sand control sediment has been routinely tested in conjunction with this species at this laboratory and results from historical testing have demonstrated acceptable organism health and sediment quality.

The polychaete bioassay was conducted as a 20-day static-renewal test, with overlying exchanges of 300 mL of water occurring every third day. Each test treatment, reference treatment, and control consisted of five replicates of 1-L glass chambers, which were filled with two centimeters of sediment (approximately 175 mL) and 775 mL of overlying water. Trickle-flow aeration was provided through glass pipettes, and care was taken to avoid disturbing the sediment surface. Test chambers were then randomly assigned positions and allowed to equilibrate to test conditions overnight.

Prior to the test initiation, water quality measurements were taken in a surrogate chamber for each test treatment and included dissolved oxygen, temperature, salinity, and pH. Ammonia and sulfide concentrations were measured in both porewater and overlying water following the schedule in Table 2-5. These measurements were made from a sacrificial surrogate chamber for each test treatment. Sediment porewater was extracted via centrifugation. During the test, water quality was monitored daily in one surrogate replicate per treatment. All water quality instruments were calibrated daily or on their recommended schedule. Records of instrument calibration were retained in the laboratory logs.

To initiate the test, organisms were randomly allocated to each of the test chambers. Initial stocking densities were five worms per test chamber. During the test, organisms were fed a diet of 40-mg of TetraMin® slurry every other day (approximately 8-mg dry weight per worm). Pre-test initial biomass was determined by taking dry weight and ash-free dry weight (AFDW) measurements of three replicates of five worms each on Day 0.

At test termination, sediment from each test chamber was sieved through a 0.5-mm screen. All worms were recovered, enumerated, rinsed in deionized water (to remove salt), and transferred to pre-weighed aluminum foil weigh boats. After drying in an oven at approximately 60°C for at least 24 hours, each weigh-boat was removed, cooled in a desiccator and weighed to obtain dry weight measurements. They were then heated to 550°C for two hours to determine the ashed weight. AFDW were calculated to correct for the influence of sediment grain size differences between treatments:

$$\text{AFDW} = \text{Dry weight} - \text{Ashed weight}$$

Both dry weight and AFDW were used to determine individual worm weight and growth rates. The dry weight growth rate is calculated using the following equation:

$$G = (\text{DWT}_2 - \text{DWT}_1) \div (t_2 - t_1)$$

Where:  $\text{DWT}_2$  = individual dry weight of surviving adults at test termination  
 $\text{DWT}_1$  = mean individual dry weight of organisms at test initiation  
 $t_2 - t_1$  = duration of test (e.g. days)

The AFDW growth rate is calculated using the following equations:

$$\text{AFDW} = (\text{Final Dry Weight} - \text{Final Ashed Weight}) \div \# \text{Survivors}$$

$$G = (\text{AFDW}_{t_2} - \text{AFDW}_{t_1}) \div (t_2 - t_1)$$

Where:  $\text{AFDW}_{t_2}$  = individual ash-free dry weight of surviving adults at test termination

$\text{AFDW}_{t_1}$  = mean individual ash-free dry weight of organisms at test initiation

$t_2 - t_1$  = duration of test (e.g. days)

## 2.6 Larval Developmental Bioassay

The bivalve larval development test was conducted with the mussel, *Mytilus galloprovincialis*. Adult organisms were obtained from Taylor Shellfish in Shelton, Washington and were held under flowing natural seawater at  $12 \pm 2^\circ\text{C}$  prior to spawning induction. Adult mussels were fed during the holding period a marine algal suspension ad libitum. The control treatment consisted of a clean seawater-only control. The reference treatment was compared against the control to determine test acceptability criteria.

The larval development bioassay was conducted as a static exposure with five replicates for each test treatment, reference treatment, and control. Approximately 18 g ( $\pm 1$  g) of sediment was placed into each 1-L glass chamber with 900 mL of overlying water. Test chambers were then shaken for 10 seconds and placed into randomly assigned positions and allowed to settle for approximately 4 hours. The larval test was aerated throughout the test. Air was provided through glass pipettes, and care was taken to avoid disturbing the sediment surface.

Prior to the test initiation, water quality measurements were taken in the surrogate chamber for each test treatment and included dissolved oxygen, temperature, salinity, and pH. Ammonia and sulfide concentrations were measured in the overlying water following the schedule in Table 2-5. These measurements were made from a sacrificial surrogate chamber for each test treatment. During the test, water quality was monitored daily in one surrogate replicate per treatment. All water quality instruments were calibrated daily or on their recommended schedule. Records of instrument calibration were retained in the laboratory logs.

To obtain gametes for testing, adult mussels to be spawned were placed in clean seawater at  $16^\circ\text{C}$  (culture temperature) for approximately 30 – 60 minutes in the presence of dense marine algal suspension. The mussels were then transferred to containers with culture water adjusted to  $20 - 23^\circ\text{C}$  for the purpose of inducing the release of gametes. The animals were held at the shocking temperature and were monitored for spawning individuals. Spawning females and males were removed from the water bath and placed in individual containers with seawater. These individuals were allowed to spawn until sufficient gametes were available to initiate the test. After the spawning period, eggs were transferred to fresh seawater and filtered through a 0.5 mm Nitex® mesh screen to remove large debris, feces, and excess gonadal matter. A composite was made of the sperm and diluted with fresh seawater. The fertilization process was initiated by adding sperm to the isolated egg containers. Egg-sperm solutions were periodically homogenized with a perforated plunger during the fertilization process and subsamples observed under the microscope for egg and sperm viability. Approximately one to one and a half hours after fertilization, embryo solutions were checked for fertilization rate. Only those embryo stocks with  $>90\%$  fertilization were used to initiate the tests. Embryo solutions were rinsed free of excess sperm and then combined to create one embryo stock solution. Density of the embryo stock solution was determined by counting the number of embryos in a subsample of homogenized stock solution. This was used to determine the volume of embryo stock solution to deliver approximately 27,000 embryos to each test chamber.

Though not strictly necessary as flocculent material was not listed as a concern in the SAP, all test treatments were resuspended prior to test termination to improve recovery of larvae trapped in the

debris. At approximately 40 hours from test initiation, the water, larvae and settled sediment were homogenized by gentle mixing using a perforated plunger. The contents were then allowed to settle until the test was terminated.

The protocol calls for test termination when 95% of the embryos in the control have reached the prodissoconch I stage (approximately 48-60 hours). At termination, the overlying seawater was decanted into a clean 1-L jar and mixed with a perforated plunger. From this container, a 10 mL subsample was transferred to a scintillation vial and preserved in 10% buffered formalin. Larvae were subsequently stained with a dilute solution of Rose Bengal in 70% ethanol to help visualization of larvae. The number of normal and abnormal larvae was enumerated on an inverted microscope. Normal larvae included all D-shaped prodissoconch I stage larvae. Abnormal larvae included abnormally shaped prodissoconch I larvae and all early-stage larvae.

## **2.7 Data Analysis and QA/QC**

All water quality and endpoint data were entered into Excel spreadsheets. Water quality parameters were summarized by calculating the mean, minimum, and maximum values for each test treatment. Endpoint data were calculated for each replicate and the mean values and standard deviations were determined for each test treatment.

All hand-entered data was reviewed for data entry errors, which were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting were reviewed for errors. Review counts were conducted on any apparent outliers.

For Sediment Cleanup Objective (SCO) and Cleanup Screening Level (CSL) suitability determinations, comparisons were made according to the Sediment Management Standards (SMS; WDOE 2019) and Fox et al. (1998), using BioStat software. Data reported as percent mortality were transformed using an arcsine square root transformation prior to statistical analysis. All data were tested for normality using the Wilk-Shapiro test and equality of variance using Levene's test. Growth data with unequal variance were log<sub>10</sub> transformed prior to analysis. Determinations of statistical significance were based on one-tailed Student's t-tests with an alpha of 0.05. A comparison of the larval endpoint relative to the reference was made using an alpha level of 0.10. For samples failing to meet assumptions of normality, a Mann-Whitney test was conducted to determine significance. For those samples failing to meet the assumptions of normality and equality of variance, a t-test on rankits was used.

To evaluate the relative sensitivity of the organisms, reference toxicity tests were performed using standard reference toxicants (Lee 1980). A water-only reference-toxicant test was conducted concurrently with the sediment tests using ammonium chloride. The ammonium chloride reference-toxicant test was used to ensure animals used in the test were healthy and of similar sensitivity to prior tests. This test also provided information on the sensitivity to ammonia concentrations that would possibly be present in the sediments.

Statistical analyses of all dose-response tests were performed using CETIS Comprehensive Toxicity Data Analysis and Database Software version 2.1.4.3. Comparisons between the lab control and each test concentration were performed following recommended USEPA decision matrices (USEPA 2002).

### 3. RESULTS

The results of the sediment testing, including a summary of test results and water quality observations are presented in this section. All data, laboratory bench sheets, and statistical analyses are provided in Appendix A while chain of custody forms and pre-test documents are supplied in Appendix B.

#### 3.1 10-day Amphipod Bioassay

The bioassay test with *E. estuarius* had 16% mortality in the native sediment control, which did not meet the performance criterion of  $\leq 10\%$  mortality for SMS evaluations. Mean mortality for CARR18-23-REF was 14% and for both CARR40-23-REF and CARR62-23-REF the mean mortality was 15%, which met the performance criteria ( $\leq 25\%$  mortality SMS) and indicated that the reference sediment was acceptable for suitability determination. Insufficient time was available to rerun the test within holding time. The results were discussed with the client and WDOE and the decision was made to not rerun the bioassay test.

All endpoint results are summarized in Table 3-1. Summaries of water quality measurements, ammonia and sulfide concentrations, and test conditions are presented in Table 3-2 through Table 3-5.

Water quality parameters were within the acceptable limits throughout the duration of the test (Table 3-2).

A reference-toxicant test (positive control) was performed on the batch of test organisms utilized for this study. The reference-toxicant test control met the test acceptability criterion and the  $LC_{50}$  value was within control chart limits for total ammonia and unionized ammonia ( $\pm 2$  standard deviations from the laboratory historical mean). Ammonia concentrations observed in the *E. estuarius* test were below the NOEC value derived from the concurrent ammonia reference-toxicant test (Table 3-3; compare to NOEC of 1.52 mg/L unionized ammonia). Therefore, ammonia concentrations within the sediment samples should not have been a contributor to any adverse biological effects if observed in the test treatments. Measured initial and final sulfide concentrations within the overlying water and porewater were below the trigger value of 0.122 mg/L hydrogen sulfide for *E. estuarius* (Inouye et al. 2015).

**Table 3-1. Test Results for *Eohaustorius estuarius***

Treatment	Replicate	Number Initiated	Number Surviving	Number Missing or Dead	Percentage Mortality	Mean Percentage Mortality	SD
Control	1	20	17	3	15	16.0	5.5
	2	20	15	5	25		
	3	20	17	3	15		
	4	20	18	2	10		
	5	20	17	3	15		
CARR18-23-REF	1	20	18	2	10	14.0	8.9
	2	20	18	2	10		
	3	20	18	2	10		
	4	20	14	6	30		
	5	20	18	2	10		

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Treatment	Replicate	Number Initiated	Number Surviving	Number Missing or Dead	Percentage Mortality	Mean Percentage Mortality	SD
CARR40-23-REF	1	20	15	5	25	15.0	7.1
	2	20	18	2	10		
	3	20	18	2	10		
	4	20	18	2	10		
	5	20	16	4	20		
CARR62-23-REF	1	20	15	5	25	15.0	9.4
	2	20	19	1	5		
	3	20	15	5	25		
	4	20	18	2	10		
	5	20	18	2	10		
SED-07G:0-10	1	20	15	5	25	22.0	14.0
	2	20	13	7	35		
	3	20	13	7	35		
	4	20	18	2	10		
	5	20	19	1	5		
SED-11G:0-10	1	20	13	7	35	18.0	10.4
	2	20	16	4	20		
	3	20	17	3	15		
	4	20	18	2	10		
	5	20	18	2	10		
SED-14G:0-10	1	20	18	2	10	20.0	7.9
	2	20	16	4	20		
	3	20	14	6	30		
	4	20	17	3	15		
	5	20	15	5	25		
SED-15G:0-10	1	20	15	5	25	13.0	10.4
	2	20	19	1	5		
	3	20	16	4	20		
	4	20	17	3	15		
	5	20	20	0	0		
SED-03G:0-10	1	20	16	4	20	28.0	13.0
	2	20	14	6	30		
	3	20	12	8	40		
	4	20	12	8	40		
	5	20	18	2	10		
SED-01G:0-10	1	20	19	1	5	13.0	9.1
	2	20	15	5	25		
	3	20	19	1	5		
	4	20	16	4	20		
	5	20	18	2	10		

Treatment	Replicate	Number Initiated	Number Surviving	Number Missing or Dead	Percentage Mortality	Mean Percentage Mortality	SD
SED-05G:0-10	1	20	17	3	15	18.0	4.5
	2	20	17	3	15		
	3	20	16	4	20		
	4	20	15	5	25		
	5	20	17	3	15		
SED-27G:0-10	1	20	17	3	15	19.0	7.4
	2	20	16	4	20		
	3	20	16	4	20		
	4	20	18	2	10		
	5	20	14	6	30		

Table 3-2. Water Quality Summary for *Eohaustorius estuarius*

Treatment	Dissolved Oxygen (mg/L) ≥5.1 mg/L			Temperature (°C) 15 ± 1°C			Salinity (ppt) 28 ± 1 ppt			pH 7 - 9 units		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	8.3	8.2	8.4	15.6	15.4	15.9	28	28	28	8.1	8.0	8.1
CARR18-23-REF	8.2	7.8	8.3	15.6	15.3	16.4	28	28	28	8.1	7.9	8.2
CARR40-23-REF	8.2	8.0	8.3	15.8	15.5	16.0	28	28	28	8.2	8.0	8.4
CARR62-23-REF	8.2	8.1	8.4	15.6	15.3	16.0	28	28	28	8.0	8.0	8.1
SED-07G:0-10	8.2	7.9	8.3	15.7	15.5	16.1	28	28	28	8.2	8.0	8.3
SED-11G:0-10	8.2	8.1	8.3	15.6	15.4	16.0	28	28	28	8.3	8.1	8.5
SED-14G:0-10	8.2	8.1	8.3	15.6	15.4	15.9	28	28	28	8.2	8.0	8.3
SED-15G:0-10	8.2	8.1	8.3	15.6	15.3	16.2	28	28	28	8.1	7.9	8.2
SED-03G:0-10	8.1	7.9	8.2	15.9	15.6	16.2	28	28	28	8.0	7.9	8.2
SED-01G:0-10	8.2	8.1	8.3	15.7	15.4	15.9	28	28	28	8.1	8.0	8.3
SED-05G:0-10	8.2	7.9	8.3	15.6	15.4	15.8	28	28	29	8.2	8.0	8.3
SED-27G:0-10	8.2	8.1	8.3	15.6	15.4	15.8	28	28	28	8.2	8.0	8.3

Table 3-3. Ammonia Summary for *Eohaustorius estuarius*

Treatment	Overlying				Porewater			
	Total Ammonia (mg/L Total) NOEC = 89.1 mg/L <sup>1</sup>		Unionized Ammonia (mg/L) NOEC = 1.52 <sup>1</sup> Trigger Value = 0.8 mg/L <sup>2</sup>		Total Ammonia (mg/L Total) NOEC = 89.1 mg/L <sup>1</sup>		Unionized Ammonia (mg/L) NOEC = 1.52 <sup>1</sup> Trigger Value = 0.8 mg/L <sup>2</sup>	
	Day		Day		Day		Day	
	0	10	0	10	0	10	0	10
Control	0.00	0.353	0.000	0.012	0.0415	0.00	0.000	0.000
CARR18-23-REF	0.690	0.190	0.015	0.008	3.28	2.49	0.028	0.022
CARR40-23-REF	0.587	0.00	0.016	0.000	2.61	3.38	0.028	0.047
CARR62-23-REF	0.00	0.00	0.000	0.000	1.18	0.648	0.010	0.009
SED-07G:0-10	3.87	5.17	0.104	0.278	14.0	8.47	0.152	0.119
SED-11G:0-10	2.75	8.65	0.092	0.714	8.56	21.7	0.116	0.601
SED-14G:0-10	1.39	2.97	0.037	0.157	2.92	5.87	0.025	0.052
SED-15G:0-10	0.536	0.607	0.011	0.026	1.83	1.70	0.010	0.019
SED-03G:0-10	0.0857	0.00	0.002	0.000	1.02	0.437	0.007	0.005
SED-01G:0-10	0.335	0.173	0.009	0.009	1.76	0.788	0.012	0.014
SED-05G:0-10	0.372	0.206	0.010	0.011	2.30	0.353	0.020	0.003
SED-27G:0-10	0.992	2.23	0.026	0.117	3.85	4.23	0.033	0.037

<sup>1</sup>NOEC derived from concurrent reference-toxicant test;

<sup>2</sup>Inouye et al. 2015.



**Table 3-4. Sulfide Summary for *Eohaustorius estuarius***

Treatment	Overlying				Porewater			
	Total Sulfides (mg/L)		Hydrogen Sulfide (mg/L) Trigger Value = 0.122 mg/L <sup>1</sup>		Total Sulfides (mg/L)		Hydrogen Sulfide (mg/L) Trigger Value = 0.122 mg/L <sup>1</sup>	
	Day		Day		Day		Day	
	0	10	0	10	0	10	0	10
Control	0.013	ND	0.0008	ND	0.002	ND	0.0004	ND
CARR18-23-REF	0.019	ND	0.0015	ND	0.020	0.033	0.0035	0.0056
CARR40-23-REF	ND	0.002	ND	0.0001	ND	NM	ND	NM
CARR62-23-REF	ND	0.000	ND	0.0000	ND	0.004	ND	0.0005
SED-07G:0-10	0.009	0.003	0.0006	0.0001	0.026	ND	0.0037	ND
SED-11G:0-10	ND	ND	ND	ND	0.002	0.009	0.0002	0.0005
SED-14G:0-10	ND	0.004	ND	0.0001	0.205	0.007	0.0354	0.0012
SED-15G:0-10	ND	0.000	ND	0.0000	0.010	0.008	0.0025	0.0011
SED-03G:0-10	ND	0.002	ND	0.0001	0.011	ND	0.0023	ND
SED-01G:0-10	ND	ND	ND	ND	0.015	ND	0.0031	ND
SED-05G:0-10	ND	ND	ND	ND	0.081	0.000	0.0140	0.0000
SED-27G:0-10	ND	0.001	ND	0.0000	ND	ND	ND	ND

<sup>1</sup>Inouye et al. 2015. Trigger value is for porewater water, overlying value not specified for *Eohaustorius*

NM = not measured; insufficient volume for analysis

ND = not detected; measurement below detection limit

**Table 3-5. Test Condition Summary for *Eohaustorius estuarius***

Test Conditions: PSEP <i>E. estuarius</i>		
Date Sampled	March 20, 21, and 23, 2023 (test samples) May 10, 2023 (reference samples)	
Date Received	March 24, 2023 (test samples) May 10, 2023 (reference samples)	
Test Dates	May 12 – 22, 2023	
Sample Storage Conditions	4°C, dark	
Days of Holding Recommended: ≤8 weeks (56 days)	53 Days (test samples) 2 Days (reference samples)	
Source of Control Sediment	Yaquina Bay, OR	
Test Species	<i>Eohaustorius estuarius</i>	
Supplier	Northwest Amphipod, Newport, OR	
Date Acquired	May 10, 2023	
Age Class	Immature: 3 – 5 mm	
Test Procedures	PSEP 1995 with SMARM revisions, SOP No. SED002.11	
Test Location	EcoAnalysts Port Gamble Laboratory	
Test Type/Duration	10-Day static	
Control Water	North Hood Canal seawater, 0.45µm filtered	
Test Lighting	50 – 100 foot candles (ambient and constant)	
Test Chamber	1-Liter Glass Chamber	
Replicates per Treatment	5 + 2 surrogates (one used for WQ measurements throughout the test)	
Organisms per Replicate	20	
Exposure Volume	175 mL sediment/ 775 mL water	
Feeding	None	
Water Renewal	None	
Test Dissolved Oxygen	Recommended: ≥ 5.1 mg/L	Observed: 7.8 – 8.4 mg/L
Test Temperature	Recommended: 15 ± 1 °C	Observed: 15.3 – 16.4°C
Test Salinity	Recommended: 28 ± 1 ppt	Observed: 28 – 29 ppt
Test pH	Recommended: 7 - 9	Observed: 7.9 – 8.5
Control Performance Standard	Recommended: Control ≤ 10% mortality (SMS)	Observed: 16% mortality; Fail
Reference Performance Standard	Recommended: Reference ≤ 25% mortality (SMS)	Observed: CARR18-23-REF, 14%; Pass CARR40-23-REF, 15%; Pass CARR62-23-REF, 15%; Pass
Reference Toxicant LC <sub>50</sub> (total ammonia)	LC <sub>50</sub> = 124.5 mg/L	
Mean; Acceptable Range (total ammonia)	161.3; 68.4 – 380 mg/L	
NOEC (total ammonia)	89.1 mg/L	
NOEC (unionized ammonia)	1.52 mg /L	
Deviations from Test Protocol	Control mortality	

### 3.2 20-day Juvenile Polychaete Bioassay

The bioassay test with *N. arenaceodentata* was validated with 0% mortality in the control, meeting the test survival acceptability criterion of  $\leq 10\%$  (WDOE 2021). Mean individual growth rate (MIG) in the control was 1.592 mg/ind/day (dry weight) and 1.251 mg/ind/day (AFDW), meeting the test acceptability criterion of  $\geq 0.38$  mg/ind/day AFDW (WDOE 2021). Mean mortality in all reference treatments was 0%. Mean individual growth rate for the reference samples was between 1.702 – 1.998 mg/ind/day (dry weight) and 1.412 – 1.648 mg/ind/day (AFDW). When compared to the control, MIG expressed as dry weight for the references was between 1.07 and 1.26 mg/ind/day. The MIG expressed as AFDW when comparing to the control to the references was between 1.13 and 1.32 mg/ind/day. These ratios met the reference performance standard of  $\geq 0.80$  (WDOE 2021). A summary of the test results for all samples is shown in Table 3-6. Summaries of water quality measurements, ammonia and sulfide concentrations, and test conditions are presented in Table 3-7 through Table 3-10.

Mortality in the project sediment was 0%. Mean individual growth (as dry weight) in the test treatments ranged between 0.885 and 1.660 mg/ind/day. Mean individual growth in the AFDW assessment, which removes variability caused by gut contents, ranged between 0.737 and 1.343 mg/ind/day as AFDW.

Water quality parameters were within the acceptable limits throughout the duration of the test (Table 3-7). Initial mean individual biomass (pretest) of the test organisms met the minimum required growth criterion of 0.25 – 1.0 mg/individual at 1.223 mg/ind dry weight and 0.529 mg/ind AFDW (Kendall 1996). Replicate 5 of reference CARR40-23-REF was inadvertently stocked with 6 animals. The organism count was adjusted for statistical analysis. This deviation should not affect the test results.

A reference-toxicant test (positive control) was performed on the batch of test organisms utilized for this study. The reference-toxicant test control met the test acceptability criterion and the  $LC_{50}$  value was within control chart limits for total ammonia and unionized ammonia ( $\pm 2$  standard deviations from the laboratory historical mean). Ammonia concentrations observed in the *N. arenaceodentata* test were below the NOEC value derived from the concurrent ammonia reference-toxicant test (Table 3-8; compare to NOEC of 2.36 mg/L unionized ammonia). Sulfide concentrations were below the trigger value of 3.4 mg/L hydrogen sulfide (Inouye et al. 2015) (Table 3-9). Neither ammonia nor sulfides contributed to mortality in the test treatment.

Table 3-6. Test Results for *Neanthes arenaceodentata*

Treatment	Rep	Number Initiated	Survivors	Mean Mortality (%)	Individual Growth Rate (mg/ind/day)					
					Dry Weight	Mean	SD	AFDW	Mean	SD
Control	1	5	5	0	1.180	1.592	0.27	0.876	1.251	0.248
	2	5	5		1.531			1.136		
	3	5	5		1.778			1.401		
	4	5	5		1.593			1.343		
	5	5	5		1.876			1.499		
CARR18-23-REF	1	5	5	0	1.421	1.702	0.41	1.207	1.412	0.410
	2	5	5		1.854			1.652		
	3	5	5		2.338			1.992		
	4	5	5		1.315			0.949		
	5	5	5		1.582			1.262		
CARR40-23-REF	1	5	5	0	1.767	1.828	0.21	1.442	1.523	0.131
	2	5	5		1.619			1.389		
	3	5	5		1.882			1.613		
	4	5	5		1.703			1.469		
	5	6	6		2.169			1.703		
CARR62-23-REF	1	5	5	0	2.162	1.998	0.29	1.770	1.648	0.283
	2	5	5		2.191			1.803		
	3	5	5		2.190			1.871		
	4	5	5		1.921			1.631		
	5	5	5		1.524			1.168		
SED-07G:0-10	1	5	5	0	1.521	1.423	0.12	1.351	1.208	0.133
	2	5	5		1.483			1.174		
	3	5	5		1.361			1.130		
	4	5	5		1.505			1.339		
	5	5	5		1.246			1.047		
SED-11G:0-10	1	5	5	0	0.726	0.885	0.28	0.562	0.737	0.258
	2	5	5		1.054			0.906		
	3	5	5		1.128			0.971		
	4	5	5		1.053			0.873		
	5	5	5		0.465			0.372		
SED-14G:0-10	1	5	5	0	0.996	1.370	0.34	0.697	1.139	0.346
	2	5	5		1.407			1.169		
	3	5	5		1.270			1.154		
	4	5	5		1.922			1.658		
	5	5	5		1.257			1.017		

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Treatment	Rep	Number Initiated	Survivors	Mean Mortality (%)	Individual Growth Rate (mg/ind/day)					
					Dry Weight	Mean	SD	AFDW	Mean	SD
SED-15G:0-10	1	5	5	0	0.834	1.556	0.60	0.611	1.280	0.574
	2	5	5		1.839			1.471		
	3	5	5		1.787			1.533		
	4	5	5		2.277			2.004		
	5	5	5		1.045			0.783		
SED-03G:0-10	1	5	5	0	1.388	1.413	0.30	1.174	1.174	0.302
	2	5	5		1.087			0.812		
	3	5	5		1.153			0.939		
	4	5	5		1.648			1.464		
	5	5	5		1.789			1.482		
SED-01G:0-10	1	5	5	0	1.476	1.660	0.33	1.214	1.343	0.338
	2	5	5		1.740			1.437		
	3	5	5		1.198			0.831		
	4	5	5		2.023			1.713		
	5	5	5		1.864			1.521		
SED-05G:0-10	1	5	5	0	1.807	1.519	0.32	1.579	1.274	0.372
	2	5	5		1.533			1.274		
	3	5	5		1.189			0.824		
	4	5	5		1.860			1.699		
	5	5	5		1.204			0.993		
SED-27G:0-10	1	5	5	0	0.808	1.261	0.46	0.602	1.035	0.435
	2	5	5		0.761			0.558		
	3	5	5		1.474			1.210		
	4	5	5		1.815			1.542		
	5	5	5		1.450			1.264		

**Table 3-7. Water Quality Summary for *Neanthes arenaceodentata***

Treatment	Dissolved Oxygen (mg/L) ≥4.6 mg/L			Temperature (°C) 20 ± 1°C			Salinity (ppt) 28 ± 2 ppt			pH 7 - 9 units		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Control	7.3	7.0	7.7	20.6	19.3	21.2	28	28	28	7.9	7.8	8.0
CARR18-23-REF	7.3	6.9	7.6	20.5	19.2	21.3	28	28	29	8.0	7.9	8.1
CARR40-23-REF	7.1	6.9	7.8	20.5	19.2	21.4	28	28	29	8.0	7.8	8.3
CARR62-23-REF	7.3	7.1	7.6	20.4	19.3	21.1	28	28	29	8.0	7.9	8.2
SED-07G:0-10	7.2	6.9	7.6	20.5	19.2	21.3	28	28	29	8.1	8.0	8.2
SED-11G:0-10	7.3	7.0	7.6	20.4	19.3	21.2	28	28	29	8.2	8.0	8.4
SED-14G:0-10	7.3	6.9	7.7	20.4	19.3	21.2	28	28	29	8.0	7.9	8.2
SED-15G:0-10	7.1	6.8	7.6	20.6	19.2	21.4	28	28	29	8.0	7.8	8.1
SED-03G:0-10	7.4	7.1	7.6	20.5	19.4	21.2	28	28	29	8.1	7.9	8.2
SED-01G:0-10	7.2	6.8	7.6	20.5	19.3	21.1	28	28	29	8.0	7.9	8.2
SED-05G:0-10	7.4	7.0	7.6	20.5	19.4	21.2	28	28	29	8.2	7.9	8.3
SED-27G:0-10	7.3	7.1	7.6	20.5	19.3	21.1	29	28	29	8.1	7.9	8.2

Table 3-8. Ammonia Summary for *Neanthes arenaceodentata*

Treatment	Overlying				Porewater			
	Total Ammonia (mg/L Total) NOEC = 130 mg/L <sup>1</sup>		Unionized Ammonia (mg/L) NOEC = 2.36 <sup>1</sup> Trigger Value = 0.46 mg/L <sup>2</sup>		Total Ammonia (mg/L Total) NOEC = 130 mg/L <sup>1</sup>		Unionized Ammonia (mg/L) NOEC = 2.36 <sup>1</sup> Trigger Value = 0.46 mg/L <sup>2</sup>	
	Day		Day		Day		Day	
	0	20	0	20	0	20	0	20
Control	0.00	1.40	0.000	0.041	0.0415	1.62	0.000	0.015
CARR18-23-REF	0.690	0.0926	0.040	0.003	3.28	0.395	0.040	0.002
CARR40-23-REF	0.587	0.139	0.040	0.005	2.61	1.61	0.040	0.024
CARR62-23-REF	0.00	0.0591	0.014	0.002	1.18	0.277	0.014	0.002
SED-07G:0-10	3.87	0.0467	0.217	0.002	14.0	1.67	0.217	0.010
SED-11G:0-10	2.75	5.78	0.165	0.329	8.56	6.82	0.165	0.065
SED-14G:0-10	1.39	0.140	0.035	0.005	2.92	3.27	0.035	0.020
SED-15G:0-10	0.536	0.153	0.014	0.005	1.83	1.17	0.014	0.004
SED-03G:0-10	0.0857	0.0238	0.010	0.001	1.02	0.894	0.010	0.007
SED-01G:0-10	0.335	0.0530	0.017	0.002	1.76	2.28	0.017	0.014
SED-05G:0-10	0.372	0.0202	0.028	0.001	2.30	0.734	0.028	0.003
SED-27G:0-10	0.992	0.0712	0.047	0.003	3.85	1.25	0.047	0.007

<sup>1</sup>NOEC (concurrent reference-toxicant test derived); <sup>2</sup>Inouye et al. 2015.

**Table 3-9. Sulfide Summary for *Neanthes arenaceodentata***

Treatment	Overlying				Porewater			
	Total Sulfides (mg/L Total)		Hydrogen Sulfide (mg/L) Trigger Value = 3.4 mg/L <sup>1</sup>		Total Sulfides (mg/L Total)		Hydrogen Sulfide (mg/L) Trigger Value = 3.4 mg/L <sup>1</sup>	
	Day		Day		Day		Day	
	0	20	0	20	0	20	0	20
Control	0.013	0.001	0.001	0.000	0.002	0.005	0.000	0.001
CARR18-23-REF	0.019	0.003	0.001	0.000	0.020	0.000	0.003	0.000
CARR40-23-REF	ND	ND	ND	ND	ND	0.012	ND	0.001
CARR62-23-REF	ND	0.001	ND	0.000	ND	0.006	ND	0.002
SED-07G:0-10	0.009	0.007	0.000	0.000	0.026	0.014	0.003	0.004
SED-11G:0-10	ND	0.003	ND	0.000	0.002	ND	0.000	ND
SED-14G:0-10	ND	0.006	ND	0.000	0.205	0.058	0.031	0.015
SED-15G:0-10	ND	0.002	ND	0.000	0.010	ND	0.002	ND
SED-03G:0-10	ND	ND	ND	ND	0.011	0.016	0.002	0.004
SED-01G:0-10	ND	0.003	ND	0.000	0.015	ND	0.003	ND
SED-05G:0-10	ND	ND	ND	ND	0.081	0.031	0.012	0.011
SED-27G:0-10	ND	0.003	ND	0.000	ND	0.008	ND	0.002

<sup>1</sup>Inouye et al. 2015. Trigger value for porewater. Overlying water trigger not available.

ND = not detected; measurement below detection limit



**Table 3-10. Test Condition Summary for *Neanthes arenaceodentata***

Test Conditions: PSEP <i>N. arenaceodentata</i>		
Date Sampled	March 20, 21, and 23, 2023 (test samples) May 10, 2023 (reference samples)	
Date Received	March 24, 2023 (test samples) May 10, 2023 (reference samples)	
Test Dates	May 12 – June 1, 2023	
Sample Storage Conditions	4°C, dark	
Days of Holding	53 Days (test sample)	
Recommended: ≤8 weeks (56 days)	2 Days (reference sample)	
Source of Control Sediment	Yaquina Bay, OR	
Test Species	<i>Neanthes arenaceodentata</i>	
Supplier	Aquatic Toxicology Support	
Date Acquired	May 11, 2023	
Age Class	Juvenile; 2 – 3 weeks post-emergence	
Test Procedures	PSEP 1995 with SMARM revisions, SOP No. SED009.09	
Test Location	EcoAnalysts Port Gamble Laboratory	
Test Type/Duration	20-Day static renewal	
Control Water	North Hood Canal seawater, 0.45µm filtered	
Test Lighting	50 – 100 foot candles (ambient and constant)	
Test Chamber	1-Liter Glass Chamber	
Replicates per Treatment	5 + 2 surrogates (one used for WQ measurements throughout the test)	
Organisms per Replicate	5	
Exposure Volume	175 mL sediment/ 775 mL water	
Feeding	40 mg/jar every other day (8 mg/ind every other day)	
Water Renewal	Water renewed every third day (1/3 volume of exposure chamber)	
Test Dissolved Oxygen	Recommended: > 4.6 mg/L	Observed: 6.8 – 7.8 mg/L
Test Temperature	Recommended: 20 ± 1 °C	Observed: 19.2 – 21.4 °C
Test Salinity	Recommended: 28 ± 2 ppt	Observed: 28 – 29 ppt
Test pH	Recommended: 7 - 9	Observed: 7.8 – 8.4
Initial Biomass	Recommended: 0.5 - 1.0 mg Minimum: 0.25 mg	1.223 mg (dry weight) 0.529 mg (AFDW)
Control Performance Standard	Recommended: Control < 10% mortality	Observed: 0%; Pass
	Recommended: ≥ 0.38 mg/ind/day (as AFDW)	Observed: 1.251 mg/ind/day: Pass
Reference Performance Standard	Recommended: MIG <sub>Reference</sub> /MIG <sub>Control</sub> ≥ 0.80 (SMS)	Observed: 1.07 – 1.26 (dry weight), 1.13 – 1.32 (AFDW): Pass
Reference Toxicant LC <sub>50</sub> (total ammonia)	LC <sub>50</sub> = 155.1 mg/L	
Mean; Acceptable Range (total ammonia)	171.6; 122 – 241 mg/L	
NOEC (total ammonia)	130 mg/L	
NOEC (unionized ammonia)	2.36 mg/L	
Deviations from Test Protocol	CARR40-23-REF Replicate 5 stocked with 6 animals	

### 3.3 Larval Development Bioassay

The larval development test with *M. galloprovincialis* was validated by 1.02 proportion normal survivorship (defined as the mean number of normal larvae within the control divided by the stocking density) in the seawater control. These values were within the control acceptability criterion of  $\geq 0.70$  (WDOE 2021). A summary of the test results for all samples is shown in Table 3-11. Summaries of water quality measurements, ammonia and sulfide concentrations, and test conditions are presented in Table 3-12 through Table 3-14.

Mean number normal for all reference treatments ranged from 0.70 – 0.76 of the seawater control response, meeting the reference acceptability criteria ( $N_R/N_C$ ) of  $\geq 0.65$  (defined as the number of normal larvae in the reference sample divided by the number of normal larvae in the control). The test mean chamber stocking density (measured at test initiation) was 27.9 embryos/mL and was within the test objective of 20 – 40 embryos/mL.

Mean number normal for all test samples ranged from 212.8 – 229.4 and 1.06 – 1.14 of the seawater control response.

Water quality parameters were within acceptable limits throughout the test.

A reference-toxicant test (positive control) was performed on the batch of test organisms utilized for this study. The reference-toxicant test control met the test acceptability criterion and the  $EC_{50}$  value was within control chart limits ( $\pm 2$  standard deviations from the laboratory historical mean). This indicates that the test organisms used in this study were of similar sensitivity to those previously tested.

While the hydrogen sulfide for sample SED-03G:0-10 (0.0038 mg/L) was slightly above the purge trigger of 0.0025 mg/L on Day 2, no observed effects in the *M. galloprovincialis* test were seen for this sample (Table 3-13), indicating that sulfides were not a likely driver of any observed toxicity (Inouye et al. 2015). All ammonia values were below the NOEC from the concurrent reference toxicant test (0.065 mg/L unionized ammonia) and the purging trigger value of 0.04 mg/L unionized ammonia.

**Table 3-11. Test Results for *Mytilus galloprovincialis***

Treatment	Rep	Number Normal	Number Abnormal	Mean # Normal (N)	SD	Control Normal Survival N <sub>c</sub> /I	Reference Normal Survival Relative to Control N <sub>R</sub> /N <sub>c</sub>	Performance Standard
Seawater Control	1	269	21	284.0	10.5	1.02		≥0.70; Meets Criterion
	2	282	2					
	3	295	2					
	4	293	2					
	5	281	3					
CARR18-23-REF	1	219	6	200.8	27.5		vs Seawater Control: 0.71	≥0.65; Meets Criterion
	2	209	1					
	3	173	1					
	4	171	4					
	5	232	1					
CARR40-23-REF	1	226	2	216.0	26.7		vs Seawater Control: 0.76	≥0.65; Meets Criterion
	2	197	0					
	3	182	4					
	4	225	3					
	5	250	2					
CARR62-23-REF	1	187	2	199.6	28.3		vs Seawater Control: 0.70	≥0.65; Meets Criterion
	2	180	1					
	3	222	2					
	4	237	1					
	5	172	0					
SED-07G:0-10	1	231	1	216.6	12.6	See Section 4.3 for Larval Test Suitability Determination		
	2	211	9					
	3	226	10					
	4	199	2					
	5	216	3					
SED-11G:0-10	1	211	0	224.2	37.5			
	2	188	4					
	3	194	1					
	4	269	2					
	5	259	0					
SED-14G:0-10	1	211	12	212.8	15.7			
	2	209	4					
	3	232	3					
	4	222	3					
	5	190	3					

Bioassay Testing Results  
Port of Friday Harbor Remedial Investigation  
Friday Harbor, Washington

Treatment	Rep	Number Normal	Number Abnormal	Mean # Normal (N)	SD	Control Normal Survival N <sub>C</sub> /I	Reference Normal Survival Relative to Control N <sub>R</sub> /N <sub>C</sub>	Performance Standard
SED-15G:0-10	1	215	13	223.4	25.1			
	2	249	1					
	3	184	2					
	4	234	2					
	5	235	2					
SED-03G:0-10	1	224	4	228.4	13.0			
	2	242	9					
	3	229	4					
	4	238	6					
	5	209	5					
SED-01G:0-10	1	239	4	229.4	15.4			
	2	229	6					
	3	231	4					
	4	244	9					
	5	204	4					
SED-05G:0-10	1	247	2	223.4	15.6			
	2	224	5					
	3	226	6					
	4	215	12					
	5	205	2					
SED-27G:0-10	1	271	0	219.4	36.8			
	2	183	10					
	3	220	5					
	4	237	2					
	5	186	3					

I = Mean Initial count (Stocking density); 279

$N_c$  = Mean Control Normal

$N_R$  = Mean Reference Normal

Table 3-12. Water Quality Summary for *Mytilus galloprovincialis*

Treatment	Dissolved Oxygen (mg/L) ≥5.0 mg/L			Temperature (°C) 16 ± 1°C			Salinity (ppt) 28 ± 1 ppt			pH 7.5 - 9 units		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Seawater Control	8.0	7.7	8.3	16.9	16.6	17.3	28	28	28	7.9	7.9	7.9
CARR18-23-REF	8.2	8.1	8.2	16.1	16.0	16.2	28	28	28	8.0	7.9	8.0
CARR40-23-REF	7.7	7.3	8.2	16.9	16.7	17.1	28	28	28	7.9	7.9	8.0
CARR62-23-REF	7.9	7.7	8.1	17.0	16.9	17.1	28	28	28	7.9	7.9	8.0
SED-07G:0-10	8.0	7.8	8.2	15.9	15.8	16.0	28	28	28	7.9	7.9	8.0
SED-11G:0-10	7.1	6.3	8.1	16.8	16.4	17.0	28	28	28	7.9	7.8	7.9
SED-14G:0-10	8.0	7.8	8.1	16.3	16.0	16.7	28	28	28	7.9	7.9	8.0
SED-15G:0-10	7.9	7.8	8.1	16.1	15.9	16.3	28	28	28	7.9	7.9	8.0
SED-03G:0-10	8.1	8.0	8.1	16.1	16.0	16.3	28	28	28	7.9	7.9	8.0
SED-01G:0-10	7.5	6.5	8.2	17.2	17.0	17.4	28	28	28	7.9	7.9	7.9
SED-05G:0-10	8.1	8.0	8.1	16.0	15.8	16.2	28	28	28	7.9	7.9	8.0
SED-27G:0-10	8.0	7.9	8.1	16.3	16.1	16.7	28	28	28	8.0	7.9	8.0

Table 3-13. Ammonia and Sulfide Summary for *Mytilus galloprovincialis*

Treatment	Total Ammonia (mg/L Total) NOEC = 3.02 mg/L <sup>1</sup>		Unionized Ammonia (mg/L) NOEC = 0.065 mg/L <sup>1</sup> Trigger Value = 0.04 mg/L <sup>2</sup>		Total Sulfides (mg/L Total)		Hydrogen Sulfide (mg/L) Trigger Value = 0.0025 mg/L <sup>2</sup>	
	Day		Day		Day		Day	
	0	2	0	2	0	2	0	2
Seawater Control	0.00	0.00	0.000	0.000	ND	0.012	ND	0.0009
CARR18-23-REF	0.00	0.00	0.000	0.000	ND	ND	ND	ND
CARR40-23-REF	0.00	0.00	0.000	0.000	0.004	ND	0.0003	ND
CARR62-23-REF	0.00	0.00	0.000	0.000	ND	0.001	ND	0.0001
SED-07G:0-10	0.0162	0.00	0.000	0.000	0.010	0.002	0.0008	0.0002
SED-11G:0-10	0.0614	0.00	0.001	0.000	0.015	ND	0.0011	ND
SED-14G:0-10	0.00	0.00	0.000	0.000	0.008	ND	0.0006	ND
SED-15G:0-10	0.00	0.00	0.000	0.000	ND	0.011	ND	0.0008
SED-03G:0-10	0.00	0.00	0.000	0.000	0.006	0.051	0.0005	<b>0.0038</b>
SED-01G:0-10	0.00	0.00	0.000	0.000	0.005	ND	0.0004	ND
SED-05G:0-10	0.00	0.00	0.000	0.000	0.013	ND	0.0010	ND
SED-27G:0-10	0.00	0.00	0.000	0.000	ND	0.000	ND	0.0000

<sup>1</sup>NOEC (concurrent reference-toxicant test derived)

<sup>2</sup>Inouye et al. 2015

ND = Non-detect

**Bold** = above trigger value

**Table 3-14. Test Condition Summary for *Mytilus galloprovincialis***

Test Conditions: PSEP <i>M. galloprovincialis</i>		
Date Sampled	March 20, 21, and 23, 2023 (test samples) May 10, 2023 (reference samples)	
Date Received	March 24, 2023 (test samples) May 10, 2023 (reference samples)	
Test Dates	May 12 – 14, 2023	
Sample Storage Conditions	4°C, dark	
Holding Time Recommended: < 8 weeks (56 days)	53 Days (test samples) 2 Days (reference samples)	
Test Species	<i>Mytilus galloprovincialis</i>	
Supplier	Taylor Shellfish, Shelton, WA	
Date Acquired	May 1, 2023	
Age Class	<4-h old embryos	
Test Procedures	PSEP 1995 with SMARM revisions, SOP No. SED005.07	
Test Location	EcoAnalysts Port Gamble Laboratory	
Test Type/Duration	48-60 Hour static test (Actual: 48 hours)	
Control Water	North Hood Canal sea water, 0.45µm filtered	
Test Lighting	50 – 100 foot candles (14hr light: 10hr dark)	
Test Chamber	1-Liter Glass Chamber	
Replicates per Treatment	5 + 1 surrogate (used for WQ measurements throughout the test)	
Exposure Volume	18 g sediment/ 900 mL water	
Feeding	None	
Water Renewal	None	
Test Dissolved Oxygen	Recommended: >5.0 mg/L	Observed: 6.3 – 8.3 mg/L
Test Temperature	Recommended: 16 ± 1 °C	Observed: 15.8 – 17.4 °C
Test Salinity	Recommended: 28 ± 1 ppt	Observed: 28 ppt
Test pH	Recommended: 7.5 – 9	Observed: 7.8 – 8.0
Stocking Density	Recommended: 20 – 40 embryos/mL	Observed: 27.9 embryos/mL
Control Performance Standard (SMS)	Recommended: Control normal survival ≥ 0.70	Observed: Seawater Control 1.02; Pass
Reference Performance Standard (SMS)	Recommended: Reference normal survival relative to controls ≥ 0.65	Observed: 0.70 – 0.76; Pass
Reference Toxicant Endpoint	Total Ammonia	Unionized Ammonia
Reference Toxicant EC <sub>50</sub>	EC <sub>50</sub> = 4.42 mg/L	EC <sub>50</sub> = 0.099 mg/L
Mean; Acceptable Range	6.88; 3.60 – 13.2 mg/L	0.131; 0.051 – 0.336 mg/L
NOEC Combined Proportion Normal	3.02 mg/L	0.065 mg/L
Deviations from Test Protocol	Resuspension	

## 4. DISCUSSION

Sediments were evaluated using SMS criteria. The SMS criteria are derived from the Washington Department of Ecology's SMS document (WDOE 2013). Comparisons were made for each treatment against the reference sample using BioStat software. Two numerical comparisons were made under SMS: the Sediment Cleanup Objectives (SCO) and the Cleanup Screening Level (CSL).

### 4.1 Amphipod Test Suitability Determination

Under the SMS program, a treatment will fail SCO if mean mortality in the test sediment is >25% and the difference between mean mortality in the treatment compared to mean mortality in the reference is statistically significant ( $p < 0.05$ ). Treatments fail the CSL if mean mortality in the test treatment  $\geq 30\%$  relative to the reference sediment and the difference is statistically significant.

Project sediments passed the SCO and CSL criteria for the amphipod test with the exception of SED-03G:0-10, which failed the SCO criteria, as shown in Table 4-1. The control survival was below the acceptability criterion for the sediment test, and was at the acceptability criterion in the concurrent *E. estuarius* reference toxicant test (90%). Because of this, the overall health of the organisms used to initiate the test is suspect and may have contributed to the SED-03G:0-10 failure. Pete Adolphson at WDOE was consulted about the results, and indicated that, based on the control performance and the larval results available at the time, the SED-03G:0-10 results may be anomalous and thus not considered an SCO failure for the amphipod test (Personal communication, WDOE, Pete Adolphson, 2023).

**Table 4-1. SMS Comparison for *Eohaustorius estuarius***

Treatment	Mean Mortality (%)	Compared To:	Statistically Less Than Reference? ( $P=0.05$ )	Mortality Comparison to Reference $M_T-M_R$ (%)	Fails SCO? <sup>1</sup> > 25 % (Absolute)	Fails CSL? <sup>2</sup> $\geq 30$ % ( $M_T-M_R$ )
Control	16					
CARR18-23-REF	14					
CARR40-23-REF	15					
CARR62-23-REF	15					
SED-07G:0-10	22	CARR18-23-REF	No	8	No	No
SED-11G:0-10	18	CARR18-23-REF	No	4	No	No
SED-01G:0-10	13	CARR40-23-REF	No	-2	No	No
SED-27G:0-10	19	CARR40-23-REF	No	4	No	No
SED-15G:0-10	13	CARR40-23-REF	No	-2	No	No
SED-03G:0-10	28	CARR40-23-REF	Yes	13	Yes <sup>3</sup>	No
SED-14G:0-10	20	CARR62-23-REF	No	5	No	No
SED-05G:0-10	18	CARR62-23-REF	No	3	No	No

<sup>1</sup>SCO: Statistical Significance and  $M_T > 25\%$

<sup>2</sup>CSL: Statistical Significance and  $M_T-M_R \geq 30\%$

<sup>3</sup>Result qualified. See text above.

$M_T$  = Treatment Mortality

$M_R$  = Reference Mortality

## 4.2 Juvenile Polychaete Test Suitability Determination

A test treatment fails SCO criteria if MIG is statistically lower in the test treatment, relative to the reference, and the ratio of the MIG in the test treatment is  $<0.70$  that of the reference. The treatments will fail CSL criteria if the MIG is significantly lower than the reference treatment and the ratio between the MIG of the treatment and the MIG of the reference is  $<0.50$ .

Project sediment passes the SCO and CSL criteria for the juvenile polychaete test with the exception of SED-11G:0-10, SED14G:0-10 and SED-27G:0-10, which failed the SCO criteria and were statistically less than the reference, as shown in Table 4-2.

**Table 4-2. SMS Comparison for *Neanthes arenaceodentata***

Treatment	MIG (mg/ind/day) AFDW	Compared To:	Statistically Less than Reference? ( $p=0.05$ )	MIG Relative to Reference $MIG_T/MIG_R$	Fails SCO? <sup>1</sup> $< 0.70$	Fails CSL? <sup>2</sup> $< 0.50$
Control	1.251					
CARR18-23-REF	1.412					
CARR40-23-REF	1.523					
CARR62-23-REF	1.648					
SED-07G:0-10	1.208	CARR18-23-REF	No	0.86	No	No
SED-11G:0-10	0.737	CARR18-23-REF	Yes	0.52	Yes	No
SED-01G:0-10	1.343	CARR40-23-REF	No	0.88	No	No
SED-27G:0-10	1.035	CARR40-23-REF	Yes	0.68	Yes	No
SED-15G:0-10	1.280	CARR40-23-REF	No	0.84	No	No
SED-03G:0-10	1.174	CARR40-23-REF	Yes	0.77	No	No
SED-14G:0-10	1.139	CARR62-23-REF	Yes	0.69	Yes	No
SED-05G:0-10	1.274	CARR62-23-REF	No	0.77	No	No

<sup>1</sup>SCO: Statistical Significance and  $MIG_T/MIG_R < 0.70$

<sup>2</sup>CSL: Statistical Significance and  $MIG_T/MIG_R < 0.50$

$MIG_T$  = Treatment Mean Individual Growth Rate

$MIG_R$  = Reference Mean Individual Growth Rate



### 4.3 Larval Test Suitability Determination

Larval test treatments fail SCO criteria if the number of normal larvae in the test treatment is significantly lower ( $p < 0.10$ ) than that of the reference and if the ratio between the normal larval development in the test treatment is less than 0.85 of the normal development in the reference. Treatments fail CSL criteria if the number of normal larvae in the test treatment is significantly lower ( $p < 0.10$ ) than that of the reference and if the ratio between the normal larval development in the test treatment is less than 0.70 of the normal development in the reference. Project sediment passes the SCO and CSL criteria for larval development, as show in (Table 4-3).

**Table 4-3. SMS Comparison for *Mytilus galloprovincialis***

Treatment	Mean Number Normal	Compared To:	Statistically Less Than Reference? ( $p=0.10$ )	Normal Survival Comparison to Reference $N_T/N_R$	Fails SCO? <sup>1</sup>	Fails CSL? <sup>2</sup>
Seawater Control	284.0					
CARR18-23-REF	200.8					
CARR40-23-REF	216.0					
CARR62-23-REF	199.6					
SED-07G:0-10	216.6	CARR18-23-REF	No	1.08	No	No
SED-11G:0-10	224.2	CARR18-23-REF	No	1.12	No	No
SED-01G:0-10	229.4	CARR40-23-REF	No	1.06	No	No
SED-27G:0-10	219.4	CARR40-23-REF	No	1.02	No	No
SED-15G:0-10	223.4	CARR40-23-REF	No	1.03	No	No
SED-03G:0-10	228.4	CARR40-23-REF	No	1.06	No	No
SED-14G:0-10	212.8	CARR62-23-REF	No	1.07	No	No
SED-05G:0-10	223.4	CARR62-23-REF	No	1.12	No	No

<sup>1</sup> SCO: Statistical Significance and  $(N_T/N_R) < 0.85$

<sup>2</sup> CSL: Statistical Significance and  $(N_T/N_R) < 0.70$

$N_T$  =Treatment Mean Number Normal

$N_R$  =Reference Mean Number Normal

## 5. SUMMARY

A summary of the biological tests conducted on the sediments evaluated under the SMS sediment quality criteria is provided in Table 5-1. Project sediment passes the SCO and CSL performance criteria for the larval tests performed. The amphipod test failed the SCO criteria for SED-03G:0-10 but passed performance criteria for CSL, though the results are qualified and WDOE's determination may differ. The polychaete test failed SCO criteria for SED-11G:0-10, SED-14:0-10 and SED-27G:0-10 but passed performance criteria for CSL. The larval test passed SCO and CSL criteria for all samples.

**Table 5-1. Summary of SMS Evaluation**

Treatment	Sediment Cleanup Objectives			Cleanup Screening Levels		
	Amphipod	Polychaete	Larval	Amphipod	Polychaete	Larval
SED-07G:0-10	Pass	Pass	Pass	Pass	Pass	Pass
SED-11G:0-10	Pass	<b>Fail</b>	Pass	Pass	Pass	Pass
SED-01G:0-10	Pass	Pass	Pass	Pass	Pass	Pass
SED-27G:0-10	Pass	<b>Fail</b>	Pass	Pass	Pass	Pass
SED-15G:0-10	Pass	Pass	Pass	Pass	Pass	Pass
SED-03G:0-10	<b>Fail<sup>1</sup></b>	Pass	Pass	Pass	Pass	Pass
SED-14G:0-10	Pass	<b>Fail</b>	Pass	Pass	Pass	Pass
SED-05G:0-10	Pass	Pass	Pass	Pass	Pass	Pass

<sup>1</sup>Results qualified, see section 4.1. WDOE determination may differ.

## 6. REFERENCES

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## **APPENDIX A. TEST DATA, STATISTICAL ANALYSES, AND REFERENCE TOXICANT TEST RESULTS**

### **1. *EOHAUSTORIUS ESTUARIUS* 10-DAY TEST**

- 1.1 *EOHAUSTORIUS ESTUARIUS* TEST DATA
- 1.2 *EOHAUSTORIUS ESTUARIUS* STATISTICAL RESULTS
- 1.3 *EOHAUSTORIUS ESTUARIUS* REFERENCE TOXICANT TEST RESULTS

### **2. *NEANTHES ARENACEODENTATA* 20-DAY SOLID-PHASE TEST**

- 2.1 *NEANTHES ARENACEODENTATA* TEST DATA
- 2.2 *NEANTHES ARENACEODENTATA* STATISTICAL RESULTS
- 2.3 *NEANTHES ARENACEODENTATA* REFERENCE TOXICANT TEST RESULTS

### **3. *MYTILUS GALLOPROVINCIALIS* WATER-COLUMN TEST**

- 3.1 *MYTILUS GALLOPROVINCIALIS* TEST DATA
- 3.2 *MYTILUS GALLOPROVINCIALIS* STATISTICAL RESULTS
- 3.3 *MYTILUS GALLOPROVINCIALIS* REFERENCE TOXICANT TEST RESULTS

## **APPENDIX B. CHAIN-OF-CUSTODY FORMS, LOGS, AND PRE-TEST DOCUMENTS**

**APPENDIX A. TEST DATA, STATISTICAL ANALYSES, AND REFERENCE  
TOXICANT TEST RESULTS**

- 1. *Eohaustorius estuarius* 10-day Test**

## 1.1 *Eohaustorius estuarius* Test Data

## GENERAL

Client	Leon Environmental
Project	Port of Friday Harbor
Project Number	PG1785
Project Manager	J. Levengood, M. Seibert
Date Oldest Sample Collected	3/20/2023
Sample Holding Time	53
Test Start Date	05/12/23
Test Species	<i>Eohaustorius estuarius</i>
Organism Supplier	Northwest Amphipod
Organism Acquired	5/10/2023
Organism Acclimation	2
Organism Age	Mature, 3 - 5 mm
Test Type/Duration	10-day solid phase
Test Protocol	PSEP 1995
Regional Protocol	SCUM 2019
Laboratory Location	Port Gamble
Test Location	Bath 3
Sample Treatment	reference sample press sieved 2mm Sample SED-11G:0-10 press sieved 2mm
Control Sediment Source	Yaquina Bay, OR
Water Batch	FSW051023.01
Test Lighting	Continuous
Test Chamber	1 L mason jars
Replicates Per Treatment	5 replicates + 2 surr
Organisms per Replicate	20
Exposure Volume	2 cm sediment/ 900 mL water
Feeding Information	None
Test Dissolved Oxygen	> 5.1
Test Temperature	15 ± 1
Test Salinity	28 ± 1
Test pH	8 ± 1
Water Renewal Info	None

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO:	5.1	
Temp:	14	16
Sal:	27	29
pH:	7	9

TEST START TIME/INITIALS:	1409 MS/MK
TEST END TIME:	0950 DM/SZ

	CLIENT SAMPLE ID	LAB ID
1	Control	P230510.01
2	CARR18-23-REF	P230510.02
3	CARR40-23-REF	P230510.03
4	CARR62-23-REF	P230510.04
5	SED-07G:0-10	P230324.01
6	SED-11G:0-10	P230324.02
7	SED-14G:0-10	P230324.03
8	SED-15G:0-10	P230324.04
9	SED-03G:0-10	P230324.05
10	SED-01G:0-10	P230324.06
11	SED-05G:0-10	P230324.07
12	SED-27G:0-10	P230324.08
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16	.	.
17	.	.
18	.	.
19	.	.
20	.	.



# 10 DAY SP WQ DATA SHEET

CLIENT Leon Environmental				PROJECT Port of Friday Harbor			SPECIES <i>Eohaustorius estuarius</i>		LOCATION Port Gamble / Bath 3		PROTOCOL PSEP 1995 , SCUM 2019	
PROJECT NUMBER PG1785				TEST TYPE/DURATION 10-day solid phase			WATER DESCRIPTION FSW051023.01		TEST START DATE May 12, 2023		TEST END DATE May 22, 2023	
WATER QUALITY DATA												
				TECHNICIAN	Date	Meter	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	Notes	
SAMPLE ID	DAY	REP	JAR #				> 5.1	14 - 16	27 - 29	7 - 9		
Control	0	Surr	18	MS	05/12/23	8	8.4	15.4	28	8.0		
Control	1	Surr	18	NL	05/13/23	9	8.3	15.6	28	8.1		
Control	2	Surr	18	LG	05/14/23	9	8.3	15.8	28	8.1		
Control	3	Surr	18	SZ	05/15/23	8	8.2	15.4	28	8.0		
Control	4	Surr	18	LG	05/16/23	8	8.3	15.4	28	8.1		
Control	5	Surr	18	SZ	05/17/23	8	8.3	15.5	28	8.1		
Control	6	Surr	18	LG	05/18/23	8	8.3	15.5	28	8.1		
Control	7	Surr	18	MS	05/19/23	9	8.2	15.9	28	8.1		
Control	8	Surr	18	NL	05/20/23	9	8.3	15.6	28	8.1		
Control	9	Surr	18	NL	05/21/23	9	8.2	15.7	28	8.1		
Control	10	Surr	18	DM	05/22/23	9	8.4	15.5	28	8.1		
CARR18-23-REF	0	Surr	4	MS	05/12/23	8	8.3	15.4	28	7.9		
CARR18-23-REF	1	Surr	4	NL	05/13/23	9	7.8	16.4	28	8.0		
CARR18-23-REF	2	Surr	4	LG	05/14/23	9	8.2	15.6	28	8.0		
CARR18-23-REF	3	Surr	4	SZ	05/15/23	8	8.2	15.3	28	8.0		
CARR18-23-REF	4	Surr	4	LG	05/16/23	8	8.2	15.4	28	8.1		
CARR18-23-REF	5	Surr	4	SZ	05/17/23	8	8.2	15.4	28	8.1		
CARR18-23-REF	6	Surr	4	LG	05/18/23	8	8.3	15.4	28	8.1		
CARR18-23-REF	7	Surr	4	MS	05/19/23	9	8.1	16.2	28	8.2		
CARR18-23-REF	8	Surr	4	NL	05/20/23	9	8.2	15.6	28	8.2		
CARR18-23-REF	9	Surr	4	NL	05/21/23	9	8.3	15.5	28	8.2		
CARR18-23-REF	10	Surr	4	DM	05/22/23	9	8.2	15.8	28	8.2		

# 10 DAY SP WQ DATA SHEET

CLIENT Leon Environmental				PROJECT Port of Friday Harbor			SPECIES <i>Eohaustorius estuarius</i>		LOCATION Port Gamble / Bath 3		PROTOCOL PSEP 1995 , SCUM 2019	
PROJECT NUMBER PG1785				TEST TYPE/DURATION 10-day solid phase			WATER DESCRIPTION FSW051023.01		TEST START DATE May 12, 2023		TEST END DATE May 22, 2023	
WATER QUALITY DATA												
				TECHNICIAN	Date	Meter	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	Notes	
SAMPLE ID	DAY	REP	JAR #				> 5.1	14 - 16	27 - 29	7 - 9		
CARR40-23-REF	0	Surr	62	MS	05/12/23	8	8.3	15.5	28	8.0		
CARR40-23-REF	1	Surr	62	NL	05/13/23	9	8.3	15.7	28	8.1		
CARR40-23-REF	2	Surr	62	LG	05/14/23	9	8.1	16.0	28	8.1		
CARR40-23-REF	3	Surr	62	SZ	05/15/23	8	8.0	15.8	28	8.0		
CARR40-23-REF	4	Surr	62	LG	05/16/23	8	8.2	15.7	28	8.1		
CARR40-23-REF	5	Surr	62	SZ	05/17/23	8	8.1	15.8	28	8.1		
CARR40-23-REF	6	Surr	62	LG	05/18/23	8	8.2	15.7	28	8.2		
CARR40-23-REF	7	Surr	62	MS	05/19/23	9	8.2	16.0	28	8.2		
CARR40-23-REF	8	Surr	62	NL	05/20/23	9	8.2	15.9	28	8.3		
CARR40-23-REF	9	Surr	62	NL	05/21/23	9	8.2	15.7	28	8.3		
CARR40-23-REF	10	Surr	62	DM	05/22/23	9	8.3	15.8	28	8.4		
CARR62-23-REF	0	Surr	28	MS	05/12/23	8	8.4	15.3	28	8.0		
CARR62-23-REF	1	Surr	28	NL	05/13/23	9	8.3	15.5	28	8.0		
CARR62-23-REF	2	Surr	28	LG	05/14/23	9	8.2	15.9	28	8.0		
CARR62-23-REF	3	Surr	28	SZ	05/15/23	8	8.1	15.5	28	8.0		
CARR62-23-REF	4	Surr	28	LG	05/16/23	8	8.3	15.4	28	8.0		
CARR62-23-REF	5	Surr	28	SZ	05/17/23	8	8.1	15.7	28	8.0		
CARR62-23-REF	6	Surr	28	LG	05/18/23	8	8.2	15.5	28	8.0		
CARR62-23-REF	7	Surr	28	MS	05/19/23	9	8.2	15.8	28	8.1		
CARR62-23-REF	8	Surr	28	NL	05/20/23	9	8.3	15.6	28	8.1		
CARR62-23-REF	9	Surr	28	NL	05/21/23	9	8.1	16.0	28	8.1		
CARR62-23-REF	10	Surr	28	DM	05/22/23	9	8.3	15.6	28	8.1		

# 10 DAY SP WQ DATA SHEET

CLIENT Leon Environmental				PROJECT Port of Friday Harbor			SPECIES <i>Eohaustorius estuarius</i>		LOCATION Port Gamble / Bath 3		PROTOCOL PSEP 1995 , SCUM 2019	
PROJECT NUMBER PG1785				TEST TYPE/DURATION 10-day solid phase			WATER DESCRIPTION FSW051023.01		TEST START DATE May 12, 2023		TEST END DATE May 22, 2023	
WATER QUALITY DATA												
				TECHNICIAN	Date	Meter	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	Notes	
SAMPLE ID	DAY	REP	JAR #				> 5.1	14 - 16	27 - 29	7 - 9		
SED-07G:0-10	0	Surr	43	MS	05/12/23	8	8.3	15.5	28	8.0		
SED-07G:0-10	1	Surr	43	NL	05/13/23	9	8.2	15.6	28	8.1		
SED-07G:0-10	2	Surr	43	LG	05/14/23	9	7.9	16.1	28	8.1		
SED-07G:0-10	3	Surr	43	SZ	05/15/23	8	8.2	15.5	28	8.1		
SED-07G:0-10	4	Surr	43	LG	05/16/23	8	8.2	15.5	28	8.2		
SED-07G:0-10	5	Surr	43	SZ	05/17/23	8	8.2	15.7	28	8.2		
SED-07G:0-10	6	Surr	43	LG	05/18/23	8	8.2	15.7	28	8.3		
SED-07G:0-10	7	Surr	43	MS	05/19/23	9	8.1	16.0	28	8.3		
SED-07G:0-10	8	Surr	43	NL	05/20/23	9	8.2	15.8	28	8.3		
SED-07G:0-10	9	Surr	43	NL	05/21/23	9	8.2	15.9	28	8.3		
SED-07G:0-10	10	Surr	43	DM	05/22/23	9	8.1	15.9	28	8.3		
SED-11G:0-10	0	Surr	37	MS	05/12/23	8	8.3	15.4	28	8.1		
SED-11G:0-10	1	Surr	37	NL	05/13/23	9	8.3	15.5	28	8.2		
SED-11G:0-10	2	Surr	37	LG	05/14/23	9	8.1	16.0	28	8.2		
SED-11G:0-10	3	Surr	37	SZ	05/15/23	8	8.2	15.4	28	8.2		
SED-11G:0-10	4	Surr	37	LG	05/16/23	8	8.3	15.5	28	8.2		
SED-11G:0-10	5	Surr	37	SZ	05/17/23	8	8.2	15.6	28	8.3		
SED-11G:0-10	6	Surr	37	LG	05/18/23	8	8.2	15.5	28	8.3		
SED-11G:0-10	7	Surr	37	MS	05/19/23	9	8.2	15.8	28	8.4		
SED-11G:0-10	8	Surr	37	NL	05/20/23	9	8.2	15.6	28	8.4		
SED-11G:0-10	9	Surr	37	NL	05/21/23	9	8.2	15.9	28	8.4		
SED-11G:0-10	10	Surr	37	DM	05/22/23	9	8.2	15.9	28	8.5		

# 10 DAY SP WQ DATA SHEET

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PROJECT NUMBER PG1785				TEST TYPE/DURATION 10-day solid phase			WATER DESCRIPTION FSW051023.01		TEST START DATE May 12, 2023		TEST END DATE May 22, 2023	
WATER QUALITY DATA												
				TECHNICIAN	Date	Meter	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	Notes	
SAMPLE ID	DAY	REP	JAR #				> 5.1	14 - 16	27 - 29	7 - 9		
SED-14G:0-10	0	Surr	45	MS	05/12/23	8	8.3	15.4	28	8.0		
SED-14G:0-10	1	Surr	45	NL	05/13/23	9	8.3	15.5	28	8.1		
SED-14G:0-10	2	Surr	45	LG	05/14/23	9	8.1	15.8	28	8.1		
SED-14G:0-10	3	Surr	45	SZ	05/15/23	8	8.2	15.4	28	8.1		
SED-14G:0-10	4	Surr	45	LG	05/16/23	8	8.2	15.4	28	8.2		
SED-14G:0-10	5	Surr	45	SZ	05/17/23	8	8.2	15.6	28	8.2		
SED-14G:0-10	6	Surr	45	LG	05/18/23	8	8.2	15.5	28	8.3		
SED-14G:0-10	7	Surr	45	MS	05/19/23	9	8.1	15.9	28	8.3		
SED-14G:0-10	8	Surr	45	NL	05/20/23	9	8.2	15.7	28	8.3		
SED-14G:0-10	9	Surr	45	NL	05/21/23	9	8.2	15.9	28	8.3		
SED-14G:0-10	10	Surr	45	DM	05/22/23	9	8.1	15.7	28	8.3		
SED-15G:0-10	0	Surr	17	MS	05/12/23	8	8.3	15.3	28	7.9		
SED-15G:0-10	1	Surr	17	NL	05/13/23	9	8.1	15.6	28	8.0		
SED-15G:0-10	2	Surr	17	LG	05/14/23	9	8.1	15.7	28	8.0		
SED-15G:0-10	3	Surr	17	SZ	05/15/23	8	8.1	15.4	28	8.0		
SED-15G:0-10	4	Surr	17	LG	05/16/23	8	8.2	15.4	28	8.0		
SED-15G:0-10	5	Surr	17	SZ	05/17/23	8	8.1	15.6	28	8.1		
SED-15G:0-10	6	Surr	17	LG	05/18/23	8	8.2	15.5	28	8.1		
SED-15G:0-10	7	Surr	17	MS	05/19/23	9	8.1	16.2	28	8.2		
SED-15G:0-10	8	Surr	17	NL	05/20/23	9	8.2	15.6	28	8.2		
SED-15G:0-10	9	Surr	17	NL	05/21/23	9	8.2	15.5	28	8.2		
SED-15G:0-10	10	Surr	17	DM	05/22/23	9	8.2	15.6	28	8.2		

# 10 DAY SP WQ DATA SHEET

CLIENT Leon Environmental				PROJECT Port of Friday Harbor			SPECIES <i>Eohaustorius estuarius</i>		LOCATION Port Gamble / Bath 3		PROTOCOL PSEP 1995 , SCUM 2019	
PROJECT NUMBER PG1785				TEST TYPE/DURATION 10-day solid phase			WATER DESCRIPTION FSW051023.01		TEST START DATE May 12, 2023		TEST END DATE May 22, 2023	
WATER QUALITY DATA												
				TECHNICIAN	Date	Meter	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	Notes	
SAMPLE ID	DAY	REP	JAR #				> 5.1	14 - 16	27 - 29	7 - 9		
SED-03G:0-10	0	Surr	67	MS	05/12/23	8	8.2	15.6	28	7.9		
SED-03G:0-10	1	Surr	67	NL	05/13/23	9	8.1	15.8	28	7.9		
SED-03G:0-10	2	Surr	67	LG	05/14/23	9	8.0	16.2	28	7.9		
SED-03G:0-10	3	Surr	67	SZ	05/15/23	8	7.9	15.9	28	7.9		
SED-03G:0-10	4	Surr	67	LG	05/16/23	8	8.0	15.8	28	8.0		
SED-03G:0-10	5	Surr	67	SZ	05/17/23	8	8.1	16.1	28	8.0		
SED-03G:0-10	6	Surr	67	LG	05/18/23	8	8.1	15.8	28	8.1		
SED-03G:0-10	7	Surr	67	MS	05/19/23	9	8.1	16.1	28	8.1		
SED-03G:0-10	8	Surr	67	NL	05/20/23	9	8.1	16.0	28	8.1		
SED-03G:0-10	9	Surr	67	NL	05/21/23	9	8.2	16.0	28	8.2		
SED-03G:0-10	10	Surr	67	DM	05/22/23	9	8.2	15.8	28	8.2		
SED-01G:0-10	0	Surr	51	MS	05/12/23	8	8.3	15.4	28	8.0		
SED-01G:0-10	1	Surr	51	NL	05/13/23	9	8.3	15.6	28	8.0		
SED-01G:0-10	2	Surr	51	LG	05/14/23	9	8.1	15.8	28	8.0		
SED-01G:0-10	3	Surr	51	SZ	05/15/23	8	8.1	15.5	28	8.0		
SED-01G:0-10	4	Surr	51	LG	05/16/23	8	8.1	15.5	28	8.1		
SED-01G:0-10	5	Surr	51	SZ	05/17/23	8	8.1	15.8	28	8.1		
SED-01G:0-10	6	Surr	51	LG	05/18/23	8	8.1	15.7	28	8.1		
SED-01G:0-10	7	Surr	51	MS	05/19/23	9	8.1	15.9	28	8.2		
SED-01G:0-10	8	Surr	51	NL	05/20/23	9	8.2	15.7	28	8.2		
SED-01G:0-10	9	Surr	51	NL	05/21/23	9	8.2	15.9	28	8.3		
SED-01G:0-10	10	Surr	51	DM	05/22/23	9	8.1	15.9	28	8.3		

# 10 DAY SP WQ DATA SHEET

CLIENT Leon Environmental				PROJECT Port of Friday Harbor			SPECIES <i>Eohaustorius estuarius</i>		LOCATION Port Gamble / Bath 3		PROTOCOL PSEP 1995 , SCUM 2019	
PROJECT NUMBER PG1785				TEST TYPE/DURATION 10-day solid phase			WATER DESCRIPTION FSW051023.01		TEST START DATE May 12, 2023		TEST END DATE May 22, 2023	
WATER QUALITY DATA												
				TECHNICIAN	Date	Meter	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	Notes	
SAMPLE ID	DAY	REP	JAR #				> 5.1	14 - 16	27 - 29	7 - 9		
SED-05G:0-10	0	Surr	35	MS	05/12/23	8	8.3	15.4	28	8.0		
SED-05G:0-10	1	Surr	35	NL	05/13/23	9	8.2	15.6	28	8.0		
SED-05G:0-10	2	Surr	35	LG	05/14/23	9	7.9	15.7	29	8.0		
SED-05G:0-10	3	Surr	35	SZ	05/15/23	8	8.1	15.4	28	8.0		
SED-05G:0-10	4	Surr	35	LG	05/16/23	8	8.3	15.4	29	8.1		
SED-05G:0-10	5	Surr	35	SZ	05/17/23	8	8.2	15.5	28	8.2		
SED-05G:0-10	6	Surr	35	LG	05/18/23	8	8.2	15.5	29	8.2		
SED-05G:0-10	7	Surr	35	MS	05/19/23	9	8.2	15.7	28	8.3		
SED-05G:0-10	8	Surr	35	NL	05/20/23	9	8.2	15.6	28	8.3		
SED-05G:0-10	9	Surr	35	NL	05/21/23	9	8.1	15.8	28	8.3		
SED-05G:0-10	10	Surr	35	DM	05/22/23	9	8.3	15.6	28	8.3		
SED-27G:0-10	0	Surr	48	MS	05/12/23	8	8.3	15.4	28	8.0		
SED-27G:0-10	1	Surr	48	NL	05/13/23	9	8.3	15.6	28	8.1		
SED-27G:0-10	2	Surr	48	LG	05/14/23	9	8.1	15.8	28	8.1		
SED-27G:0-10	3	Surr	48	SZ	05/15/23	8	8.2	15.4	28	8.1		
SED-27G:0-10	4	Surr	48	LG	05/16/23	8	8.3	15.4	28	8.2		
SED-27G:0-10	5	Surr	48	SZ	05/17/23	8	8.1	15.7	28	8.2		
SED-27G:0-10	6	Surr	48	LG	05/18/23	8	8.2	15.5	28	8.2		
SED-27G:0-10	7	Surr	48	MS	05/19/23	9	8.1	15.8	28	8.2		
SED-27G:0-10	8	Surr	48	NL	05/20/23	9	8.3	15.5	28	8.3		
SED-27G:0-10	9	Surr	48	NL	05/21/23	9	8.2	15.7	28	8.3		
SED-27G:0-10	10	Surr	48	DM	05/22/23	9	8.2	15.6	28	8.3		

# 10-DAY SP TEST OBSERVATION DATA SHEET

CLIENT Leon Environmental					PROJECT Port of Friday Harbor		PROJECT NO. PG1785	PROJECT MANAGER J. Levengood, M. Seibert		LOCATION Port Gamble / Bath 3		PROTOCOL PSEP 1995		SPECIES <i>Eohaustorius estuarius</i>		
<b>Observation Key</b> #FOS = Num Floating on Water Surface #E = Num Emerged from Sediment #M = Number of Mortalities or Molts L = Anoxic Surface G = Growth D = No Air Flow (Measure DO) N = Normal TC = Too Cloudy to Observe					<b>ENDPOINT DATA &amp; OBSERVATIONS</b>											
					Technician	NL	LG	SZ	LG	SZ	LG	MS	NL	NL	DM	
					Date	05/13/23	05/14/23	05/15/23	05/16/23	05/17/23	05/18/23	05/19/23	05/20/23	05/21/23	05/22/23	
CLIENT ID	REP	Jar #	Initial #	Day	1	2	3	4	5	6	7	8	9	10	Comments	
Control	1	64	20		N	2M	2M	2M	1M	1M	2M	N	N	N		
Control	2	72	20		N	1E	2M	1M	1M	1M	2M	2M	2M	2M		
Control	3	1	20		1E	1E	N	1M	N	2M	1M,1E	1M	N	N		
Control	4	38	20		N	N	N	N	N	N	N	N	N	N		
Control	5	22	20		N	2E	2M	3M	3M	3M	3M	N	N	N		
CARR18-23-REF	1	54	20		N	N	N	N	N	N	N	N	N	N		
CARR18-23-REF	2	24	20		N	1FOS	N	N	N	N	N	N	N	N		
CARR18-23-REF	3	30	20		N	1E	1M	1M	1M	1M	1M	N	N	N		
CARR18-23-REF	4	65	20		N	2FOS	1M	N	N	N	1M	N	N	N		
CARR18-23-REF	5	7	20		N	1E	1FOS	1M	1M	1M	1M	1M	N	N		
CARR40-23-REF	1	53	20		N	N	N	N	N	1M	N	N	N	N		
CARR40-23-REF	2	8	20		N	N	N	N	N	N	N	N	N	N		
CARR40-23-REF	3	39	20		N	N	N	N	N	N	N	N	N	N		
CARR40-23-REF	4	10	20		N	N	N	N	1M	1M	1M	1M	N	N		
CARR40-23-REF	5	14	20		N	1E	N	1M	4M	4M	4M	4M	N	N		

# 10-DAY SP TEST OBSERVATION DATA SHEET

CLIENT Leon Environmental					PROJECT Port of Friday Harbor		PROJECT NO. PG1785		PROJECT MANAGER J. Levengood, M. Seibert		LOCATION Port Gamble / Bath 3			PROTOCOL PSEP 1995		SPECIES <i>Eohaustorius estuarius</i>		
<b>Observation Key</b> #FOS = Num Floating on Water Surface #E = Num Emerged from Sediment #M = Number of Mortalities or Molts L = Anoxic Surface G = Growth D = No Air Flow (Measure DO) N = Normal TC = Too Cloudy to Observe					<b>ENDPOINT DATA &amp; OBSERVATIONS</b>													
					Technician	NL	LG	SZ	LG	SZ	LG	MS	NL	NL	DM			
					Date	05/13/23	05/14/23	05/15/23	05/16/23	05/17/23	05/18/23	05/19/23	05/20/23	05/21/23	05/22/23			
					CLIENT ID	REP	Jar #	Initial #	Day	1	2	3	4	5	6	7	8	
CARR62-23-REF	1	26	20		N	1E	N	N	N	N	N	N	N	N				
CARR62-23-REF	2	19	20		N	N	N	N	N	N	N	N	N	N				
CARR62-23-REF	3	3	20		N	2E	N	2M	N	N	N	1M	N	N				
CARR62-23-REF	4	40	20		N	1FOS	N	1M	N	N	N	N	N	N				
CARR62-23-REF	5	15	20		N	N	N	N	N	N	N	N	N	N				
SED-07G:0-10	1	63	20		N	1M	N	1M	N	1M	N	N	1FOS	N				
SED-07G:0-10	2	55	20		N	N	N	1E	N	N	N	N	N	N				
SED-07G:0-10	3	50	20		N	N	N	N	N	1FOS	N	1FOS	N	N				
SED-07G:0-10	4	46	20		N	N	N	N	N	N	1FOS	N	1FOS	N				
SED-07G:0-10	5	20	20		N	3FOS	N	N	N	N	2FOS	N	N	N				
SED-11G:0-10	1	16	20		4FOS	4FOS, 2M	2FOS, 3M	2M	2M, 1FOS, L	1FOS, 2M, L	1M	1FOS, 1M	1M	1M				
SED-11G:0-10	2	47	20		3FOS	4FOS, 1M	1FOS, 1M	N	1FOS	2FOS	3FOS	1M, 1FOS	1FOS	N				
SED-11G:0-10	3	61	20		N	N	N	N	N	N	N	N	N	N				
SED-11G:0-10	4	56	20		N	N	N	N	N	N	N	N	N	N				
SED-11G:0-10	5	44	20		N	N	N	N	N	N	N	N	N	N				



# 10-DAY SP TEST OBSERVATION DATA SHEET

<b>CLIENT</b> Leon Environmental					<b>PROJECT</b> Port of Friday Harbor		<b>PROJECT NO.</b> PG1785	<b>PROJECT MANAGER</b> J. Levengood, M. Seibert		<b>LOCATION</b> Port Gamble / Bath 3		<b>PROTOCOL</b> PSEP 1995		<b>SPECIES</b> <i>Eohaustorius estuarius</i>		
<b>Observation Key</b> #FOS = Num Floating on Water Surface #E = Num Emerged from Sediment #M = Number of Mortalities or Molts L = Anoxic Surface G = Growth D = No Air Flow (Measure DO) N = Normal TC = Too Cloudy to Observe					<b>ENDPOINT DATA &amp; OBSERVATIONS</b>											
					<b>Technician</b>	<b>NL</b>	<b>LG</b>	<b>SZ</b>	<b>LG</b>	<b>SZ</b>	<b>LG</b>	<b>MS</b>	<b>NL</b>	<b>NL</b>	<b>DM</b>	
					<b>Date</b>	05/13/23	05/14/23	05/15/23	05/16/23	05/17/23	05/18/23	05/19/23	05/20/23	05/21/23	05/22/23	
					<b>CLIENT ID</b>	<b>REP</b>	<b>Jar #</b>	<b>Initial #</b>	<b>Day</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	
SED-14G:0-10	1	41	20		N	4FOS	N	N	G	N	N	N	N	1FOS		
SED-14G:0-10	2	9	20		N	N	N	N	G	N	N	N	N	N		
SED-14G:0-10	3	33	20		N	N	N	N	N	N	N	N	N	N		
SED-14G:0-10	4	68	20		N	4FOS	1FOS	N	N	N	N	N	N	N		
SED-14G:0-10	5	58	20		N	N	N	N	G	N	N	N	N	N		
SED-15G:0-10	1	71	20		N	1FOS	N	N	N	N	N	N	1FOS	2FOS		
SED-15G:0-10	2	66	20		N	N	N	N	N	N	N	N	1FOS	N		
SED-15G:0-10	3	36	20		N	N	N	1M	1M	1M	N	N	N	N		
SED-15G:0-10	4	23	20		N	5FOS	N	N	N	N	N	N	N	N		
SED-15G:0-10	5	49	20		N	N	1M	N	N	N	N	N	N	N		
SED-03G:0-10	1	31	20		N	N	N	N	1M	N	N	N	N	N		
SED-03G:0-10	2	13	20		N	1FOS	N	N	N	N	N	N	N	1FOS		
SED-03G:0-10	3	21	20		N	1E	N	N	N	N	N	N	N	N		
SED-03G:0-10	4	32	20		1FOS	1FOS	N	N	N	N	N	N	N	N		
SED-03G:0-10	5	57	20		N	N	N	N	N	N	N	N	N	N		

# 10-DAY SP TEST OBSERVATION DATA SHEET

CLIENT Leon Environmental					PROJECT Port of Friday Harbor		PROJECT NO. PG1785	PROJECT MANAGER J. Levengood, M. Seibert		LOCATION Port Gamble / Bath 3		PROTOCOL PSEP 1995		SPECIES <i>Eohaustorius estuarius</i>		
<b>Observation Key</b> #FOS = Num Floating on Water Surface #E = Num Emerged from Sediment #M = Number of Mortalities or Molts L = Anoxic Surface G = Growth D = No Air Flow (Measure DO) N = Normal TC = Too Cloudy to Observe					<b>ENDPOINT DATA &amp; OBSERVATIONS</b>											
					Technician	NL	LG	SZ	LG	SZ	LG	MS	NL	NL	DM	
					Date	05/13/23	05/14/23	05/15/23	05/16/23	05/17/23	05/18/23	05/19/23	05/20/23	05/21/23	05/22/23	
CLIENT ID	REP	Jar #	Initial #	Day	1	2	3	4	5	6	7	8	9	10	Comments	
SED-01G:0-10	1	59	20		N	1M	1M	N	N	1M	1M	N	N	N		
SED-01G:0-10	2	5	20		N	1E	N	1M	N	N	N	N	N	N		
SED-01G:0-10	3	12	20		N	N	N	N	N	N	N	N	N	N		
SED-01G:0-10	4	27	20		N	N	N	N	N	N	N	N	N	N		
SED-01G:0-10	5	42	20		N	N	N	N	N	N	N	N	N	N		
SED-05G:0-10	1	11	20		1FOS	N	G	N	G	N	1FOS	N	N	N		
SED-05G:0-10	2	70	20		N	N	N	N	G	G	G	G	N	N		
SED-05G:0-10	3	29	20		N	1FOS	1FOS, G	G	G	G	1FOS	N	N	N		
SED-05G:0-10	4	52	20		N	1FOS	G	G	G	G	G	G	G,1E	G,1FOS		
SED-05G:0-10	5	2	20		N	N	N	1M	N	N	1FOS, 1E	N	N	N		
SED-27G:0-10	1	34	20		N	N	N	N	N	1M	1M	N	N	N		
SED-27G:0-10	2	6	20		N	1FOS	N	1E	N	1FOS, 1E	1FOS	N	N	N		
SED-27G:0-10	3	25	20		N	1FOS	N	N	N	1M	N	N	1M	N		
SED-27G:0-10	4	69	20		N	N	N	N	N	N	N	N	N	N		
SED-27G:0-10	5	60	20		N	2FOS	1FOS	N	N	N	N	N	N	2FOS		

CLIENT Leon Environmental		PROJECT Port of Friday Harbor			PROJECT NUMBER PG1785			
PROJECT MANAGER J. Levengood, M. Seibert		SPECIES <i>Eohaustorius estuarius</i>						
Sample ID	Rep	Jar #	# Initiated	Date Recovered:		5/22/2023		
				# Alive	# Dead	Initials	# Missing or Dead	
Control	1	64	20	17	0	SZ	3	
Control	2	72	20	15	1	DM	5	
Control	3	1	20	17	0	SZ	3	
Control	4	38	20	18	0	DM	2	
Control	5	22	20	17	0	SZ	3	
CARR18-23-REF	1	54	20	18	0	DM	2	
CARR18-23-REF	2	24	20	18	0	SZ	2	
CARR18-23-REF	3	30	20	18	0	DM	2	
CARR18-23-REF	4	65	20	14	0	SZ	6	
CARR18-23-REF	5	7	20	18	0	DM	2	
CARR40-23-REF	1	53	20	15	0	SZ	5	
CARR40-23-REF	2	8	20	18	0	DM	2	
CARR40-23-REF	3	39	20	18	1	SZ	2	
CARR40-23-REF	4	10	20	18	0	DM	2	
CARR40-23-REF	5	14	20	16	0	SZ	4	
CARR62-23-REF	1	26	20	15	2	DM	5	
CARR62-23-REF	2	19	20	19	0	SZ	1	
CARR62-23-REF	3	3	20	15	0	SZ	5	
CARR62-23-REF	4	40	20	18	0	SZ	2	
CARR62-23-REF	5	15	20	18	0	DM	2	
SED-07G:0-10	1	63	20	15	0	SZ	5	
SED-07G:0-10	2	55	20	13	0	DM	7	
SED-07G:0-10	3	50	20	13	2	SZ	7	
SED-07G:0-10	4	46	20	18	0	DM	2	
SED-07G:0-10	5	20	20	19	0	SZ	1	
SED-11G:0-10	1	16	20	13	1	DM	7	
SED-11G:0-10	2	47	20	16	1	DM	4	
SED-11G:0-10	3	61	20	17	0	SZ	3	
SED-11G:0-10	4	56	20	18	0	SZ	2	
SED-11G:0-10	5	44	20	18	0	DM	2	

CLIENT Leon Environmental		PROJECT Port of Friday Harbor			PROJECT NUMBER PG1785			
PROJECT MANAGER J. Levengood, M. Seibert		SPECIES <i>Eohaustorius estuarius</i>						
Sample ID	Rep	Jar #	# Initiated	Date Recovered:		5/22/2023		
				# Alive	# Dead	Initials	# Missing or Dead	
SED-14G:0-10	1	41	20	18	0	SZ	2	
SED-14G:0-10	2	9	20	16	0	SZ	4	
SED-14G:0-10	3	33	20	14	0	DM	6	
SED-14G:0-10	4	68	20	17	0	DM	3	
SED-14G:0-10	5	58	20	15	0	SZ	5	
SED-15G:0-10	1	71	20	15	0	SZ	5	
SED-15G:0-10	2	66	20	19	0	SZ	1	
SED-15G:0-10	3	36	20	16	0	SZ	4	
SED-15G:0-10	4	23	20	17	1	DM	3	
SED-15G:0-10	5	49	20	20	0	SZ	0	
SED-03G:0-10	1	31	20	16	1	SZ	4	
SED-03G:0-10	2	13	20	14	0	DM	6	
SED-03G:0-10	3	21	20	12	1	SZ	8	
SED-03G:0-10	4	32	20	12	1	DM	8	
SED-03G:0-10	5	57	20	18	0	SZ	2	
SED-01G:0-10	1	59	20	19	0	SZ	1	
SED-01G:0-10	2	5	20	15	0	DM	5	
SED-01G:0-10	3	12	20	19	0	SZ	1	
SED-01G:0-10	4	27	20	16	0	SZ	4	
SED-01G:0-10	5	42	20	18	0	DM	2	
SED-05G:0-10	1	11	20	17	0	SZ	3	
SED-05G:0-10	2	70	20	17	0	DM	3	
SED-05G:0-10	3	29	20	16	0	SZ	4	
SED-05G:0-10	4	52	20	15	0	DM	5	
SED-05G:0-10	5	2	20	17	0	SZ	3	
SED-27G:0-10	1	34	20	17	0	SZ	3	
SED-27G:0-10	2	6	20	16	0	DM	4	
SED-27G:0-10	3	25	20	16	0	SZ	4	
SED-27G:0-10	4	69	20	18	0	DM	2	
SED-27G:0-10	5	60	20	14	2	SZ	6	

CLIENT		PROJECT			PROJECT NUMBER		SPECIES		
Leon Environmental		Port of Friday Harbor			PG1785		<i>Eohaustorius estuarius</i>		
Sample ID	Rep	# Initiated		Survival Statistics			Mortality Statistics		
			# Alive	% Survival	Mean Survival (%)	SD	% Mortality	Mean Mortality (%)	SD
Control	1	20	17	85			15		
Control	2	20	15	75			25		
Control	3	20	17	85			15		
Control	4	20	18	90			10		
Control	5	20	17	85	84.0	5.5	15	16.0	5.5
CARR18-23-REF	1	20	18	90			10		
CARR18-23-REF	2	20	18	90			10		
CARR18-23-REF	3	20	18	90			10		
CARR18-23-REF	4	20	14	70			30		
CARR18-23-REF	5	20	18	90	86.0	8.9	10	14.0	8.9
CARR40-23-REF	1	20	15	75			25		
CARR40-23-REF	2	20	18	90			10		
CARR40-23-REF	3	20	18	90			10		
CARR40-23-REF	4	20	18	90			10		
CARR40-23-REF	5	20	16	80	85.0	7.1	20	15.0	7.1
CARR62-23-REF	1	20	15	75			25		
CARR62-23-REF	2	20	19	95			5		
CARR62-23-REF	3	20	15	75			25		
CARR62-23-REF	4	20	18	90			10		
CARR62-23-REF	5	20	18	90	85.0	9.4	10	15.0	9.4
SED-07G:0-10	1	20	15	75			25		
SED-07G:0-10	2	20	13	65			35		
SED-07G:0-10	3	20	13	65			35		
SED-07G:0-10	4	20	18	90			10		
SED-07G:0-10	5	20	19	95	78.0	14.0	5	22.0	14.0
SED-11G:0-10	1	20	13	65			35		
SED-11G:0-10	2	20	16	80			20		
SED-11G:0-10	3	20	17	85			15		
SED-11G:0-10	4	20	18	90			10		
SED-11G:0-10	5	20	18	90	82.0	10.4	10	18.0	10.4
SED-14G:0-10	1	20	18	90			10		
SED-14G:0-10	2	20	16	80			20		
SED-14G:0-10	3	20	14	70			30		
SED-14G:0-10	4	20	17	85			15		
SED-14G:0-10	5	20	15	75	80.0	7.9	25	20.0	7.9
SED-15G:0-10	1	20	15	75			25		
SED-15G:0-10	2	20	19	95			5		
SED-15G:0-10	3	20	16	80			20		
SED-15G:0-10	4	20	17	85			15		
SED-15G:0-10	5	20	20	100	87.0	10.4	0	13.0	10.4

SED-03G:0-10	1	20	16	80			20		
SED-03G:0-10	2	20	14	70			30		
SED-03G:0-10	3	20	12	60			40		
SED-03G:0-10	4	20	12	60			40		
SED-03G:0-10	5	20	18	90	72.0	13.0	10	28.0	13.0
SED-01G:0-10	1	20	19	95			5		
SED-01G:0-10	2	20	15	75			25		
SED-01G:0-10	3	20	19	95			5		
SED-01G:0-10	4	20	16	80			20		
SED-01G:0-10	5	20	18	90	87.0	9.1	10	13.0	9.1
SED-05G:0-10	1	20	17	85			15		
SED-05G:0-10	2	20	17	85			15		
SED-05G:0-10	3	20	16	80			20		
SED-05G:0-10	4	20	15	75			25		
SED-05G:0-10	5	20	17	85	82.0	4.5	15	18.0	4.5
SED-27G:0-10	1	20	17	85			15		
SED-27G:0-10	2	20	16	80			20		
SED-27G:0-10	3	20	16	80			20		
SED-27G:0-10	4	20	18	90			10		
SED-27G:0-10	5	20	14	70	81.0	7.4	30	19.0	7.4

[illegible]

[illegible]



[illegible]

1 of 1

## 1.2 *Eohaustorius estuarius* Statistical Results

Project Name: Port of Friday Harbor Eohs

Sample:	x1
Samp ID:	SED-07G:0-10
Alias:	P230324.01
Replicates:	5
Mean:	0.22
SD:	0.14
Tr Mean:	2.549
Trans SD:	0.958

Ref Samp:	x2
Ref ID:	CARR18-23-REF
Alias:	P230510.02
Replicates:	5
Mean:	0.14
SD:	0.089
Tr Mean:	2.078
Trans SD:	0.594

Shapiro-Wilk Results:

Levene's Results:

Test Results:

Residual Mean:	0
Residual SD:	0.517
SS:	5.081
K:	5
b:	2.165

Test Residual Mean:	0.801
Test Residual SD:	0.339
Ref. Residual Mean:	0.425
Ref. Residual SD:	0.356
Deg. of Freedom:	8

Statistic:	Student's t
Balanced Design:	Yes
Transformation:	ArcSin

Alpha Level:	0.05
Calculated Value:	0.9228
Critical Value:	$\leq 0.842$

Alpha Level:	0.1
Calculated Value:	1.7131
Critical Value:	$\geq 1.860$

	Experimental Hypothesis
Null:	$x_1 \leq x_2$
Alternate:	$x_1 > x_2$

Normally Distributed:	Yes
Override Option:	N/A

Variances  
Homogeneous: Yes

Degrees of Freedom:	8
Experimental Alpha Level:	0.05
Calculated Value:	0.9342
Critical Value:	$\geq 1.860$
Accept Null Hypothesis:	Yes

Power:  
Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor Eohs

Sample:	x1
Samp ID:	SED-11G:0-10
Alias:	P230324.02
Replicates:	5
Mean:	0.18
SD:	0.104
Tr Mean:	N/A
Trans SD:	N/A

Ref Samp:	x2	
Ref ID:	CARR18-23-REF	
Alias:	P230510.02	
Replicates:		5
Mean:		0.14
SD:		0.089
Tr Mean:	N/A	
Trans SD:	N/A	

Shapiro-Wilk Results:

Levene's Results:

Test Results:

Residual Mean:	0
Residual SD:	0.406
SS:	3.136
K:	5
b:	1.559

Test Residual Mean:	0.494
Test Residual SD:	0.355
Ref. Residual Mean:	0.425
Ref. Residual SD:	0.356
Deg. of Freedom:	8

Statistic:	Mann-Whitney
Balanced Design:	Yes
Transformation:	rank-order

Alpha Level: 0.05  
Calculated Value: 0.7755  
Critical Value:  $\leq 0.842$

Alpha Level:	0.1
Calculated Value:	0.308
Critical Value:	$\geq 1.860$

	Experimental Hypothesis
Null:	$x_1 \leq x_2$
Alternate:	$x_1 > x_2$

Normally Distributed:	No
Override Option:	Not Invoked

Variances  
Homogeneous: Yes

Mann-Whitney N1:	5
Mann-Whitney N2:	5
Degrees of Freedom:	
Experimental Alpha Level:	0.05
Calculated Value:	17
Critical Value:	$\geq 21.000$
Accept Null Hypothesis:	Yes

Power:  
Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor- Eoh

Sample:	x1
Samp ID:	SED-01G:0-10
Alias:	P230324.06
Replicates:	5
Mean:	0.13
SD:	0.091
Tr Mean:	1.961
Trans SD:	0.729

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	0.15
SD:	0.071
Tr Mean:	2.173
Trans SD:	0.506

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.407 SS: 3.151 K: 5 b: 1.668  Alpha Level: 0.05 Calculated Value: 0.8827 Critical Value: $\leq 0.842$	Test Residual Mean: 0.603 Test Residual SD: 0.278 Ref. Residual Mean: 0.433 Ref. Residual SD: 0.146 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 1.2097 Critical Value: $\geq 1.860$	Statistic: Student's t Balanced Design: Yes Transformation: ArcSin  Experimental Hypothesis Null: $x_1 \leq x_2$ Alternate: $x_1 > x_2$
Normally Distributed: Yes  Override Option: N/A	Variances Homogeneous: Yes	Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: -0.535 Critical Value: $\geq 1.860$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor Eohs

Sample:	x1
Samp ID:	SED-27G:0-10
Alias:	P230324.08
Replicates:	5
Mean:	0.19
SD:	0.074
Tr Mean:	2.46
Trans SD:	0.49

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	0.15
SD:	0.071
Tr Mean:	2.173
Trans SD:	0.506

Shapiro-Wilk Results:

Levene's Results:

**Test Results:**

Residual Mean:	0
Residual SD:	0.323
SS:	1.984
K:	5
b:	1.343

Test Residual Mean:	0.355
Test Residual SD:	0.288
Ref. Residual Mean:	0.433
Ref. Residual SD:	0.146
Deg. of Freedom:	8

Statistic:	Student's t
Balanced Design:	Yes
Transformation:	ArcSin

Alpha Level:	0.05
Calculated Value:	0.9094
Critical Value:	$\leq 0.842$

Alpha Level:	0.1
Calculated Value:	0.5424
Critical Value:	$\geq 1.860$

Null:  $x1 \leq x2$   
Alternate:  $x1 > x2$

Normally Distributed: Yes

Variances  
Homogeneous: Yes

Degrees of Freedom:	8
Experimental Alpha Level:	0.05
Calculated Value:	0.9095
Critical Value:	$\geq 1.860$
Accept Null Hypothesis:	Yes

Override Option: N/A

Power:  
Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor Eohs

Sample:	x1
Samp ID:	SED-15G:0-10
Alias:	P230324.04
Replicates:	5
Mean:	0.13
SD:	0.104
Tr Mean:	1.786
Trans SD:	1.162

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	0.15
SD:	0.071
Tr Mean:	2.173
Trans SD:	0.506

Shapiro-Wilk Results:

Levene's Results:

Test Results:

Residual Mean:	0
Residual SD:	0.582
SS:	6.426
K:	5
b:	2.42

Test Residual Mean:	0.916
Test Residual SD:	0.549
Ref. Residual Mean:	0.433
Ref. Residual SD:	0.146
Deg. of Freedom:	8

Statistic:	Approximate t
Balanced Design:	Yes
Transformation:	ArcSin

Alpha Level:	0.05
Calculated Value:	0.911
Critical Value:	$\leq 0.842$

Alpha Level:	0.1
Calculated Value:	1.9024
Critical Value:	$\geq 1.860$

	Experimental Hypothesis
Null:	$x_1 \leq x_2$
Alternate:	$x_1 > x_2$

Normally Distributed:	Yes
Override Option:	N/A

Variances  
Homogeneous: No

Degrees of Freedom:	5
Experimental Alpha Level:	0.05
Calculated Value:	-0.6829
Critical Value:	$\geq 2.015$
Accept Null Hypothesis:	Yes

Power:  
Min. Difference for Power:

[illegible]



Project Name: Port of Friday Harbor Eohs

Sample:	x1
Samp ID:	SED-03G:0-10
Alias:	P230324.05
Replicates:	5
Mean:	0.28
SD:	0.13
Tr Mean:	2.953
Trans SD:	0.774

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	0.15
SD:	0.071
Tr Mean:	2.173
Trans SD:	0.506

Shapiro-Wilk Results:

Levene's Results:

**Test Results:**

Residual Mean:	0
Residual SD:	0.424
SS:	3.418
K:	5
b:	1.738

Test Residual Mean:	0.613
Test Residual SD:	0.36
Ref. Residual Mean:	0.433
Ref. Residual SD:	0.146
Deg. of Freedom:	8

Statistic:	Student's t
Balanced Design:	Yes
Transformation:	ArcSin

Alpha Level:	0.05
Calculated Value:	0.8833
Critical Value:	$\leq 0.842$

Alpha Level:	0.1
Calculated Value:	1.0337
Critical Value:	$\geq 1.860$

	Experimental Hypothesis
Null:	$x_1 \leq x_2$
Alternate:	$x_1 > x_2$

Normally Distributed: Yes

Variances  
Homogeneous: Yes

Degrees of Freedom:	8
Experimental Alpha Level:	0.05
Calculated Value:	1.8877
Critical Value:	$\geq 1.860$
Accept Null Hypothesis:	No

Power:  
Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor- Eoh

Sample:	x1
Samp ID:	SED-14G:0-10
Alias:	P230324.03
Replicates:	5
Mean:	0.2
SD:	0.079
Tr Mean:	2.52
Trans SD:	0.524

Ref Samp:	x2
Ref ID:	CARR62-23-REF
Alias:	P230510.04
Replicates:	5
Mean:	0.15
SD:	0.094
Tr Mean:	2.128
Trans SD:	0.708

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.404 SS: 3.103 K: 5 b: 1.68  Alpha Level: 0.05 Calculated Value: 0.91 Critical Value: $\leq 0.842$  Normally Distributed: Yes  Override Option: N/A	Test Residual Mean: 0.403 Test Residual SD: 0.266 Ref. Residual Mean: 0.591 Ref. Residual SD: 0.255 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 1.1362 Critical Value: $\geq 1.860$  Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: ArcSin  Experimental Hypothesis Null: $x_1 \leq x_2$ Alternate: $x_1 > x_2$  Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: 0.9969 Critical Value: $\geq 1.860$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor Eohs

Sample:	x1
Samp ID:	SED-05G:0-10
Alias:	P230324.07
Replicates:	5
Mean:	0.18
SD:	0.045
Tr Mean:	2.418
Trans SD:	0.291

Ref Samp:	x2
Ref ID:	CARR62-23-REF
Alias:	P230510.04
Replicates:	5
Mean:	0.15
SD:	0.094
Tr Mean:	2.128
Trans SD:	0.708

Shapiro-Wilk Results:

Levene's Results:

Test Results:

Residual Mean:	0
Residual SD:	0.351
SS:	2.346
K:	5
b:	1.462

Test Residual Mean:	0.238
Test Residual SD:	0.12
Ref. Residual Mean:	0.591
Ref. Residual SD:	0.255
Deg. of Freedom:	8

Statistic:	Approximate t
Balanced Design:	Yes
Transformation:	ArcSin

Alpha Level:	0.05
Calculated Value:	0.9111
Critical Value:	$\leq 0.842$

Alpha Level:	0.1
Calculated Value:	2.8001
Critical Value:	$\geq 1.860$

	Experimental Hypothesis
Null:	$x_1 \leq x_2$
Alternate:	$x_1 > x_2$

Normally Distributed: Yes

Variances  
Homogeneous: No

Degrees of Freedom:	5
Experimental Alpha Level:	0.05
Calculated Value:	0.8471
Critical Value:	$\geq 2.015$
Accept Null Hypothesis:	Yes

Override Option: N/A

Power:  
Min. Difference for Power:

[illegible]

### 1.3 *Eohaustorius estuarius* Reference Toxicant Test Results

## Reference Toxicant 96-h Acute Survival Test

All Matching Labs

Test Type: Survival

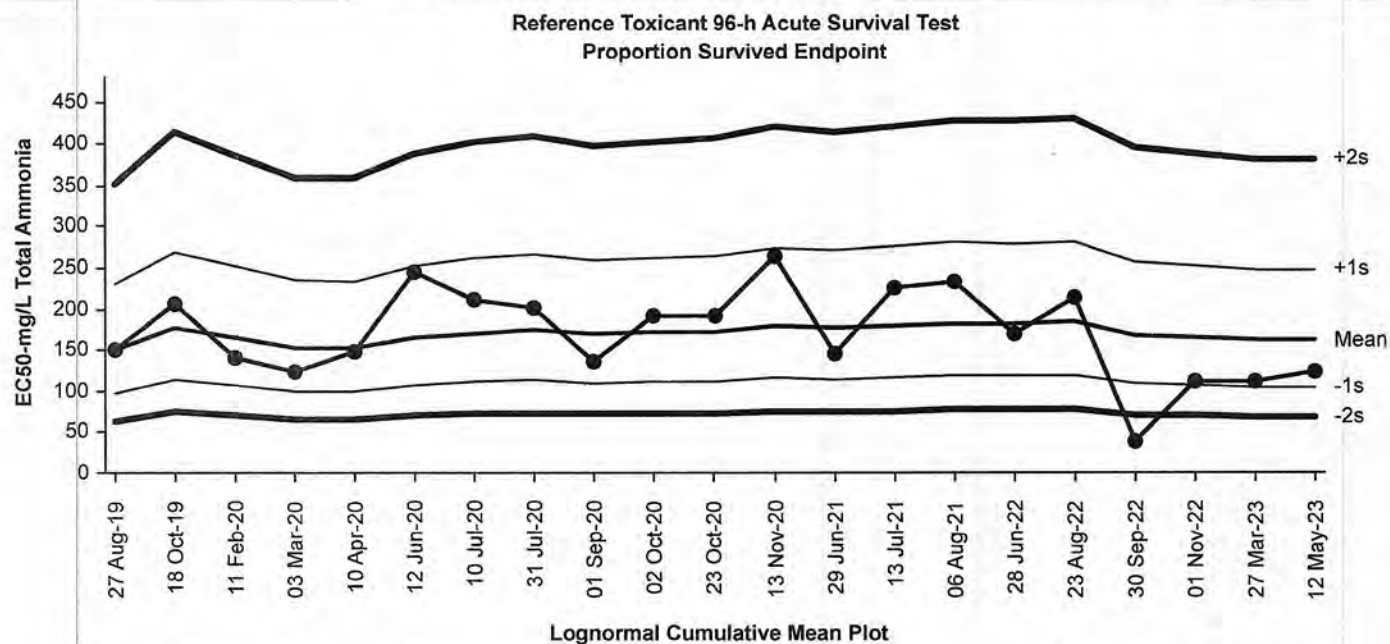
Organism: Eohaustorius estuarius

Material: Total Ammonia

Protocol: EPA/600/R-94/025 (1994)

Endpoint: Proportion Survived

Source: Reference Toxicant-REF



Mean: 161.3

Count: 20

-1s Warning Limit: 105

-2s Action Limit: 68.4

Sigma: NA

CV: 44.90%

+1s Warning Limit: 248

+2s Action Limit: 380

## Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2019	Aug	27	14:25	149.5	-11.76	-0.1766			16-1141-7710	18-3800-2446	EcoAnalysts
2		Oct	18	14:40	206.9	45.69	0.5817			10-1139-9672	04-6029-5418	EcoAnalysts
3	2020	Feb	11	11:30	141.8	-19.48	-0.3001			09-3507-3124	12-7490-3807	EcoAnalysts
4		Mar	3	10:50	123.7	-37.52	-0.6175			01-7038-2185	20-8945-2710	EcoAnalysts
5		Apr	10	14:35	148.2	-13.02	-0.1963			15-3093-6458	02-4348-5257	EcoAnalysts
6		Jun	12	14:25	244.4	83.19	0.9699			17-6535-4072	07-7299-7620	EcoAnalysts
7		Jul	10	14:19	210.4	49.12	0.62			21-2712-7178	15-5267-1443	EcoAnalysts
8			31	13:31	200.1	38.86	0.5033			19-0166-9324	03-8578-2577	EcoAnalysts
9		Sep	1	11:50	136.2	-25.01	-0.393			02-9531-4833	11-6890-2134	EcoAnalysts
10		Oct	2	13:55	191.4	30.16	0.3998			10-8959-5787	01-2752-4170	EcoAnalysts
11			23	10:35	190.7	29.48	0.3914			09-8256-6636	12-6068-0049	EcoAnalysts
12		Nov	13	15:42	264.7	103.5	1.156	(+)		12-1804-8521	19-5275-8749	EcoAnalysts
13	2021	Jun	29	16:15	144.4	-16.86	-0.2574			07-6082-1836	05-1033-0371	EcoAnalysts
14		Jul	13	13:45	225.7	64.41	0.7835			15-1767-0094	07-8834-3646	EcoAnalysts
15		Aug	6	15:40	233.3	72.08	0.8615			13-1244-3040	10-3339-7021	EcoAnalysts
16	2022	Jun	28	14:55	169.4	8.142	0.1148			02-4574-4479	16-9394-6529	EcoAnalysts
17		Aug	23	15:40	214.4	53.14	0.664			13-3970-9795	17-9108-7239	EcoAnalysts
18		Sep	30	14:50	37.97	-123.3	-3.372	(-)	(-)	13-9618-4863	18-1195-4011	EcoAnalysts
19		Nov	1	15:42	112	-49.24	-0.8495			18-4264-0694	03-2825-9179	EcoAnalysts
20	2023	Mar	27	14:52	110.7	-50.52	-0.8763			00-4639-3907	16-8783-1532	EcoAnalysts
21		May	12	14:18	124.5	-36.81	-0.6041			01-7728-8936	00-8061-3184	EcoAnalysts

## Reference Toxicant 96-h Acute Survival Test

All Matching Labs

Test Type: Survival

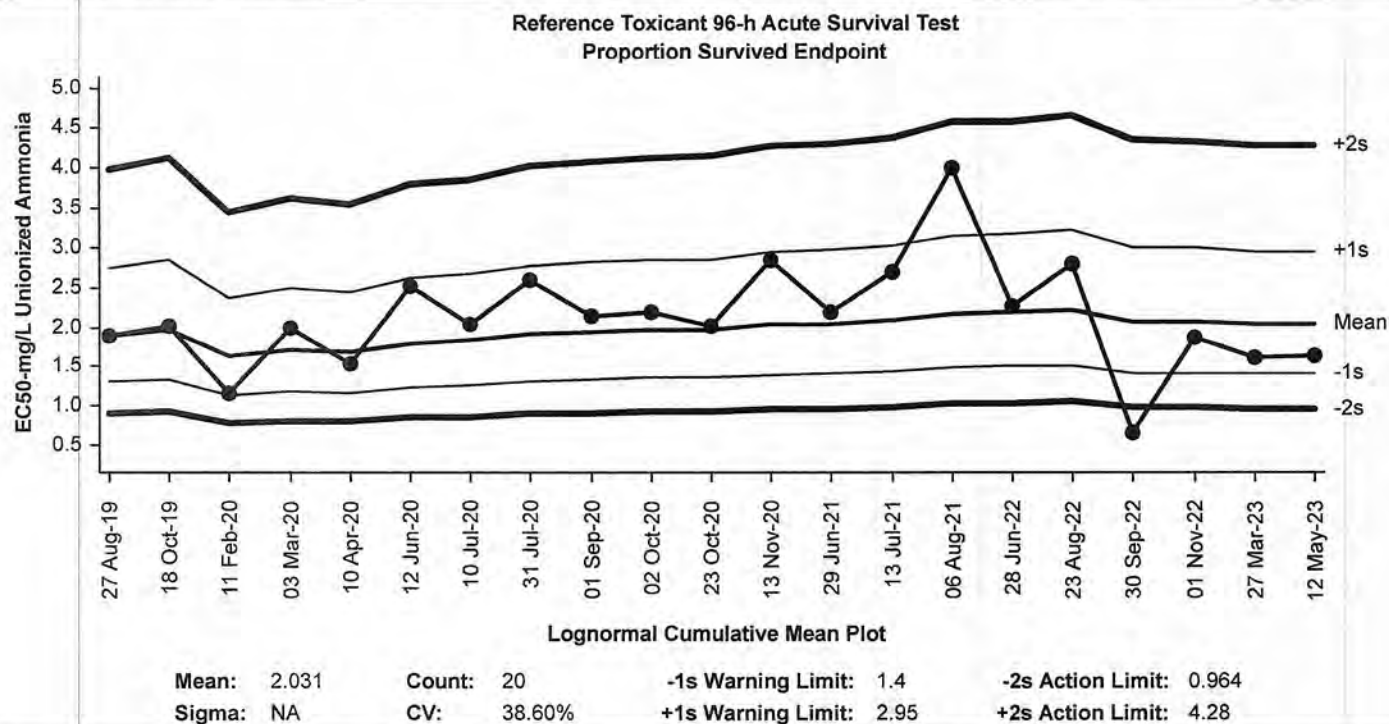
Organism: Eohaustorius estuarius

Material: Unionized Ammonia

Protocol: EPA/600/R-94/025 (1994)

Endpoint: Proportion Survived

Source: Reference Toxicant-REF



## Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2019	Aug	27	14:25	1.888	-0.1433	-0.1962			18-5396-1387	02-7188-5010	EcoAnalysts
2		Oct	18	14:40	2.025	-0.00655	-0.00867			01-8678-8913	10-6861-7593	EcoAnalysts
3	2020	Feb	11	11:30	1.153	-0.8779	-1.519	(-)		20-1044-3853	15-7919-2486	EcoAnalysts
4		Mar	3	10:50	2	-0.03156	-0.04202			02-4224-7585	10-8613-3376	EcoAnalysts
5		Apr	10	14:35	1.532	-0.4992	-0.7568			03-6100-0320	17-4770-7985	EcoAnalysts
6		Jun	12	14:25	2.515	0.4838	0.5733			12-0347-2631	12-6656-5651	EcoAnalysts
7		Jul	10	14:19	2.036	0.004736	0.00625			19-2747-2298	11-5216-9625	EcoAnalysts
8			31	13:31	2.604	0.5731	0.6668			00-3001-9040	12-3550-9219	EcoAnalysts
9		Sep	1	11:50	2.15	0.1185	0.1521			20-8197-8748	01-8924-2597	EcoAnalysts
10		Oct	2	13:55	2.192	0.1613	0.205			11-7285-1322	05-0028-0196	EcoAnalysts
11			23	10:35	2.014	-0.01758	-0.02333			06-9998-7880	19-7260-2994	EcoAnalysts
12		Nov	13	15:42	2.846	0.8147	0.9049			18-9101-0953	11-1982-6601	EcoAnalysts
13	2021	Jun	29	16:15	2.193	0.1616	0.2054			19-9925-7350	18-0084-5381	EcoAnalysts
14		Jul	13	13:45	2.706	0.6751	0.7699			19-3066-6785	16-4976-7245	EcoAnalysts
15		Aug	6	15:40	3.994	1.963	1.815	(+)		10-9355-9036	08-0099-3943	EcoAnalysts
16	2022	Jun	28	14:55	2.257	0.2256	0.2825			15-1320-8403	14-1461-4017	EcoAnalysts
17		Aug	23	15:40	2.789	0.7575	0.8505			19-4730-2098	12-6509-7347	EcoAnalysts
18		Sep	30	14:50	0.6533	-1.378	-3.043	(-)	(-)	18-5906-4045	01-7092-5166	EcoAnalysts
19		Nov	1	15:42	1.868	-0.1634	-0.2251			18-6321-3380	11-1992-4810	EcoAnalysts
20	2023	Mar	27	14:52	1.614	-0.4169	-0.6163			00-2990-2720	12-5002-5640	EcoAnalysts
21		May	12	14:18	1.648	-0.3831	-0.5608			02-8407-4452	15-9902-1861	EcoAnalysts

# CETIS Summary Report

Report Date: 16 May-23 12:42 (p 1 of 1)  
Test Code/ID: P220819.48 / 01-7728-8936

## Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID: 16-8633-5109	Test Type: Survival	Analyst: Marisa Seibert
Start Date: 12 May-23 14:18	Protocol: EPA/600/R-94/025 (1994)	Diluent: Laboratory Seawater
Ending Date: 16 May-23 12:18	Species: Eohaustorius estuarius	Brine: Not Applicable
Test Length: 94h	Taxon: Malacostraca	Source: Northwest Amphipod, OR Age:
Sample ID: 04-9038-3513	Code: P220819.48	Project: Reference Toxicant
Sample Date: 19 Aug-22	Material: Total Ammonia	Source: Reference Toxicant
Receipt Date: 19 Aug-22	CAS (PC):	Station: P220819.48
Sample Age: 266d 14h	Client: Internal Lab	

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
17-7082-1184	Proportion Survived	Fisher Exact Test	89.1	163	120.5	---	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
00-8061-3184	Proportion Survived	Linear Interpolation (ICPIN)	EC15	92.13	12.92	105.6	1
			EC20	96.18	28.73	109.6	
			EC25	100.4	41.82	113.8	
			EC40	114.2	77.83	127.3	
			EC50	124.5	93.8	137.1	

## Proportion Survived Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	3	0.9000	0.9000	0.9000	0.9000	0.9000	0.0000	0.0000	0.00%	0.00%
23.7		3	0.9000	0.6516	1.1480	0.8000	1.0000	0.0577	0.1000	11.11%	0.00%
42.7		3	0.8667	0.7232	1.0100	0.8000	0.9000	0.0333	0.0577	6.66%	3.70%
89.1		3	0.8000	0.3697	1.2300	0.6000	0.9000	0.1000	0.1732	21.65%	11.11%
163		3	0.1667	0.0232	0.3101	0.1000	0.2000	0.0333	0.0577	34.64%	81.48%
330		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

## Proportion Survived Detail

MD5: C5020D62E0F69D537AA7A7BF0392DF77

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	0.9000	0.9000	0.9000
23.7		0.9000	0.8000	1.0000
42.7		0.9000	0.8000	0.9000
89.1		0.9000	0.6000	0.9000
163		0.2000	0.2000	0.1000
330		0.0000	0.0000	0.0000

## Proportion Survived Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	9/10	9/10	9/10
23.7		9/10	8/10	10/10
42.7		9/10	8/10	9/10
89.1		9/10	6/10	9/10
163		2/10	2/10	1/10
330		0/10	0/10	0/10

## CETIS Summary Report

Report Date: 16 May-23 12:43 (p 1 of 1)

Test Code/ID: P220819.48UIA / 02-8407-4452

## Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID: 16-8633-5109	Test Type: Survival	Analyst: Marisa Seibert
Start Date: 12 May-23 14:18	Protocol: EPA/600/R-94/025 (1994)	Diluent: Laboratory Seawater
Ending Date: 16 May-23 12:18	Species: Eohaustorius estuarius	Brine: Not Applicable
Test Length: 94h	Taxon: Malacostraca	Source: Northwest Amphipod, OR Age:
Sample ID: 01-4528-0220	Code: P220819.48UIA	Project: Reference Toxicant
Sample Date: 19 Aug-22	Material: Unionized Ammonia	Source: Reference Toxicant
Receipt Date: 19 Aug-22	CAS (PC):	Station: P220819.48UIA
Sample Age: 266d 14h	Client: Internal Lab	

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
16-0735-8629	Proportion Survived	Fisher Exact Test	1.516	1.76	1.633	---	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
15-9902-1861	Proportion Survived	Linear Interpolation (ICPIN)	EC15	1.529	0.6088	1.583	1
			EC20	1.546	0.8459	1.596	
			EC25	1.562	1.071	1.609	
			EC40	1.613	1.472	1.654	
			EC50	1.648	1.543	1.685	

## Proportion Survived Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	3	0.9000	0.9000	0.9000	0.9000	0.9000	0.0000	0.0000	0.00%	0.00%
0.642		3	0.9000	0.6516	1.1480	0.8000	1.0000	0.0577	0.1000	11.11%	0.00%
0.91		3	0.8667	0.7232	1.0100	0.8000	0.9000	0.0333	0.0577	6.66%	3.70%
1.516		3	0.8000	0.3697	1.2300	0.6000	0.9000	0.1000	0.1732	21.65%	11.11%
1.76		3	0.1667	0.0232	0.3101	0.1000	0.2000	0.0333	0.0577	34.64%	81.48%
2.815		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

## Proportion Survived Detail

MD5: 71C2F4569F3CC4A4A1E4625366BC0A26

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	0.9000	0.9000	0.9000
0.642		0.9000	0.8000	1.0000
0.91		0.9000	0.8000	0.9000
1.516		0.9000	0.6000	0.9000
1.76		0.2000	0.2000	0.1000
2.815		0.0000	0.0000	0.0000

## Proportion Survived Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	9/10	9/10	9/10
0.642		9/10	8/10	10/10
0.91		9/10	8/10	9/10
1.516		9/10	6/10	9/10
1.76		2/10	2/10	1/10
2.815		0/10	0/10	0/10



# CETIS Test Data Worksheet

Report Date: 16 May-23 12:42 (p 1 of 1)  
Test Code/ID: P220819.48 / 01-7728-8936

Reference Toxicant 96-h Acute Survival Test					EcoAnalysts		
Start Date: 12 May-23 14:18		Species: Eohaustorius estuarius		Sample Code: P220819.48			
End Date: 16 May-23 12:18		Protocol: EPA/600/R-94/025 (1994)		Sample Source: Reference Toxicant			
Sample Date: 19 Aug-22		Material: Total Ammonia		Sample Station: P220819.48			

Conc-mg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	1	10	9	
0	D	2	15	10	9	
0	D	3	14	10	9	
23.7		1	11	10	9	
23.7		2	10	10	8	
23.7		3	6	10	10	
42.7		1	18	10	9	
42.7		2	13	10	8	
42.7		3	2	10	9	
89.1		1	7	10	9	
89.1		2	5	10	6	
89.1		3	8	10	9	
163		1	3	10	2	
163		2	4	10	2	
163		3	17	10	1	
330		1	9	10	0	
330		2	16	10	0	
330		3	12	10	0	

# CETIS Test Data Worksheet

Report Date: 16 May-23 12:43 (p 1 of 1)  
 Test Code/ID: P220819.48UIA / 02-8407-4452

Reference Toxicant 96-h Acute Survival Test					EcoAnalysts		
Start Date: 12 May-23 14:18		Species: Eohaustorius estuarius		Sample Code: P220819.48UIA			
End Date: 16 May-23 12:18		Protocol: EPA/600/R-94/025 (1994)		Sample Source: Reference Toxicant			
Sample Date: 19 Aug-22		Material: Unionized Ammonia		Sample Station: P220819.48UIA			
Conc-mg/L	Code	Rep	Pos	# Exposed	# Survived	Notes	
0	D	1	17	10	9		
0	D	2	5	10	9		
0	D	3	6	10	9		
0.642		1	11	10	9		
0.642		2	3	10	8		
0.642		3	14	10	10		
0.91		1	9	10	9		
0.91		2	2	10	8		
0.91		3	7	10	9		
1.516		1	15	10	9		
1.516		2	13	10	6		
1.516		3	16	10	9		
1.76		1	18	10	2		
1.76		2	12	10	2		
1.76		3	4	10	1		
2.815		1	1	10	0		
2.815		2	8	10	0		
2.815		3	10	10	0		

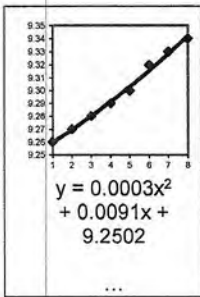
# Un-ionized Ammonia Calculator

CLIENT:	Leon Environmental	Date of Test:	May 12, 2023
PROJECT:	Port of Friday Harbor	Test Type:	Eohaustorius estuarius
COMMENTS:	P220819.48 48 ①		

To convert Total Ammonia (mg/L) to Free (un-ionized) Ammonia (mg/L) enter the corresponding total ammonia, salinity, temperature, and pH.

Sample		Mod	NH3T (mg/L)	salinity (ppt)	pH	temp (C)	temp (K)	pKa <sup>s</sup>	NH <sub>3</sub> U (mg/L)
Target / Sample Name			Actual	Actual	Actual	Actual	Calculated	Calculated	Calculated
Example 3.5			2.000	10.0	7.5	5.0	278.15	9.2520	0.008
1									
2	20		23.7	28	8.0	15.6	288.75	9.2555	0.642
3	40		42.7	28	7.9	15.4	288.55	9.2555	0.910
4	80		89.1	28	7.8	15.4	288.55	9.2555	1.516
5	160		163	29	7.6	15.4	288.55	9.2557	1.760
6	320		330	29	7.5	15.3	288.45	9.2557	2.815
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
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27									
28									
29									
30									
31									
32									
33									
34									
35									
36									
37									
38									
39									
40									
41									
42									
43									
44									
45									

Ionic strength:pKa <sup>s</sup>	
1	9.26
2	9.27
3	9.28
4	9.29
5	9.30
6	9.32
7	9.33
8	9.34



① incorrect number - ms 5/16

QA ✓  
ms

CLIENT Leon Environmental	PROJECT Port of Friday Harbor	Eohaustorius estuarius		Laboratory Port Gamble .	PROTOCOL PSEP, SCUM
TEST ID p220819.48	LOT #: 22E3156086	DILUTION PREP INITIALS: MK			
CHAMBER SIZE/TYPE Pint Jar	EXPOSURE VOLUME 250 ml	TEST START DATE 5/12/23	INITIALS MK/MS	TIME 1418	TEST END DATE 5/16/23
					TIME 1218 CG

WATER QUALITY DATA

TEST CONDITIONS				DO (mg/L)		TEMP(C)		SAL (ppt)		pH		TECHNICIAN	AMMONIA					
				> 5.1		15 ± 1		28 ± 1		7 - 9								
SAMPLE ID	CONCENTRATION		DAY	REP	D.O.		TEMP.		SALINITY		pH		WQ TECH/ DATE	AMMONIA		Tech		
	value	units			meter	mg/L	meter	°C	meter	ppt	meter	unit		METER	mg/L			
Ref.Tox.-ammonia	0	mg/L	0	Stock	8	7.9	8	15.6	8	28	8	8.0	MS 5/12	10		MK		
			4	1	9	7.1	9	16.0	9	28	9	7.8	MS 5/16					
Ref.Tox.-ammonia	20	mg/L	0	Stock	8	7.9	8	15.9	8	28	8	8.0	MS 5/12	10	23.7	MK		
			4	1	9	7.1	9	16.0	9	28	9	7.9	MS 5/16					
Ref.Tox.-ammonia	40	mg/L	0	Stock	8	8.0	8	15.4	8	28	8	7.9	MS 5/12	10	42.7	MK		
			4	1	9	7.5	9	15.8	9	28	9	7.9	MS 5/16					
Ref.Tox.-ammonia	80	mg/L	0	Stock	8	8.0	8	15.4	8	28	8	7.8	MS 5/12	10	89.1	MK		
			4	1	9	7.6	9	15.7	9	28	9	7.9	MS 5/16					
Ref.Tox.-ammonia	160	mg/L	0	Stock	8	7.9	8	15.4	8	29	8	7.6	MS 5/12	10	163	MK		
			4	1	9	7.8	9	15.6	9	28	9	7.9	MS 5/16					
Ref.Tox.-ammonia	320	mg/L	0	Stock	8	7.9	8	15.3	8	29	8	7.5	MS 5/12	10	330	MK		
			4	1	—	—	—	—	—	—	—	—	—					

①MR-MS 5/12

8220819.48

SPECIES <i>Eohaustorius estuarius</i>		
PROJECT MANAGER J. Levengood/ M. Seibert	LABORATORY Port Gamble	PROTOCOL PSEP, SCUM

CLIENT Leon Environmental	PROJECT Port of Friday Harbor
------------------------------	----------------------------------

**SURVIVAL & BEHAVIOR DATA**

OBSERVATION KEY N = Normal LOE = Loss of equilibrium Q = Quiescent DC = Discoloration NB = No body F = Floating on surface  INITIAL # OF ORGANISMS 10				DAY 1			DAY 2			DAY 3			DAY 4		
				DATE 5/13/23			DATE 5/14/23			DATE 5/15/23			DATE 5/16/23		
				TECHNICIAN NL			TECHNICIAN UG			TECHNICIAN SZ			TECHNICIAN UG		
SAMPLE ID	CONC. value units	REP	INITIAL NUMBER	#ALIVE	#DEAD	OBS	#ALIVE	#DEAD	OBS	#ALIVE	#DEAD	OBS	#ALIVE	#DEAD	OBS
Ref.Tox.- Ammonia	0 mg/L	1	10	9	1	3FOS	9	0	N	9	0	N	9	0	N
		2	10	9	1	2FOS	9	0	3FOS	9	0	N	9	0	1FOS
		3	10	9	1	2FOS	9	0	2FOS	9	0	1FOS	9	0	1FOS
Ref.Tox.- Ammonia	20 mg/L	1	10	10	0	3FOS	9	1	4FOS	9	0	2FOS	9	0	11FOS
		2	10	9	1	1FOS	9	0	N	8	1	2FOS	8	0	1FOS
		3	10	10	0	2FOS	10	0	1FOS	10	0	N	10	0	N
Ref.Tox.- Ammonia	40 mg/L	1	10	10	0	4FOS	9	1	2FOS	9	0	1FOS	9	0	N
		2	10	9	1	4FOS	9	0	3FOS	9	0	3FOS	8	1	3FOS
		3	10	10	0	3FOS	9	1	3FOS	9	0	2FOS	9	0	2FOS
Ref.Tox.- Ammonia	80 mg/L	1	10	10	0	3FOS	9	1	4FOS	9	0	1FOS	9	0	1FOS
		2	10	9	1	5FOS	9	0	3FOS	7	2	5FOS	6	1	2FOS
		3	10	9	1	5FOS	9	0	4FOS	9	0	5FOS	9	0	1FOS
Ref.Tox.- Ammonia	160 mg/L	1	10	8	2	1FOS	5	3	1Q	2	3	1Q	2	0	2Q
		2	10	7	3	3Q	5	2	3Q	3	2	3Q	2	1	2Q
		3	10	8	2	1FOS	4	4	1Q	4	0	N	1	3	1Q
Ref.Tox.- Ammonia	320 mg/L	1	10	0	10	-									
		2	10	0	10	-									
		3	10	1	9	1Q	0	1	-						



# Ammonia Reference Toxicant Spiking Worksheet

Reference Toxicant ID: P220819.48  
 Date Prepared: 5/12/23  
 Technician Initials: ML

## Eoh NH<sub>3</sub> RT

Assumptions in Model  
 Stock ammonia concentration is 10,000 mg/L = 10 mg/mL

Date: 5/11/2023  
 Measurement: 8,340

Test Solutions			Volume of stock to reach desired concentration	
Measured Concentration	Desired Concentration	Volume		
mg/L	mg/L	mL	mL stock to increase	
				SALT WATER
23.7	20	750		2.698
42.7	40	750		5.396
89.1	80	750		10.791
163	160	750		21.583
330	320	750		43.165

1	16
2	1
3	2
4	10
5	11
6	6
7	14
8	13
9	12
10	15
11	18
12	9
13	8
14	4
15	7
16	17
17	3
18	5

P 220819-48  
 Foh NH<sub>3</sub> RT

**2.     *Neanthes arenaceodentata* 20-Day Solid-Phase Test**



## 2.1 *Neanthes arenaceodentata* Test Data

## GENERAL

<b>Client</b>	Leon Environmental			<b>Test Parameters</b>		
<b>Project</b>	Port of Friday Harbor				<b>Min</b>	<b>Max</b>
<b>Project Number</b>	PG1785			<b>DO:</b>	4.6	
<b>Project Manager</b>	Julia Levengood/Marisa Seibert	Note: input lowest and highest decimal for temp		<b>Temp:</b>	18.5	21.4
<b>Date Oldest Sample Collected</b>	3/20/2023			<b>Sal:</b>	26	30
<b>Sample Holding Time</b>	53			<b>pH:</b>	7	9
<b>Test Start Date</b>	05/12/23					
<b>Test Species</b>	<i>Neanthes arenaceodentata</i>					
<b>Organism Supplier</b>	Aquatic Toxicology Support	<b>Temp Hobo ID</b>		<b>TEST START TIME/INITIALS:</b> 0940 MK		
<b>Organism Acquired</b>	5/11/2023	NA		<b>TEST END TIME:</b> 1028 SZ/MS		
<b>Organism Acclimation</b>	1					
<b>Organism Age</b>	Juvenile (2-3 weeks post emergence)					
<b>Test Type/Duration</b>	20-Day Solid Phase					
<b>Test Protocol</b>	PSEP 1995					
<b>Regional Protocol</b>	SCUM 2019					
<b>Laboratory Location</b>	Port Gamble					
<b>Test Location</b>	Bath 2					
<b>Sample Treatment</b>	reference sed press sieved 2mm Sample SED-11G:0-10 press sieved 2mm					
<b>Control Sediment Source</b>	Yaquina Bay, OR					
<b>Water Batch</b>	FSW051023.01					
<b>Test Lighting</b>	Continuous					
<b>Test Chamber</b>	1 L glass jars					
<b>Replicates Per Treatment</b>	5 replicates + 2 surrogates					
<b>Organisms per Replicate</b>	5					
<b>Exposure Volume</b>	2 cm sediment/ 775 mL water	<b>Food Batch ID</b>				
<b>Feeding Information</b>	40 mg Tetramin/Replicate every other day	251523 454GR				
<b>Test Dissolved Oxygen</b>	> 4.6					
<b>Test Temperature</b>	20 ± 1					
<b>Test Salinity</b>	28 ± 2					
<b>Test pH</b>	8 ± 1					
<b>Water Renewal Info</b>	Renewed every third day					

	CLIENT SAMPLE ID	LAB ID
1	Control	P230510.01
2	CARR18-23-REF	P230510.02
3	CARR40-23-REF	P230510.03
4	CARR62-23-REF	P230510.04
5	SED-07G:0-10	P230324.01
6	SED-11G:0-10	P230324.02
7	SED-14G:0-10	P230324.03
8	SED-15G:0-10	P230324.04
9	SED-03G:0-10	P230324.05
10	SED-01G:0-10	P230324.06
11	SED-05G:0-10	P230324.07
12	SED-27G:0-10	P230324.08
13	.	.
14	.	.
15	.	.
16	.	.
17	.	.
18	.	.
19	.	.
20	.	.

## 2.2 *Neanthes arenaceodentata* Statistical Results

Project Name: Port of Friday Harbor - Neanthes Growth

Sample:	x1
Samp ID:	SED-07G:0-10
Alias:	P230324.01
Replicates:	5
Mean:	1.208
SD:	0.133
Tr Mean:	0.343
Trans SD:	0.026

Ref Samp:	x2
Ref ID:	CARR18-23-REF
Alias:	P230510.02
Replicates:	5
Mean:	1.412
SD:	0.41
Tr Mean:	0.378
Trans SD:	0.073

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.035 SS: 0.024 K: 5 b: 0.152  Alpha Level: 0.05 Calculated Value: 0.9697 Critical Value: $\leq 0.842$	Test Residual Mean: 0.021 Test Residual SD: 0.011 Ref. Residual Mean: 0.058 Ref. Residual SD: 0.033 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 2.3202 Critical Value: $\geq 1.860$	Statistic: Approximate t Balanced Design: Yes Transformation: $\text{Log}_{10}(x + 1.0)$  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$
Normally Distributed: Yes  Override Option: N/A	Variances Homogeneous: No	Degrees of Freedom: 5 Experimental Alpha Level: 0.05 Calculated Value: 0.9868 Critical Value: $\geq 2.015$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor - Neanthes Growth

Sample: x1
Samp ID: SED-11G:0-10
Alias: P230324.02
Replicates: 5
Mean: 0.737
SD: 0.258
Tr Mean: 0.737
Trans SD: 0.258

Ref Samp:	x2
Ref ID:	CARR18-23-REF
Alias:	P230510.02
Replicates:	5
Mean:	1.412
SD:	0.41
Tr Mean:	1.412
Trans SD:	0.41

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.222 SS: 0.939 K: 5 b: 0.945  Alpha Level: 0.05 Calculated Value: 0.9518 Critical Value: $\leq 0.842$  Normally Distributed: Yes  Override Option: N/A	Test Residual Mean: 0.216 Test Residual SD: 0.09 Ref. Residual Mean: 0.328 Ref. Residual SD: 0.184 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 1.2176 Critical Value: $\geq 1.860$  Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$  Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: 3.1187 Critical Value: $\geq 1.860$ Accept Null Hypothesis: No  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor- Neanthes Growth

Sample: x1
Samp ID: SED-01G:0-10
Alias: P230324.06
Replicates: 5
Mean: 1.343
SD: 0.338
Tr Mean: 1.343
Trans SD: 0.338

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	1.523
SD:	0.13
Tr Mean:	1.523
Trans SD:	0.13

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.166 SS: 0.524 K: 5 b: 0.702  Alpha Level: 0.05 Calculated Value: 0.9413 Critical Value: $\leq 0.842$  Normally Distributed: Yes  Override Option: N/A	Test Residual Mean: 0.257 Test Residual SD: 0.178 Ref. Residual Mean: 0.108 Ref. Residual SD: 0.049 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 1.7977 Critical Value: $\geq 1.860$  Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$  Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: 1.1119 Critical Value: $\geq 1.860$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor - Neanthes Growth

Sample:	x1
Samp ID:	SED-27G:0-10
Alias:	P230324.08
Replicates:	5
Mean:	1.035
SD:	0.434
Tr Mean:	0.3
Trans SD:	0.096

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	1.523
SD:	0.13
Tr Mean:	0.401
Trans SD:	0.022

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.045 SS: 0.039 K: 5 b: 0.192  Alpha Level: 0.05 Calculated Value: 0.9562 Critical Value: $\leq 0.842$	Test Residual Mean: 0.081 Test Residual SD: 0.03 Ref. Residual Mean: 0.018 Ref. Residual SD: 0.008 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 4.5396 Critical Value: $\geq 1.860$	Statistic: Approximate t Balanced Design: Yes Transformation: $\text{Log}_{10}(x + 1.0)$  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$
Normally Distributed: Yes  Override Option: N/A	Variances Homogeneous: No	Degrees of Freedom: 4 Experimental Alpha Level: 0.05 Calculated Value: 2.3011 Critical Value: $\geq 2.132$ Accept Null Hypothesis: No  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor - Neanthes Growth

Sample:	x1
Samp ID:	SED-15G:0-10
Alias:	P230324.04
Replicates:	5
Mean:	1.28
SD:	0.574
Tr Mean:	0.346
Trans SD:	0.113

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	1.523
SD:	0.13
Tr Mean:	0.401
Trans SD:	0.022

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.053 SS: 0.053 K: 5 b: 0.227  Alpha Level: 0.05 Calculated Value: 0.9679 Critical Value: $\leq 0.842$  Normally Distributed: Yes  Override Option: N/A	Test Residual Mean: 0.094 Test Residual SD: 0.042 Ref. Residual Mean: 0.018 Ref. Residual SD: 0.008 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 3.9361 Critical Value: $\geq 1.860$  Variances Homogeneous: No	Statistic: Approximate t Balanced Design: Yes Transformation: $\text{Log}_{10}(x + 1.0)$  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$  Degrees of Freedom: 4 Experimental Alpha Level: 0.05 Calculated Value: 1.0672 Critical Value: $\geq 2.132$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]



Project Name: Port of Friday Harbor - Neanthes Growth

Sample:	x1
Samp ID:	SED-03G:0-10
Alias:	P230324.05
Replicates:	5
Mean:	1.174
SD:	0.302
Tr Mean:	0.334
Trans SD:	0.061

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	1.523
SD:	0.13
Tr Mean:	0.401
Trans SD:	0.022

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0	Test Residual Mean: 0.049	Statistic: Approximate t
Residual SD: 0.03	Test Residual SD: 0.027	Balanced Design: Yes
SS: 0.017	Ref. Residual Mean: 0.018	Transformation: Log10 (x + 1.0)
K: 5	Ref. Residual SD: 0.008	
b: 0.128	Deg. of Freedom: 8	
		Experimental Hypothesis
Alpha Level: 0.05	Alpha Level: 0.1	Null: $x_1 \geq x_2$
Calculated Value: 0.9723	Calculated Value: 2.3655	Alternate: $x_1 < x_2$
Critical Value: $\leq 0.842$	Critical Value: $\geq 1.860$	
		Degrees of Freedom: 5
Normally	Variances	Experimental Alpha Level: 0.05
Distributed: Yes	Homogeneous: No	Calculated Value: 2.3247
		Critical Value: $\geq 2.015$
Override Option: N/A		Accept Null Hypothesis: No
		Power:
		Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor- Neanthes Growth

Sample: x1
Samp ID: SED-14G:0-10
Alias: P230324.03
Replicates: 5
Mean: 1.139
SD: 0.347
Tr Mean: 1.139
Trans SD: 0.347

Ref Samp:	x2
Ref ID:	CARR62-23-REF
Alias:	P230510.04
Replicates:	5
Mean:	1.649
SD:	0.283
Tr Mean:	1.649
Trans SD:	0.283

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.205 SS: 0.8 K: 5 b: 0.869  Alpha Level: 0.05 Calculated Value: 0.9441 Critical Value: $\leq 0.842$  Normally Distributed: Yes  Override Option: N/A	Test Residual Mean: 0.226 Test Residual SD: 0.238 Ref. Residual Mean: 0.199 Ref. Residual SD: 0.174 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 0.1998 Critical Value: $\geq 1.860$  Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$  Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: 2.5479 Critical Value: $\geq 1.860$ Accept Null Hypothesis: No  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor - Neanthes Growth

Sample:	x1
Samp ID:	SED-05G:0-10
Alias:	P230324.07
Replicates:	5
Mean:	1.274
SD:	0.373
Tr Mean:	1.274
Trans SD:	0.373

Ref Samp:	x2
Ref ID:	CARR62-23-REF
Alias:	P230510.04
Replicates:	5
Mean:	1.649
SD:	0.283
Tr Mean:	1.649
Trans SD:	0.283

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.215 SS: 0.874 K: 5 b: 0.902  Alpha Level: 0.05 Calculated Value: 0.9303 Critical Value: $\leq 0.842$  Normally Distributed: Yes  Override Option: N/A	Test Residual Mean: 0.292 Test Residual SD: 0.179 Ref. Residual Mean: 0.199 Ref. Residual SD: 0.174 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 0.8334 Critical Value: $\geq 1.860$  Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$  Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: 1.7925 Critical Value: $\geq 1.860$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

### **2.3      *Neanthes arenaceodentata* Reference Toxicant Test Results**

## Reference Toxicant 96-h Acute Survival Test

All Matching Labs

Test Type: Survival

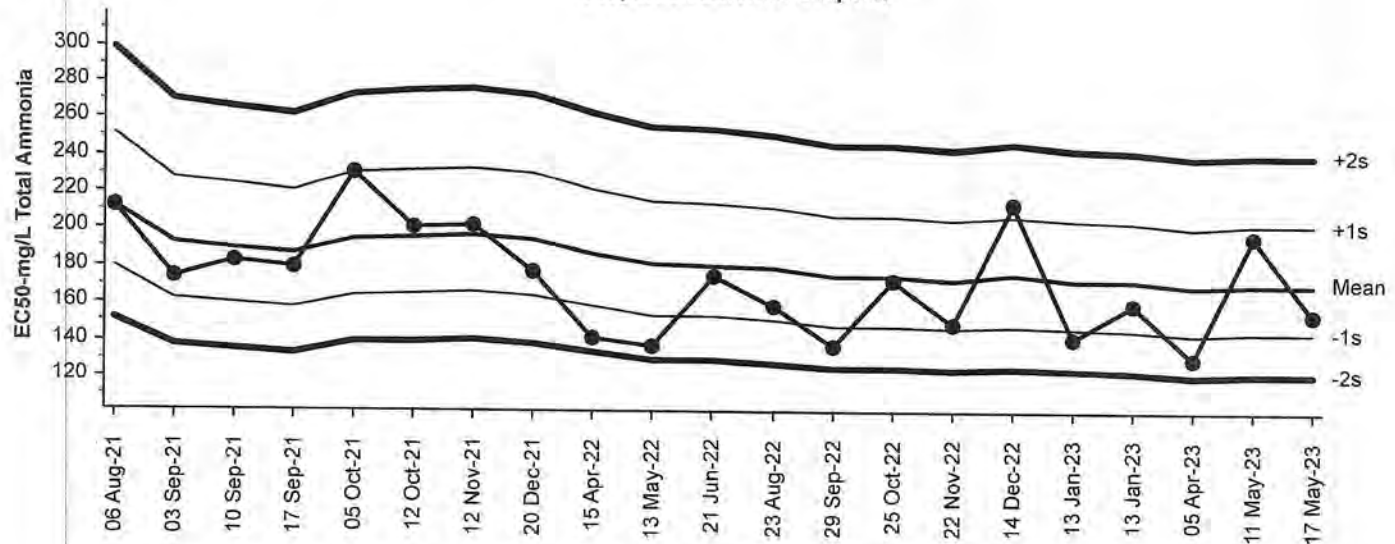
Organism: *Neanthes arenaceodentata*

Material: Total Ammonia

Protocol: All Protocols

Endpoint: Proportion Survived

Source: Reference Toxicant-REF

Reference Toxicant 96-h Acute Survival Test  
Proportion Survived Endpoint

## Lognormal Cumulative Mean Plot

Mean: 171.6

Count: 20

-1s Warning Limit: 145

-2s Action Limit: 122

Sigma: NA

CV: 17.10%

+1s Warning Limit: 203

+2s Action Limit: 241

## Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2021	Aug	6	14:30	212.3	40.73	1.257	(+)		11-8491-1883	13-1741-3410	EcoAnalysts
2		Sep	3	14:56	174.6	3.015	0.1028			12-1099-5516	08-9597-1962	EcoAnalysts
3			10	14:57	183	11.4	0.3797			16-5963-7853	20-6870-7441	EcoAnalysts
4			17	15:48	180.3	8.693	0.2916			03-0971-6137	04-8745-8681	EcoAnalysts
5		Oct	5	15:15	231.4	59.81	1.764	(+)		14-0576-1976	00-2140-6841	EcoAnalysts
6			12	13:47	201.4	29.88	0.9471			15-6333-4141	18-2584-6722	EcoAnalysts
7		Nov	12	11:27	203	31.43	0.9923			05-6077-9773	15-7918-2076	EcoAnalysts
8		Dec	20	11:01	177.1	5.556	0.188			09-3034-4608	10-2366-5665	EcoAnalysts
9	2022	Apr	15	15:04	141.8	-29.74	-1.123	(-)		16-6288-4681	11-6627-6169	EcoAnalysts
10		May	13	15:24	137.2	-34.32	-1.317	(-)		01-1963-8976	03-9703-0259	EcoAnalysts
11		Jun	21	15:45	174.9	3.377	0.115			10-2538-6283	14-3666-1506	EcoAnalysts
12		Aug	23	14:08	159.1	-12.46	-0.4449			17-0235-4010	05-7032-3472	EcoAnalysts
13		Sep	29	15:28	137.1	-34.43	-1.322	(-)		03-3712-7673	03-3541-8621	EcoAnalysts
14		Oct	25	13:00	172.8	1.264	0.04332			16-3471-2888	14-4985-7148	EcoAnalysts
15		Nov	22	18:45	149.4	-22.12	-0.8145			09-5667-8954	07-0185-4884	EcoAnalysts
16		Dec	14	15:25	214.8	43.28	1.327	(+)		18-3309-9140	14-3636-0481	EcoAnalysts
17	2023	Jan	13	13:15	141.2	-30.39	-1.15	(-)		11-5034-4123	16-2148-2454	EcoAnalysts
18			13	16:33	159.7	-11.91	-0.4243			17-7595-8780	05-3063-2004	EcoAnalysts
19		Apr	5	14:55	130.4	-41.22	-1.621	(-)		11-7289-8211	18-7983-9604	EcoAnalysts
20		May	11	15:18	196.6	25.06	0.8042			01-5896-6597	13-5560-2707	EcoAnalysts
21			17	15:25	155.1	-16.49	-0.5962			09-5506-2214	14-8576-5468	EcoAnalysts

## Reference Toxicant 96-h Acute Survival Test

All Matching Labs

Test Type: Survival

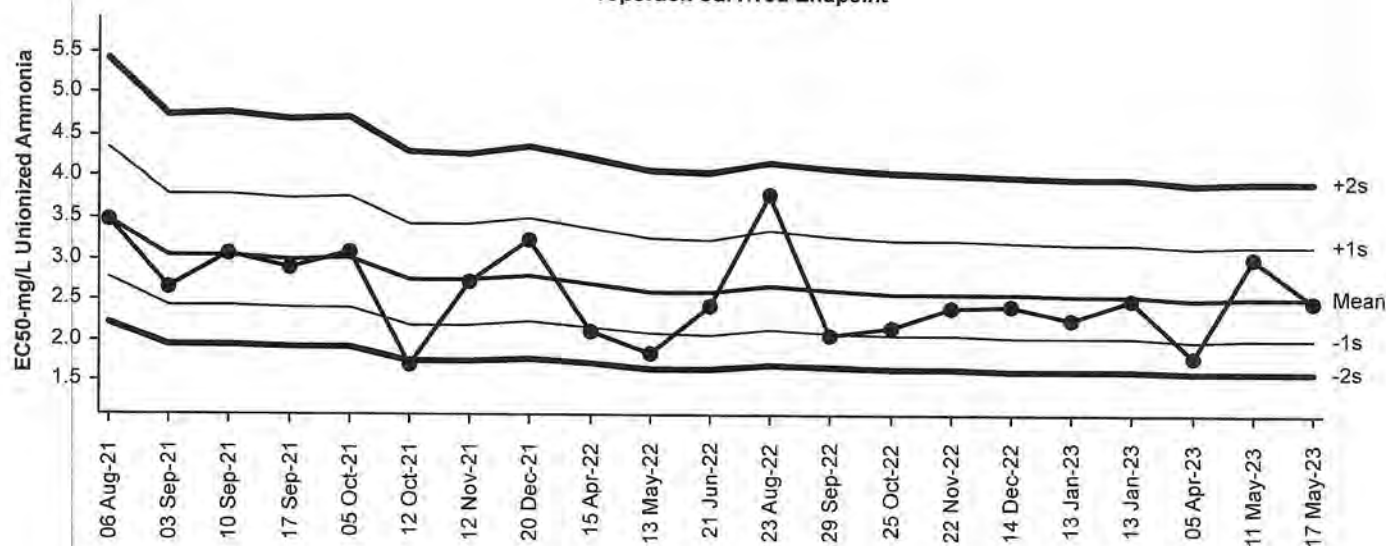
Organism: *Neanthes arenaceodentata*

Material: Unionized Ammonia

Protocol: All Protocols

Endpoint: Proportion Survived

Source: Reference Toxicant-REF

Reference Toxicant 96-h Acute Survival Test  
Proportion Survived Endpoint

## Lognormal Cumulative Mean Plot

Mean: 2.523

Count: 20

-1s Warning Limit: 2.02

-2s Action Limit: 1.61

Sigma: NA

CV: 22.70%

+1s Warning Limit: 3.16

+2s Action Limit: 3.95

## Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2021	Aug	6	14:30	3.47	0.9467	1.423	(+)		17-3928-7243	16-3530-0376	EcoAnalysts
2		Sep	3	14:56	2.643	0.1205	0.2084			00-9699-3848	20-5821-4212	EcoAnalysts
3			10	14:57	3.06	0.5369	0.8617			10-0722-2402	10-1825-7993	EcoAnalysts
4			17	15:48	2.883	0.3599	0.5956			02-1817-2962	14-0572-3774	EcoAnalysts
5		Oct	5	15:15	3.097	0.5737	0.9152			04-8116-7560	08-2878-7495	EcoAnalysts
6			12	13:47	1.696	-0.827	-1.774	(-)		00-1874-7376	16-5756-9380	EcoAnalysts
7		Nov	12	11:27	2.719	0.1963	0.3347			10-4242-2850	01-8554-2307	EcoAnalysts
8		Dec	20	11:01	3.244	0.7209	1.122	(+)		05-2220-5478	10-7291-5872	EcoAnalysts
9	2022	Apr	15	15:04	2.108	-0.4153	-0.8033			11-0641-2289	03-7517-0691	EcoAnalysts
10		May	13	15:24	1.848	-0.6745	-1.389	(-)		03-9000-1315	01-4943-0350	EcoAnalysts
11		Jun	21	15:45	2.443	-0.08029	-0.1445			19-7550-0810	15-1612-6650	EcoAnalysts
12		Aug	23	14:08	3.798	1.276	1.828	(+)		07-0648-9473	01-5439-6552	EcoAnalysts
13		Sep	29	15:28	2.077	-0.4457	-0.8681			01-5105-1781	06-0424-1990	EcoAnalysts
14		Oct	25	13:00	2.154	-0.3687	-0.7056			14-7727-6578	02-8812-8427	EcoAnalysts
15		Nov	22	18:45	2.416	-0.1065	-0.1927			07-9444-9163	00-9445-7280	EcoAnalysts
16		Dec	14	15:25	2.43	-0.09238	-0.1666			09-0689-0078	04-1997-4862	EcoAnalysts
17	2023	Jan	13	13:15	2.265	-0.2582	-0.4822			13-5395-4986	19-0621-5617	EcoAnalysts
18			13	16:33	2.497	-0.026	-0.04627			14-7632-0871	11-6659-5325	EcoAnalysts
19		Apr	5	14:55	1.792	-0.7306	-1.527	(-)		09-4366-1463	19-4497-2018	EcoAnalysts
20		May	11	15:18	3.026	0.5027	0.8115			07-4046-9049	21-4632-4439	EcoAnalysts
21			17	15:25	2.482	-0.04118	-0.0735			00-3123-8899	04-4158-4695	EcoAnalysts

# CETIS Summary Report

Report Date: 22 May-23 16:32 (p 1 of 1)  
Test Code/ID: P220819.51 / 09-5506-2214

## Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID: 09-6886-3005	Test Type: Survival	Analyst: Marisa Seibert
Start Date: 17 May-23 15:25	Protocol: PSEP (1995)	Diluent: Laboratory Seawater
Ending Date: 21 May-23 13:48	Species: Neanthes arenaceodentata	Brine: Not Applicable
Test Length: 94h	Taxon: Polychaeta	Source: Aquatic Toxicology Support Age:
Sample ID: 17-4036-7086	Code: P220819.51	Project: Reference Toxicant
Sample Date: 19 Aug-22	Material: Total Ammonia	Source: Reference Toxicant
Receipt Date: 19 Aug-22	CAS (PC):	Station: P220819.51
Sample Age: 271d 15h	Client: Internal Lab	

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
00-4239-2449	Proportion Survived	Fisher Exact Test	130	185	155.1	---	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
14-8576-5468	Proportion Survived	Binomial/Graphical	EC50	155.1	130	185	1

## Proportion Survived Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	3	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
69.4		3	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
130		3	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
185		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
294		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
451		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

## Proportion Survived Detail

MD5: 9653D55DAB0D98FDFACA78C0947AC831

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	1.0000	1.0000	1.0000
69.4		1.0000	1.0000	1.0000
130		1.0000	1.0000	1.0000
185		0.0000	0.0000	0.0000
294		0.0000	0.0000	0.0000
451		0.0000	0.0000	0.0000

## Proportion Survived Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	10/10	10/10	10/10
69.4		10/10	10/10	10/10
130		10/10	10/10	10/10
185		0/10	0/10	0/10
294		0/10	0/10	0/10
451		0/10	0/10	0/10

# CETIS Test Data Worksheet

Report Date: 22 May-23 16:32 (p 1 of 1)  
 Test Code/ID: P220819.51 / 09-5506-2214

## Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Start Date: 17 May-23 15:25 Species: Neanthes arenaceodentata Sample Code: P220819.51  
 End Date: 21 May-23 13:48 Protocol: PSEP (1995) Sample Source: Reference Toxicant  
 Sample Date: 19 Aug-22 Material: Total Ammonia Sample Station: P220819.51

Conc-mg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	8	10	10	
0	D	2	3	10	10	
0	D	3	13	10	10	
69.4		1	6	10	10	
69.4		2	17	10	10	
69.4		3	2	10	10	
130		1	14	10	10	
130		2	11	10	10	
130		3	10	10	10	
185		1	7	10	0	
185		2	1	10	0	
185		3	12	10	0	
294		1	9	10	0	
294		2	16	10	0	
294		3	4	10	0	
451		1	5	10	0	
451		2	18	10	0	
451		3	15	10	0	



## CETIS Summary Report

Report Date: 22 May-23 16:39 (p 1 of 1)  
 Test Code/ID: P220819.51UIA / 00-3123-8899

## Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID: 09-6886-3005	Test Type: Survival	Analyst: Julia Levengood
Start Date: 17 May-23 15:25	Protocol: PSEP (1995)	Diluent: Laboratory Seawater
Ending Date: 21 May-23 13:48	Species: Neanthes arenaceodentata	Brine: Not Applicable
Test Length: 94h	Taxon: Polychaeta	Source: Aquatic Toxicology Support Age:

Sample ID: 02-5299-8094	Code: P220819.51UIA	Project: Reference Toxicant
Sample Date: 19 Aug-22	Material: Unionized Ammonia	Source: Reference Toxicant
Receipt Date: 19 Aug-22	CAS (PC):	Station: P220819.51UIA
Sample Age: 271d 15h	Client: Internal Lab	

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
04-7169-2923	Proportion Survived	Fisher Exact Test	2.357	2.613	2.482	—	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
04-4158-4695	Proportion Survived	Binomial/Graphical	EC50	2.482	2.357	2.613	1

## Proportion Survived Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	3	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
1.764		3	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
2.357		3	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
2.613		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	—	100.00%
3.02		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	—	100.00%
3.688		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	—	100.00%

## Proportion Survived Detail

MD5: 4849E379B8690C2C32EDFE37044689F9

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	1.0000	1.0000	1.0000
1.764		1.0000	1.0000	1.0000
2.357		1.0000	1.0000	1.0000
2.613		0.0000	0.0000	0.0000
3.02		0.0000	0.0000	0.0000
3.688		0.0000	0.0000	0.0000

## Proportion Survived Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	10/10	10/10	10/10
1.764		10/10	10/10	10/10
2.357		10/10	10/10	10/10
2.613		0/10	0/10	0/10
3.02		0/10	0/10	0/10
3.688		0/10	0/10	0/10

# CETIS Test Data Worksheet

Report Date: 22 May-23 16:39 (p 1 of 1)  
 Test Code/ID: P220819.51UIA / 00-3123-8899

## Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Start Date: 17 May-23 15:25 Species: Neanthes arenaceodentata Sample Code: P220819.51UIA  
 End Date: 21 May-23 13:48 Protocol: PSEP (1995) Sample Source: Reference Toxicant  
 Sample Date: 19 Aug-22 Material: Unionized Ammonia Sample Station: P220819.51UIA

Conc-mg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	17	10	10	
0	D	2	10	10	10	
0	D	3	4	10	10	
1.764		1	12	10	10	
1.764		2	6	10	10	
1.764		3	9	10	10	
2.357		1	14	10	10	
2.357		2	5	10	10	
2.357		3	18	10	10	
2.613		1	15	10	0	
2.613		2	16	10	0	
2.613		3	1	10	0	
3.02		1	11	10	0	
3.02		2	13	10	0	
3.02		3	2	10	0	
3.688		1	3	10	0	
3.688		2	7	10	0	
3.688		3	8	10	0	

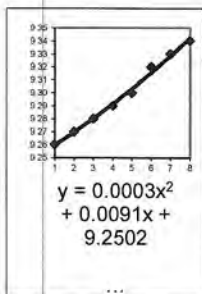
# Un-ionized Ammonia Calculator

CLIENT:	Leon Environmental	Date of Test:	May 17, 2023
PROJECT:	Port of Friday Harbor	Test Type:	<i>Neanthes arenaceodentata</i>
COMMENTS:	P220819.51		

To convert Total Ammonia (mg/L) to Free (un-ionized) Ammonia (mg/L) enter the corresponding total ammonia, salinity, temperature, and pH.

Sample	Mod	NH3T (mg/L)	salinity (ppt)	pH	temp (C)	temp (K)	pKa <sup>s</sup>	NH <sub>3</sub> U (mg/L)
Target / Sample Name		Actual	Actual	Actual	Actual	Calculated	Calculated	Calculated
Example 3.5		2.000	10.0	7.5	5.0	278.15	9.2520	0.008
1								
2	50	69.4	28	7.8	20.9	294.05	9.2555	1.764
3	100	130	28	7.7	20.9	294.05	9.2555	2.357
4	200	185	29	7.5	20.9	294.05	9.2557	2.613
5	300	294	29	7.4	20.9	294.05	9.2557	3.020
6	400	451	29	7.3	20.9	294.05	9.2557	3.688
7								
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42								
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44								
45								

Ionic strength:pKa <sup>s</sup>	
1	9.26
2	9.27
3	9.28
4	9.29
5	9.30
6	9.32
7	9.33
8	9.34



*Q. J. J.*

## Ammonia Reference Toxicant Test Survival Data Sheet

CLIENT Leon Environmental	PROJECT Port of Friday Harbor	SPECIES <i>Neanthes arenaceodentata</i>	LABORATORY Port Gamble	PROTOCOL PSEP, SCUM
TEST ID P220819.51	LOT #: 22E3156086	TEST START DATE 17May23	TIME 1525 DM	4-DAY END DATE 21May23
CHAMBER SIZE/TYPE Glass pint jar	EXPOSURE VOLUME 250 mL			

### WATER QUALITY DATA

TEST CONDITIONS				DO (mg/L)		TEMP(C)		SAL (ppt)		pH		TECHNICIAN	AMMONIA		SULFIDES				
				> 4.6		20 ± 1		30 ± 2		7 - 9									
CLIENT/ENVIRON ID	CONCENTRATION		DAY	REP	D.O.		TEMP.		SALINITY		pH		WQ TECH/ DATE	AMMONIA		Tech	SULFIDES		Tech
	value	units			meter	mg/L	meter	°C	meter	ppt	meter	unit		METER	mg/L		meter	mg/L	
Ref.Tox.-ammonia	0	mg/L	0	Stock	8	7.6	8	21.0	8	28	8	7.9	MS 5/17	10	0.466	NL			
			4	1	9	6.8	9	21.0	9	28	9	7.9	NL 5/21						
Ref.Tox.-ammonia	50	mg/L	0	Stock	8	7.5	8	20.9	8	28	8	7.8	MS 5/17	10	69.4	NL			
			4	1	9	6.7	9	21.1	9	28	9	7.9	NL 5/21						
Ref.Tox.-ammonia	100	mg/L	0	Stock	8	7.5	8	20.9	8	28	8	7.7	MS 5/17	10	130	NL			
			4	1	9	6.7	9	21.0	9	29	9	7.8	NL 5/21						
Ref.Tox.-ammonia	200	mg/L	0	Stock	8	7.5	8	20.9	8	29	8	7.5	MS 5/17	10	185	NL			
			4	1	9	5.5	9	21.0	9	29	9	7.6	NL 5/21						
Ref.Tox.-ammonia	300	mg/L	0	Stock	8	7.5	8	20.9	8	29	8	7.4	MS 5/17	10	294	NL			
			4	1															
Ref.Tox.-ammonia	400	mg/L	0	Stock	8	7.5	8	20.9	8	29	8	7.3	MS 5/17	10	451	NL			
			4	1															

# Ammonia Reference Toxicant Test Survival Data Sheet

SPECIES <i>Neanthes arenaceodentata</i>		
CLIENT Leon Environmental	PROJECT Port of Friday Harbor	TEST ID P220819.51
PROJECT MANAGER J. Levensgood/ M. Seibert	LABORATORY Port Gamble	PROTOCOL PSEP, SCUM

## SURVIVAL & BEHAVIOR DATA

OBSERVATION KEY N = Normal LOE = Loss of equilibrium Q = Quiescent DC = Discoloration NB = No body F = Floating on surface <div style="border: 1px solid black; padding: 2px; margin-top: 5px; display: inline-block;">INITIAL # OF ORGANISMS 10</div>				DAY 1			DAY 2			DAY 3			DAY 4			
				DATE 5/18/23			DATE 5/19/23			DATE 5/20/23			DATE 5/21/23			
				TECHNICIAN LG			TECHNICIAN MS			TECHNICIAN NL			TECHNICIAN NL			
CLIENT/ENVIRON ID	CONC.		REP	INITIAL NUMBER	#ALIVE: #DEAD: OBS			#ALIVE: #DEAD: OBS			#ALIVE: #DEAD: OBS			#ALIVE: #DEAD: OBS		
	value	units			#ALIVE	#DEAD	OBS	#ALIVE	#DEAD	OBS	#ALIVE	#DEAD	OBS	#ALIVE	#DEAD	OBS
Ref.Tox.- Ammonia	0 mg/L	1	10	10	0	N	10	0	N	10	0	N	10	0	N	
		2	10	10	0	N	10	0	N	10	0	N	10	0	N	
		3	10	10	0	N	10	0	N	10	0	N	10	0	N	
Ref.Tox.- Ammonia	50 mg/L	1	10	10	0	N	10	0	N	10	0	N	10	0	N	
		2	10	10	0	N	10	0	N	10	0	N	10	0	N	
		3	10	10	0	N	10	0	N	10	0	N	10	0	N	
Ref.Tox.- Ammonia	100 mg/L	1	10	10	0	N	10	0	N	10	0	N	10	0	N	
		2	10	10	0	N	10	0	N	10	0	N	10	0	N	
		3	10	10	0	N	10	0	N	10	0	N	10	0	N	
Ref.Tox.- Ammonia	200 mg/L	1	10	10	0	Q	9	1	Q	5	4	Q	0	5	-	
		2	10	10	0	Q	10	0	Q	6	4	Q	0	6	-	
		3	10	10	0	Q	10	0	Q	5	5	Q	0	5	-	
Ref.Tox.- Ammonia	300 mg/L	1	10	10	0	Q	0	10	-	-	-	-	-	-	-	
		2	10	10	0	Q	3	7	Q	0	3	-	-	-	-	
		3	10	10	0	Q	0	10	-	-	-	-	-	-	-	
Ref.Tox.- Ammonia	400 mg/L	1	10	10	0	Q	0	5	-	-	-	-	-	-	-	
		2	10	10	0	Q	0	10	-	-	-	-	-	-	-	
		3	10	10	0	Q	0	10	-	-	-	-	-	-	-	

① IE - LG 5/18/23



**Ammonia Reference Toxicant  
Spiking Worksheet**

Reference Toxicant ID: P220819.51  
 Date Prepared: 5/17/23  
 Technician Initials: ms

## Neanthes NH<sub>3</sub> RT

Assumptions in Model

Stock ammonia concentration is 10,000 mg/L = 10 mg/mL

Date:

5/11/2023

Measurement:

8340

Test Solutions			Volume of stock to reach desired concentration	
Measured Concentration	Desired Concentration	Volume		
mg/L	mg/L	mL	mL stock to increase	
			FRESH WATER (mL)	SALT WATER (mL)
0.46e	0.00			
69.4	50	750		6.74
130	100	750		13.49
<del>249</del> 185 ①	200	750		26.98
294	300	750		40.47
451	400	750		53.96

① diluted concentration - new value 185 mg/L - ms 5/17

1	7
2	12
3	15
4	16
5	3
6	8
7	5
8	14
9	18
10	10
11	1
12	4
13	11
14	17
15	9
16	13
17	6
18	2

N.a. M<sub>H3</sub> RT  
P22.0819.51

### 3. *Mytilus galloprovincialis* Water-Column Test



### 3.1 *Mytilus galloprovincialis* Test Data

## GENERAL

Client	Leon Environmental
Project	Port of Friday Harbor
Project Number	PG1785
Project Manager	J. Levengood/ M. Seibert
Date Sample Collected (Oldest Sample)	3/20/2023
Sample Holding Time	53
Test Start Date	05/12/23
Test End Date	05/14/23
Test Species	<i>Mytilus galloprovincialis</i>
Organism Supplier	Taylor Shellfish
Date Organism Acquired	5/1/2023
Organism Acclimation Time (Days)	11
Organism Batch	TS050123.01
Test Type	Larval Sediment Test
Test Protocol	PSEP 1995
Regional Protocol	SCUM 2019
Laboratory Location	Port Gamble
Test Location	Bath 1
Sample Treatment	reference sed press sieved 2mm and SED-11G:0-10 press sieved 2mm
UV Light Treatment	No
Resuspension	Yes
Aeration from test initiation	Yes
Control Sediment Source (if applicable)	Yaquina Bay, OR
Water Batch	FSW051223.01
Test Lighting	14:10 L:D
Test Chamber	1L glass chamber
Replicates Per Treatment	5
Exposure Volume	18g sediment/900 mL seawater
Test Dissolved Oxygen	> 5
Test Temperature	16 ± 1
Test Salinity	28 ± 1
Test pH	8.25 ± 0.75

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO:	5	
Temp:	15	17
Sal:	27	29
pH:	7.5	9

TEST START TIME:	15:35
TEST END TIME:	16:13

	CLIENT SAMPLE ID	LAB ID
Control 1	Control	P230510.01
Control 2 (if used) or reference	CARR18-23-REF	P230510.02
3	CARR40-23-REF	P230510.03
4	CARR62-23-REF	P230510.04
5	SED-07G:0-10	P230324.01
6	SED-11G:0-10	P230324.02
7	SED-14G:0-10	P230324.03
8	SED-15G:0-10	P230324.04
9	SED-03G:0-10	P230324.05
10	SED-01G:0-10	P230324.06
11	SED-05G:0-10	P230324.07
12	SED-27G:0-10	P230324.08
13	.	.
14	.	.
15	.	.
16	.	.
17	.	.
18	.	.
19	.	.
20	.	.
21	.	.

<b>CLIENT</b> Leon Environmental	<b>PROJECT</b> Port of Friday Harbor	<b>JOB NUMBER</b> PG1785	<b>PROJECT MANAGER</b> J. Levengood/ M. Seibert	<b>LABORATORY</b> Port Gamble , Bath 1	<b>PROTOCOL</b> PSEP 1995 , SCUM 2019
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## TEST ORGANISM SPAWNING DATA

SPECIES			TEST START DATE			SEDIMENT TREATMENT		
Mytilus galloprovincialis			12-May-23			reference sed press sieved 2mm and SED-11G:0-10 press sieved 2mm		
SUPPLIER			ORGANISM BATCH			TEST CHAMBERS		
Taylor Shellfish			TS050123.01			1L glass chamber		
SPAWNING METHOD		INITIAL SPAWNING TIME		FINAL SPAWNING TIME		EXPOSURE VOLUME		INITIALS
Heat Shock		1149		1335		18g sediment/900 mL seawater		SZ/MS
MALES	FEMALES	SPERM VIABILITY		EGG CONDITION		TIME OF SHAKE		INITIALS
2	6	Good		Good		1034		SZ/MS
BEGIN FERTILIZATION	END FERTILIZATION		CONDITION OF EMBRYOS			TIME OF INITIATION		INITIALS
1335	1535		Good			15:35		NL/DM/MS
ROSE BENGAL LOT #						FORMALIN LOT #		
5135						220304-50		

## SPECIAL CONDITIONS

<b>UV LIGHT EXPOSURE (YES/NO)</b> No	<b>AERATION FROM TEST INITIATION (YES/NO)</b> Yes
<b>RESUSPENSION (YES/NO)</b> Yes	<b>OTHER (EXPLAIN)</b>

## EMBRYO DENSITY CALCULATIONS

<b>No. of embryos in 1 mL of 100x diluted stock</b> 190.3
<b>mL of original embryo stock added to each test chamber</b> 1.42

## Sediment Larval Test WQ Data Sheet

<b>CLIENT</b> Leon Environmental		<b>PROJECT</b> Port of Friday Harbor		<b>SPECIES</b> <i>Mytilus galloprovincialis</i>		<b>LOCATION</b> Port Gamble / Bath 1		<b>PROTOCOL</b> PSEP 1995 , SCUM 2019							
<b>PROJECT MANAGER</b> J. Levengood/ M. Seibert		<b>TEST TYPE</b> Larval Sediment Test		<b>Project Number</b> PG1785		<b>TEST START DATE</b> May 12, 2023		<b>TIME</b> 15:35		<b>TEST END DATE</b> May 14, 2023		<b>TIME</b> 16:13		<b>INITIALS</b> NL	
<b>WATER QUALITY DATA</b>															
SAMPLE ID	DAY	REP	JAR #	TECHNICIAN	Date	Meter	DO (mg/L) > 5	TEMP (°C) 15 - 17	SALINITY (ppt) 27 - 29	pH 7.5 - 9	Resuspension Time	Notes			
Control	0	Surr	8	MS	05/12/23	8	8.3	16.8	28	7.9					
Control	1	Surr	8	NL	05/13/23	9	8.0	16.6	28	7.9					
Control	2	Surr	8	NL	05/14/23	8	7.7	17.3	28	7.9	8:30				
CARR18-23-REF	0	Surr	63	MS	05/12/23	8	8.2	16.0	28	7.9					
CARR18-23-REF	1	Surr	63	NL	05/13/23	9	8.2	16.1	28	8.0					
CARR18-23-REF	2	Surr	63	NL	05/14/23	8	8.1	16.2	28	8.0	8:30				
CARR40-23-REF	0	Surr	37	MS	05/12/23	8	8.2	16.7	28	7.9					
CARR40-23-REF	1	Surr	37	NL	05/13/23	9	7.3	16.9	28	8.0					
CARR40-23-REF	2	Surr	37	NL	05/14/23	8	7.7	17.1	28	7.9	8:30				
CARR62-23-REF	0	Surr	46	MS	05/12/23	8	8.1	16.9	28	7.9					
CARR62-23-REF	1	Surr	46	NL	05/13/23	9	7.7	17.0	28	8.0					
CARR62-23-REF	2	Surr	46	NL	05/14/23	8	8.0	17.1	28	7.9	8:30				
SED-07G:0-10	0	Surr	21	MS	05/12/23	8	8.1	15.8	28	7.9					
SED-07G:0-10	1	Surr	21	NL	05/13/23	9	8.2	16.0	28	8.0					
SED-07G:0-10	2	Surr	21	NL	05/14/23	8	7.8	16.0	28	7.9	8:30				

## Sediment Larval Test WQ Data Sheet

<b>CLIENT</b> Leon Environmental		<b>PROJECT</b> Port of Friday Harbor		<b>SPECIES</b> <i>Mytilus galloprovincialis</i>		<b>LOCATION</b> Port Gamble / Bath 1		<b>PROTOCOL</b> PSEP 1995 , SCUM 2019							
<b>PROJECT MANAGER</b> J. Levengood/ M. Seibert		<b>TEST TYPE</b> Larval Sediment Test		<b>Project Number</b> PG1785		<b>TEST START DATE</b> May 12, 2023		<b>TIME</b> 15:35		<b>TEST END DATE</b> May 14, 2023		<b>TIME</b> 16:13		<b>INITIALS</b> NL	
<b>WATER QUALITY DATA</b>															
SAMPLE ID	DAY	REP	JAR #	TECHNICIAN	Date	Meter	DO (mg/L) > 5	TEMP (°C) 15 - 17	SALINITY (ppt) 27 - 29	pH 7.5 - 9	Resuspension Time	Notes			
SED-11G:0-10	0	Surr	11	MS	05/12/23	8	8.1	16.9	28	7.9					
SED-11G:0-10	1	Surr	11	NL	05/13/23	9	6.3	16.4	28	7.9					
SED-11G:0-10	2	Surr	11	NL	05/14/23	8	7.0	17.0	28	7.8	8:30				
SED-14G:0-10	0	Surr	38	MS	05/12/23	8	8.1	16.0	28	7.9					
SED-14G:0-10	1	Surr	38	NL	05/13/23	9	8.0	16.2	28	8.0					
SED-14G:0-10	2	Surr	38	NL	05/14/23	8	7.8	16.7	28	7.9	8:30				
SED-15G:0-10	0	Surr	59	MS	05/12/23	8	8.1	15.9	28	7.9					
SED-15G:0-10	1	Surr	59	NL	05/13/23	9	7.8	16.2	28	8.0					
SED-15G:0-10	2	Surr	59	NL	05/14/23	8	7.9	16.3	28	7.9	8:30				
SED-03G:0-10	0	Surr	23	MS	05/12/23	8	8.1	16.0	28	7.9					
SED-03G:0-10	1	Surr	23	NL	05/13/23	9	8.1	16.1	28	8.0					
SED-03G:0-10	2	Surr	23	NL	05/14/23	8	8.0	16.3	28	7.9	8:30				
SED-01G:0-10	0	Surr	53	MS	05/12/23	8	8.2	17.0	28	7.9					
SED-01G:0-10	1	Surr	53	NL	05/13/23	9	6.5	17.2	28	7.9					
SED-01G:0-10	2	Surr	53	NL	05/14/23	8	7.9	17.4	28	7.9	8:30				
SED-05G:0-10	0	Surr	24	MS	05/12/23	8	8.1	15.8	28	7.9					
SED-05G:0-10	1	Surr	24	NL	05/13/23	9	8.1	16.1	28	8.0					
SED-05G:0-10	2	Surr	24	NL	05/14/23	8	8.0	16.2	28	7.9	8:30				
SED-27G:0-10	0	Surr	52	MS	05/12/23	8	8.1	16.1	28	7.9					
SED-27G:0-10	1	Surr	52	NL	05/13/23	9	7.9	16.7	28	8.0					
SED-27G:0-10	2	Surr	52	NL	05/14/23	8	8.0	16.2	28	8.0	8:30				

## Sediment Larval Test Summary

CLIENT Leon Environmental			PROJECT Port of Friday Harbor				PROJECT NUMBER PG1785	
PROJECT MANAGER J. Levengood/ M. Seibert			TEST TYPE Larval Sediment Test				SPECIES <i>Mytilus galloprovincialis</i>	
LARVAL ENDPOINT DATA								
Sample ID	Rep	# Normal	# Abnormal	Total	Date	Tech	Comments / QA Counts	
Stocking Density	1			304	05/15/23	MK		
Stocking Density	2			275	05/15/23	MK		
Stocking Density	3			284	05/15/23	MK		
Stocking Density	4			256	05/15/23	MK		
Stocking Density	5			276	05/15/23	MK		
Control	1	269	21	290	05/15/23	MK		
Control	2	282	2	284	05/15/23	MK		
Control	3	295	2	297	05/15/23	MK		
Control	4	293	2	295	05/15/23	MK		
Control	5	281	3	284	05/15/23	MK	QA: N:273, A:2, %diff: 0.33 SZ	
CARR18-23-REF	1	219	6	225	05/15/23	MK		
CARR18-23-REF	2	209	1	210	05/15/23	MK		
CARR18-23-REF	3	173	1	174	05/15/23	MK		
CARR18-23-REF	4	171	4	175	05/15/23	MK		
CARR18-23-REF	5	232	1	233	05/15/23	MK		
CARR40-23-REF	1	226	2	228	05/15/23	MK		
CARR40-23-REF	2	197	0	197	05/15/23	MK		
CARR40-23-REF	3	182	4	186	05/15/23	MK	QA: N:176, A:5, %diff: 0.61 SZ	
CARR40-23-REF	4	225	3	228	05/15/23	MK		
CARR40-23-REF	5	250	2	252	05/15/23	MK		
CARR62-23-REF	1	187	2	189	05/15/23	MK		
CARR62-23-REF	2	180	1	181	05/15/23	MK		
CARR62-23-REF	3	222	2	224	05/15/23	MK		
CARR62-23-REF	4	237	1	238	05/15/23	MK		
CARR62-23-REF	5	172	0	172	05/15/23	MK		
SED-07G:0-10	1	231	1	232	05/15/23	MK		
SED-07G:0-10	2	211	9	220	05/15/23	MK		
SED-07G:0-10	3	226	10	236	05/15/23	MK		
SED-07G:0-10	4	199	2	201	05/15/23	MK	QA: N:198, A:1, %diff: 0.49 SZ	
SED-07G:0-10	5	216	3	219	05/15/23	MK		
SED-11G:0-10	1	211	0	211	05/15/23	MK		
SED-11G:0-10	2	188	4	192	05/15/23	MK		
SED-11G:0-10	3	194	1	195	05/15/23	MK		
SED-11G:0-10	4	269	2	271	05/15/23	MK		
SED-11G:0-10	5	259	0	259	05/15/23	MK		

## Sediment Larval Test Summary

CLIENT Leon Environmental			PROJECT Port of Friday Harbor				PROJECT NUMBER PG1785	
PROJECT MANAGER J. Levengood/ M. Seibert			TEST TYPE Larval Sediment Test				SPECIES <i>Mytilus galloprovincialis</i>	
LARVAL ENDPOINT DATA								
Sample ID	Rep	# Normal	# Abnormal	Total	Date	Tech	Comments / QA Counts	
SED-14G:0-10	1	211	12	223	05/15/23	MK		
SED-14G:0-10	2	209	4	213	05/15/23	MK		
SED-14G:0-10	3	232	3	235	05/15/23	MK		
SED-14G:0-10	4	222	3	225	05/15/23	MK		
SED-14G:0-10	5	190	3	193	05/15/23	MK	QA: N:186, A:5, %diff: 1.1 SZ	
SED-15G:0-10	1	215	13	228	05/15/23	MK		
SED-15G:0-10	2	249	1	250	05/15/23	MK		
SED-15G:0-10	3	184	2	186	05/15/23	MK		
SED-15G:0-10	4	234	2	236	05/15/23	MK		
SED-15G:0-10	5	235	2	237	05/15/23	MK		
SED-03G:0-10	1	224	4	228	05/15/23	MK	QA: N:223, A:4, %diff: 0.01 SZ	
SED-03G:0-10	2	242	9	251	05/16/23	MK		
SED-03G:0-10	3	229	4	233	05/16/23	MK		
SED-03G:0-10	4	238	6	244	05/16/23	MK		
SED-03G:0-10	5	209	5	214	05/16/23	MK		
SED-01G:0-10	1	239	4	243	05/15/23	MK		
SED-01G:0-10	2	229	6	235	05/16/23	MK		
SED-01G:0-10	3	231	4	235	05/16/23	MK		
SED-01G:0-10	4	244	9	253	05/16/23	MK		
SED-01G:0-10	5	204	4	208	05/16/23	MK		
SED-05G:0-10	1	247	2	249	05/15/23	MK		
SED-05G:0-10	2	224	5	229	05/16/23	MK		
SED-05G:0-10	3	226	6	232	05/16/23	MK		
SED-05G:0-10	4	215	12	227	05/16/23	MK		
SED-05G:0-10	5	205	2	207	05/16/23	MK	QA: N:201, A:6, %diff: 1.9 SZ	
SED-27G:0-10	1	271	0	271	05/15/23	MK		
SED-27G:0-10	2	183	10	193	05/16/23	MK		
SED-27G:0-10	3	220	5	225	05/16/23	MK		
SED-27G:0-10	4	237	2	239	05/16/23	MK		
SED-27G:0-10	5	186	3	189	05/16/23	MK		

## Sediment Larval Test Summary

CLIENT					PROJECT			PROJECT NUMBER		
Leon Environmental					Port of Friday Harbor			PG1785		
PROJECT MANAGER					TEST TYPE			SPECIES		
J. Levengood/ M. Seibert					Larval Sediment Test			Mytilus galloprovincialis		
LARVAL ENDPOINT DATA										
Sample ID	Rep	Normal	Abnormal	Total	Mean # Normal	SD	Average Control Normal Survival (Nc/I)	# Normal Relative to Control	Average # Normal Relative to Control	Average # Normal Relative to Reference (1)
Stocking Density	1			304						
Stocking Density	2			275						
Stocking Density	3			284						
Stocking Density	4			256						
Stocking Density	5			276						
Control	1	269	21	290						
Control	2	282	2	284						
Control	3	295	2	297						
Control	4	293	2	295						
Control	5	281	3	284						
CARR18-23-REF	1	219	6	225				0.77		
CARR18-23-REF	2	209	1	210				0.74		
CARR18-23-REF	3	173	1	174				0.61		
CARR18-23-REF	4	171	4	175				0.60		
CARR18-23-REF	5	232	1	233				200.8		
CARR40-23-REF	1	226	2	228				0.80		
CARR40-23-REF	2	197	0	197				0.69		
CARR40-23-REF	3	182	4	186				0.64		
CARR40-23-REF	4	225	3	228				0.79		
CARR40-23-REF	5	250	2	252				216.0		
CARR62-23-REF	1	187	2	189				0.66		
CARR62-23-REF	2	180	1	181				0.63		
CARR62-23-REF	3	222	2	224				0.78		
CARR62-23-REF	4	237	1	238				0.83		
CARR62-23-REF	5	172	0	172				199.6		
SED-07G:0-10	1	231	1	232				0.81		
SED-07G:0-10	2	211	9	220				0.74		
SED-07G:0-10	3	226	10	236				0.80		
SED-07G:0-10	4	199	2	201				0.70		
SED-07G:0-10	5	216	3	219				216.6		
SED-11G:0-10	1	211	0	211				0.74		
SED-11G:0-10	2	188	4	192				0.66		
SED-11G:0-10	3	194	1	195				0.68		
SED-11G:0-10	4	269	2	271				0.95		
SED-11G:0-10	5	259	0	259				224.2		



# Sediment Larval Test Summary

CLIENT					PROJECT			PROJECT NUMBER		
Leon Environmental					Port of Friday Harbor			PG1785		
PROJECT MANAGER					TEST TYPE			SPECIES		
J. Levengood/ M. Seibert					Larval Sediment Test			Mytilus galloprovincialis		
LARVAL ENDPOINT DATA										
Sample ID	Rep	Normal	Abnormal	Total	Mean # Normal	SD	Average Control Normal Survival (Nc/I)	# Normal Relative to Control	Average # Normal Relative to Control	Average # Normal Relative to Reference (1)
SED-14G:0-10	1	211	12	223				0.74		
SED-14G:0-10	2	209	4	213				0.74		
SED-14G:0-10	3	232	3	235				0.82		
SED-14G:0-10	4	222	3	225				0.78		
SED-14G:0-10	5	190	3	193	212.8	15.7		0.67	0.75	1.06
SED-15G:0-10	1	215	13	228				0.76		
SED-15G:0-10	2	249	1	250				0.88		
SED-15G:0-10	3	184	2	186				0.65		
SED-15G:0-10	4	234	2	236				0.82		
SED-15G:0-10	5	235	2	237	223.4	25.1		0.83	0.79	1.11
SED-03G:0-10	1	224	4	228				0.79		
SED-03G:0-10	2	242	9	251				0.85		
SED-03G:0-10	3	229	4	233				0.81		
SED-03G:0-10	4	238	6	244				0.84		
SED-03G:0-10	5	209	5	214	228.4	13.0		0.74	0.80	1.14
SED-01G:0-10	1	239	4	243				0.84		
SED-01G:0-10	2	229	6	235				0.81		
SED-01G:0-10	3	231	4	235				0.81		
SED-01G:0-10	4	244	9	253				0.86		
SED-01G:0-10	5	204	4	208	229.4	15.4		0.72	0.81	1.14
SED-05G:0-10	1	247	2	249				0.87		
SED-05G:0-10	2	224	5	229				0.79		
SED-05G:0-10	3	226	6	232				0.80		
SED-05G:0-10	4	215	12	227				0.76		
SED-05G:0-10	5	205	2	207	223.4	15.6		0.72	0.79	1.11
SED-27G:0-10	1	271	0	271				0.95		
SED-27G:0-10	2	183	10	193				0.64		
SED-27G:0-10	3	220	5	225				0.77		
SED-27G:0-10	4	237	2	239				0.83		
SED-27G:0-10	5	186	3	189	219.4	36.8		0.65	0.77	1.09

# Sediment Larval Test Day 0 Ammonia

CLIENT			PROJECT				PROJECT NUMBER				SPECIES									
Leon Environmental			Port of Friday Harbor				PG1785				Mytilus galloprovincialis									
Calibration Standards Temperature: 20.6			Date: 5/12/2023	Tech: DM/SZ		Ammonia Meter: 10				Sulfide Meter: 1										
NH <sub>3</sub> sample temperature should be within +/- 1°C of standard temp at time and date of analysis																				
measured																				
Laureateae																				
Total																				
Dissolved Sulfide																				
Sample Volume (ml)																				
Sulfide Multiplier																				
Dissolved Sulfide (µg/L as S)																				
Test salinity (ppt)																				
Test pH																				
Test temp (C)																				
Temp (K)																				
pKa <sup>s</sup>																				
Unionized NH <sub>3</sub> (mg/L)																				
H <sub>2</sub> S pKa*																				
Undissociated Sulfide (µg/L as H <sub>2</sub> S)																				
Sample	Day	Overlying/ Porewater	Total NH <sub>3</sub> (mg/L)	NH <sub>3</sub> sample temp	WQ/Temp Meter	Dissolved Sulfide (µg/L as S)	Sulfide Sample Volume (ml)	Sulfide Multiplier	Dissolved Sulfide (µg/L as S)	Test salinity (ppt)	Test pH	Test temp (C)	Temp (K)	Calculated	Calculated	Calculated	Calculated	Notes		
Sourced	Sourced		Record	Record	Record	Record	Record	Record	Calculated	Sourced	Sourced	Sourced	Calculated	Calculated	Calculated	Calculated	Calculated			
Control	0	Overlying	0.00	20.5	T17	ND	10	1	ND	28	7.9	16.8	289.95	9.26	0.000	6.65	ND			
CARR18-23-REF	0	Overlying	0.00	20.5	T17	ND	10	1	ND	28	7.9	16.0	289.15	9.26	0.000	6.66	ND			
CARR40-23-REF	0	Overlying	0.00	21.6	T17	4	10	1	4	28	7.9	16.7	289.85	9.26	0.000	6.65	0.294			
CARR62-23-REF	0	Overlying	0.00	21.5	T17	ND	10	1	ND	28	7.9	16.9	290.05	9.26	0.000	6.65	ND			
SED-07G:0-10	0	Overlying	0.0162	20.3	T17	10	10	1	10	28	7.9	15.8	288.95	9.26	0.000	6.67	0.760			
SED-11G:0-10	0	Overlying	0.0614	20.4	T17	15	10	1	15	28	7.9	16.9	290.05	9.26	0.001	6.65	1.096			
SED-14G:0-10	0	Overlying	0.00	22.8	T17	8	10	1	8	28	7.9	16.0	289.15	9.26	0.000	6.66	0.604			
SED-15G:0-10	0	Overlying	0.00	21.3	T17	ND	10	1	ND	28	7.9	15.9	289.05	9.26	0.000	6.66	ND			
SED-03G:0-10	0	Overlying	0.00	20.5	T17	6	10	1	6	28	7.9	16.0	289.15	9.26	0.000	6.66	0.453			
SED-01G:0-10	0	Overlying	0.00	20.3	T17	5	10	1	5	28	7.9	17.0	290.15	9.26	0.000	6.64	0.364			
SED-05G:0-10	0	Overlying	0.00	21.6	T17	13	10	1	13	28	7.9	15.8	288.95	9.26	0.000	6.67	0.988			
SED-27G:0-10	0	Overlying	0.00	21.1	T17	ND	10	1	ND	28	7.9	16.1	289.25	9.26	0.000	6.66	ND			

# Sediment Larval Test Day 2 Ammonia

CLIENT			PROJECT			PROJECT NUMBER				SPECIES									
Leon Environmental			Port of Friday Harbor			PG1785				Mytilus galloprovincialis									
Calibration Standards Temperature: 21.1			Date: 5/14/2023		Tech: NL/LG		Ammonia Meter: 10				Sulfide Meter: 1								
NH <sub>3</sub> sample temperature should be within +/- 1°C of standard temp at time and date of analysis																			
Sample	Day	Overlying/ Porewater	Total NH <sub>3</sub> (mg/L)	NH <sub>3</sub> sample temp	WQ/Temp Meter	measured Total Dissolved Sulfide (µg/L as S)	Sulfide Sample Volume (ml)	Sulfide Multiplier	Calculated Total Dissolved Sulfide (µg/L as S)	Test salinity (ppt)	Test pH	Test temp (C)	Temp (K)	pKa <sup>s</sup>	Unionized NH <sub>3</sub> (mg/L)	Undissociated Sulfide H <sub>2</sub> S pKa * (µg/L as H <sub>2</sub> S)			
Sourced	Sourced		Record	Record	Record	Record	Record	Record	Calculated	Sourced	Sourced	Sourced	Calculated	Calculated	Calculated	Calculated	Calculated	Notes	
Control	2	Overlying	0.00	20.1	T17	12	10	1	12	28	7.9	17.3	290.45	9.26	0.000	6.64	0.864		
CARR18-23-REF	2	Overlying	0.00	20.1	T17	ND	10	1	ND	28	8.0	16.2	289.35	9.26	0.000	6.66	ND		
CARR40-23-REF	2	Overlying	0.00	20.1	T17	ND	10	1	ND	28	7.9	17.1	290.25	9.26	0.000	6.64	ND		
CARR62-23-REF	2	Overlying	0.00	20.2	T17	1	10	1	1	28	7.9	17.1	290.25	9.26	0.000	6.64	0.073		
SED-07G:0-10	2	Overlying	0.00	20.1	T17	2	10	1	2	28	7.9	16.0	289.15	9.26	0.000	6.66	0.151		
SED-11G:0-10	2	Overlying	0.00	20.1	T17	ND	10	1	ND	28	7.8	17.0	290.15	9.26	0.000	6.64	ND		
SED-14G:0-10	2	Overlying	0.00	20.1	T17	ND	10	1	ND	28	7.9	16.7	289.85	9.26	0.000	6.65	ND		
SED-15G:0-10	2	Overlying	0.00	20.2	T17	11	10	1	11	28	7.9	16.3	289.45	9.26	0.000	6.66	0.821		
SED-03G:0-10	2	Overlying	0.00	20.1	T17	51	10	1	51	28	7.9	16.3	289.45	9.26	0.000	6.66	3.806		
SED-01G:0-10	2	Overlying	0.00	20.1	T17	ND	10	1	ND	28	7.9	17.4	290.55	9.26	0.000	6.64	ND		
SED-05G:0-10	2	Overlying	0.00	20.1	T17	ND	10	1	ND	28	7.9	16.2	289.35	9.26	0.000	6.66	ND		
SED-27G:0-10	2	Overlying	0.00	20.1	T17	0	10	1	0	28	8.0	16.2	289.35	9.26	0.000	6.66	0.000		

### 3.2 *Mytilus galloprovincialis* Statistical Results

Project Name: Port of Friday Harbor: Larval

Sample:	x1
Samp ID:	SED-07G
Alias:	P230324.01
Replicates:	5
Mean:	216.6
SD:	12.621
Tr Mean:	216.6
Trans SD:	12.621

Ref Samp:	x2
Ref ID:	CARR18-23-REF
Alias:	P230510.02
Replicates:	5
Mean:	200.8
SD:	27.535
Tr Mean:	200.8
Trans SD:	27.535

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0	Test Residual Mean: 9.52	Statistic: Approximate t
Residual SD: 13.898	Test Residual SD: 6.783	Balanced Design: Yes
SS: 3670	Ref. Residual Mean: 23.04	Transformation: No Transformation
K: 5	Ref. Residual SD: 9.729	
b: 59.185	Deg. of Freedom: 8	
		Experimental Hypothesis
Alpha Level: 0.05	Alpha Level: 0.1	Null: $x_1 \geq x_2$
Calculated Value: 0.9545	Calculated Value: 2.549	Alternate: $x_1 < x_2$
Critical Value: $\leq 0.842$	Critical Value: $\geq 1.860$	
		Degrees of Freedom: 6
Normally	Variances	Experimental Alpha Level: 0.1
Distributed: Yes	Homogeneous: No	Calculated Value: -1.1664
		Critical Value: $\geq 1.440$
Override Option: N/A		Accept Null Hypothesis: Yes
		Power:
		Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor: Larval

Sample:	x1
Samp ID:	SED-11G
Alias:	P230324.02
Replicates:	5
Mean:	224.2
SD:	37.466
Tr Mean:	224.2
Trans SD:	37.466

Ref Samp:	x2
Ref ID:	CARR18-23-REF
Alias:	P230510.02
Replicates:	5
Mean:	200.8
SD:	27.535
Tr Mean:	200.8
Trans SD:	27.535

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 21.334 SS: 8647.6 K: 5 b: 87.422  Alpha Level: 0.05 Calculated Value: 0.8838 Critical Value: $\leq 0.842$  Normally Distributed: Yes  Override Option: N/A	Test Residual Mean: 31.84 Test Residual SD: 11.682 Ref. Residual Mean: 23.04 Ref. Residual SD: 9.729 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 1.2944 Critical Value: $\geq 1.860$  Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$  Degrees of Freedom: 8 Experimental Alpha Level: 0.1 Calculated Value: -1.1253 Critical Value: $\geq 1.397$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor- Larval

Sample:	x1
Samp ID:	SED-01G:0-10
Alias:	P230324.06
Replicates:	5
Mean:	229.4
SD:	15.437
Tr Mean:	229.4
Trans SD:	15.437

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	216
SD:	26.711
Tr Mean:	216
Trans SD:	26.711

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 14.156 SS: 3807.2 K: 5 b: 59.917  Alpha Level: 0.05 Calculated Value: 0.943 Critical Value: $\leq 0.842$	Test Residual Mean: 10.32 Test Residual SD: 10.255 Ref. Residual Mean: 21.2 Ref. Residual SD: 12.317 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 1.5179 Critical Value: $\geq 1.860$	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$
Normally Distributed: Yes  Override Option: N/A	Variances Homogeneous: Yes	Degrees of Freedom: 8 Experimental Alpha Level: 0.1 Calculated Value: -0.9712 Critical Value: $\geq 1.397$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor: Larval

Sample: x1
Samp ID: SED-27G
Alias: P230324.08
Replicates: 5
Mean: 219.4
SD: 36.787
Tr Mean: 219.4
Trans SD: 36.787

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	216
SD:	26.711
Tr Mean:	216
Trans SD:	26.711

Shapiro-Wilk Results:

Residual Mean:	0
Residual SD:	20.859
SS:	8267.2
K:	5
b:	87.686
Alpha Level:	0.05
Calculated Value:	0.93
Critical Value:	$\leq 0.842$
Normally	
Distributed:	Yes
Override Option:	N/A

Levene's Results:

Test Residual Mean:	27.92
Test Residual SD:	19.465
Ref. Residual Mean:	21.2
Ref. Residual SD:	12.317
Deg. of Freedom:	8
Alpha Level:	0.1
Calculated Value:	0.6523
Critical Value:	$\geq 1.860$
Variances	
Homogeneous:	Yes

Test Results:

Statistic: Student's t

Balanced Design: Yes

Transformation: No Transformation

Experimental Hypothesis

Null:  $x_1 \geq x_2$

Alternate:  $x_1 < x_2$

Degrees of Freedom: 8

Experimental Alpha Level: 0.1

Calculated Value: -0.1672

Critical Value:  $\geq 1.397$

Accept Null Hypothesis: Yes

Power:

Min. Difference for Power:

[illegible]



Project Name: Port of Friday Harbor: Larval

Sample:	x1
Samp ID:	SED-15G
Alias:	P230324.04
Replicates:	5
Mean:	223.4
SD:	25.126
Tr Mean:	223.4
Trans SD:	25.126

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	216
SD:	26.711
Tr Mean:	216
Trans SD:	26.711

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 16.826 SS: 5379.2 K: 5 b: 70.656  Alpha Level: 0.05 Calculated Value: 0.9281 Critical Value: $\leq 0.842$	Test Residual Mean: 19.12 Test Residual SD: 13.203 Ref. Residual Mean: 21.2 Ref. Residual SD: 12.317 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 0.2576 Critical Value: $\geq 1.860$	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$
Normally Distributed: Yes  Override Option: N/A	Variances Homogeneous: Yes	Degrees of Freedom: 8 Experimental Alpha Level: 0.1 Calculated Value: -0.4512 Critical Value: $\geq 1.397$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor: Larval

Sample: x1
Samp ID: SED-03G
Alias: P230324.05
Replicates: 5
Mean: 228.4
SD: 12.973
Tr Mean: 228.4
Trans SD: 12.973

Ref Samp:	x2
Ref ID:	CARR40-23-REF
Alias:	P230510.03
Replicates:	5
Mean:	216
SD:	26.711
Tr Mean:	216
Trans SD:	26.711

Shapiro-Wilk Results:

Residual Mean:	0
Residual SD:	13.625
SS:	3527.2
K:	5
b:	58.143
Alpha Level:	0.05
Calculated Value:	0.9584
Critical Value:	$\leq 0.842$
Normally	
Distributed:	Yes
Override Option:	N/A

Levene's Results:

Test Residual Mean:	9.52
Test Residual SD:	7.417
Ref. Residual Mean:	21.2
Ref. Residual SD:	12.317
Deg. of Freedom:	8
Alpha Level:	0.1
Calculated Value:	1.8165
Critical Value:	$\geq 1.860$
Variances	
Homogeneous:	Yes

Test Results:

Statistic: Student's t  
Balanced Design: Yes  
Transformation: No Transformation

Experimental Hypothesis  
Null:  $x_1 \geq x_2$   
Alternate:  $x_1 < x_2$

Degrees of Freedom: 8  
Experimental Alpha Level: 0.1  
Calculated Value: -0.9337  
Critical Value:  $\geq 1.397$   
Accept Null Hypothesis: Yes

Power:  
Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor- Larval

Sample:	x1
Samp ID:	SED-14G:0-10
Alias:	P230324.03
Replicates:	5
Mean:	212.8
SD:	15.738
Tr Mean:	212.8
Trans SD:	15.738

Ref Samp:	x2
Ref ID:	CARR62-23-REF
Alias:	P230510.04
Replicates:	5
Mean:	199.6
SD:	28.307
Tr Mean:	199.6
Trans SD:	28.307

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 14.861 SS: 4196 K: 5 b: 63.234  Alpha Level: 0.05 Calculated Value: 0.9529 Critical Value: $\leq 0.842$	Test Residual Mean: 11.36 Test Residual SD: 9.295 Ref. Residual Mean: 23.92 Ref. Residual SD: 9.279 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 2.1385 Critical Value: $\geq 1.860$	Statistic: Approximate t Balanced Design: Yes Transformation: No Transformation  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$
Normally Distributed: Yes  Override Option: N/A	Variances Homogeneous: No	Degrees of Freedom: 6 Experimental Alpha Level: 0.1 Calculated Value: -0.9113 Critical Value: $\geq 1.440$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

Project Name: Port of Friday Harbor: Larval

Sample: x1
Samp ID: SED-05G
Alias: P230324.07
Replicates: 5
Mean: 223.4
SD: 15.598
Tr Mean: 223.4
Trans SD: 15.598

Ref Samp:	x2
Ref ID:	CARR62-23-REF
Alias:	P230510.04
Replicates:	5
Mean:	199.6
SD:	28.307
Tr Mean:	199.6
Trans SD:	28.307

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 14.83 SS: 4178.4 K: 5 b: 62.475  Alpha Level: 0.05 Calculated Value: 0.9341 Critical Value: $\leq 0.842$  Normally Distributed: Yes  Override Option: N/A	Test Residual Mean: 10.72 Test Residual SD: 9.983 Ref. Residual Mean: 23.92 Ref. Residual SD: 9.279 Deg. of Freedom: 8  Alpha Level: 0.1 Calculated Value: 2.1657 Critical Value: $\geq 1.860$  Variances Homogeneous: No	Statistic: Approximate t Balanced Design: Yes Transformation: No Transformation  Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$  Degrees of Freedom: 6 Experimental Alpha Level: 0.1 Calculated Value: -1.6466 Critical Value: $\geq 1.440$ Accept Null Hypothesis: Yes  Power: Min. Difference for Power:

[illegible]

### 3.3 *Mytilus galloprovincialis* Reference Toxicant Test Results

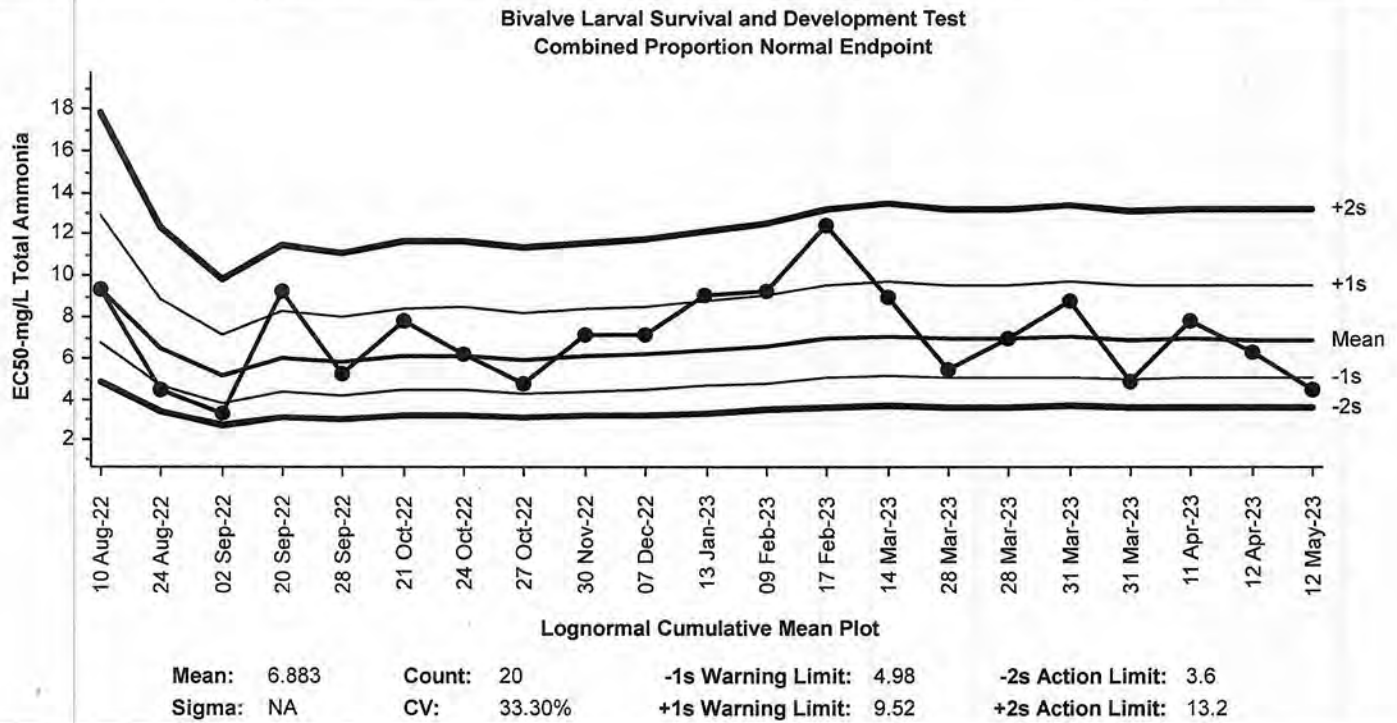
## Bivalve Larval Survival and Development Test

All Matching Labs

Test Type: Development-Survival  
Protocol: EPA/600/R-95/136 (1995)

Organism: *Mytilus galloprovincialis*  
Endpoint: Combined Proportion Normal

Material: Total Ammonia  
Source: Reference Toxicant-REF



## Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	Aug	10	16:57	9.323	2.44	0.936			20-5736-9281	08-2934-0504	EcoAnalysts
2			24	16:43	4.439	-2.444	-1.353	(-)		10-4871-9595	11-0042-4049	EcoAnalysts
3		Sep	2	14:54	3.311	-3.571	-2.257	(-)	(-)	16-0701-8534	00-0124-1152	EcoAnalysts
4			20	16:02	9.267	2.385	0.9176			11-7896-9547	00-7476-6700	EcoAnalysts
5			28	16:31	5.182	-1.701	-0.8756			10-3818-0354	11-9896-8834	EcoAnalysts
6		Oct	21	14:16	7.804	0.9218	0.3877			05-2022-4267	03-4308-3965	EcoAnalysts
7			24	15:17	6.15	-0.7328	-0.3472			01-4864-2336	19-5269-5566	EcoAnalysts
8			27	17:02	4.776	-2.107	-1.127	(-)		12-4527-0974	13-7457-7890	EcoAnalysts
9		Nov	30	14:32	7.166	0.2831	0.1243			11-2220-4195	10-4569-3704	EcoAnalysts
10		Dec	7	17:43	7.159	0.2762	0.1214			19-4874-8030	20-9525-0017	EcoAnalysts
11	2023	Jan	13	15:30	9.078	2.195	0.8538			14-2219-3979	18-3945-1944	EcoAnalysts
12		Feb	9	15:28	9.246	2.364	0.9106			00-8572-7368	10-5325-0783	EcoAnalysts
13			17	14:30	12.4	5.515	1.815	(+)		20-3891-7103	06-7296-3936	EcoAnalysts
14		Mar	14	15:15	8.955	2.073	0.8119			00-9622-9067	21-3408-3763	EcoAnalysts
15			28	15:46	5.455	-1.428	-0.7171			02-2233-3890	16-3797-4494	EcoAnalysts
16			28	15:47	6.941	0.05798	0.02588			01-6969-0938	06-4639-7696	EcoAnalysts
17			31	16:52	8.774	1.891	0.7489			21-2826-5425	10-8042-3972	EcoAnalysts
18			31	16:54	4.818	-2.065	-1.1	(-)		13-8989-7877	05-5295-3514	EcoAnalysts
19		Apr	11	16:37	7.809	0.9266	0.3896			14-1713-1401	15-2064-5147	EcoAnalysts
20			12	15:13	6.298	-0.5842	-0.2736			21-2394-6995	12-4981-2785	EcoAnalysts
21		May	12	15:35	4.42	-2.463	-1.366	(-)		02-3839-1595	05-0285-3181	EcoAnalysts

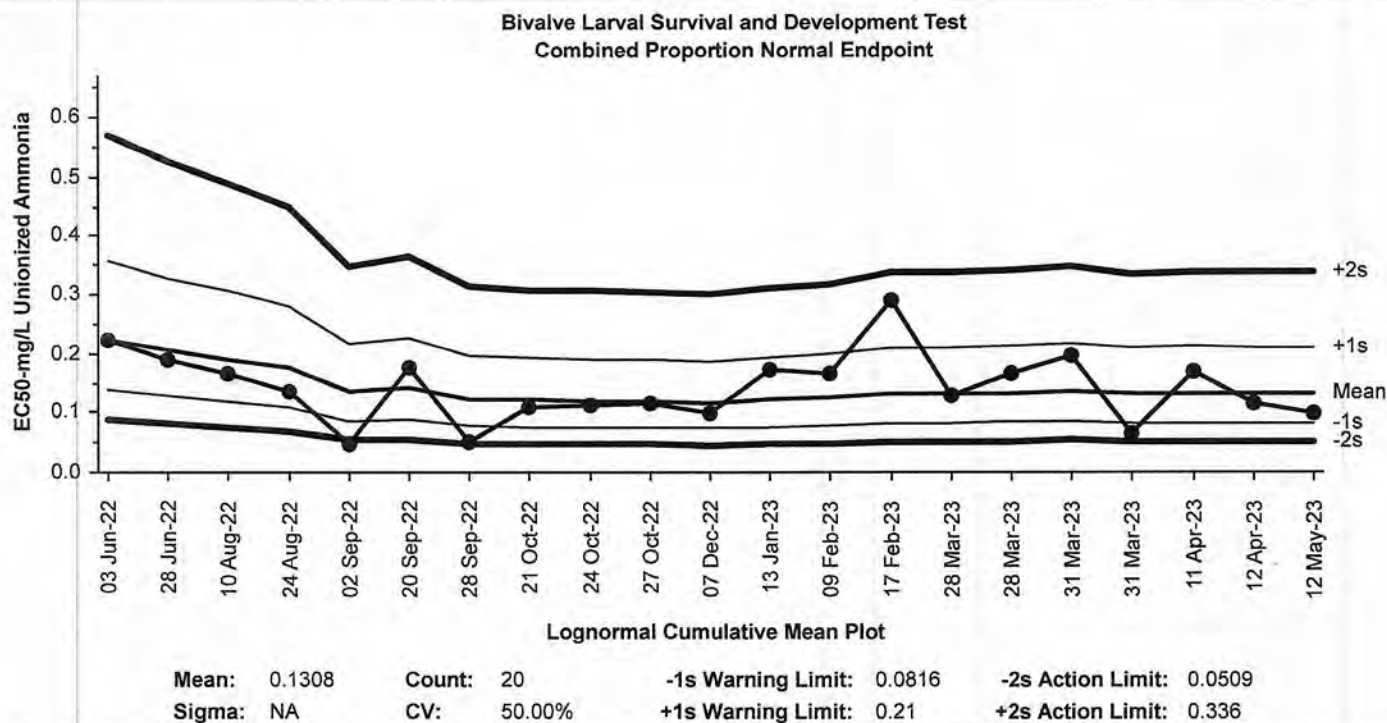
## Bivalve Larval Survival and Development Test

All Matching Labs

Test Type: Development-Survival  
Protocol: EPA/600/R-95/136 (1995)

Organism: *Mytilus galloprovincialis*  
Endpoint: Combined Proportion Normal

Material: Unionized Ammonia  
Source: Reference Toxicant-REF



## Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	Jun	3	16:12	0.2219	0.09112	1.12	(+)		21-4199-4121	20-5427-8206	EcoAnalysts
2			28	16:05	0.188	0.05722	0.7685			19-3785-6817	00-8378-9623	EcoAnalysts
3		Aug	10	16:57	0.1651	0.03433	0.4937			09-3839-8015	12-5640-2017	EcoAnalysts
4			24	16:43	0.1359	0.005056	0.08034			00-7678-9875	07-1760-4646	EcoAnalysts
5		Sep	2	14:54	0.04851	-0.0823	-2.101	(-)	(-)	13-9573-6141	09-4475-1376	EcoAnalysts
6			20	16:02	0.1767	0.04593	0.6374			13-8303-2046	02-4939-5521	EcoAnalysts
7			28	16:31	0.04973	-0.08108	-2.049	(-)	(-)	14-4835-8902	06-7637-8760	EcoAnalysts
8		Oct	21	14:16	0.1071	-0.02372	-0.4239			20-9426-4253	15-1656-6246	EcoAnalysts
9			24	15:17	0.1096	-0.02122	-0.3749			18-7734-9147	06-4748-9707	EcoAnalysts
10			27	17:02	0.1156	-0.01522	-0.262			01-3898-0369	19-9850-5740	EcoAnalysts
11		Dec	7	17:43	0.09634	-0.03448	-0.648			15-6747-3203	15-5237-0673	EcoAnalysts
12	2023	Jan	13	15:30	0.1703	0.03951	0.559			14-6111-3358	19-5184-9524	EcoAnalysts
13		Feb	9	15:28	0.1664	0.03555	0.5092			11-1705-9064	00-9866-2896	EcoAnalysts
14			17	14:30	0.2912	0.1604	1.695	(+)		05-8051-1741	00-4535-0428	EcoAnalysts
15		Mar	28	15:46	0.1275	-0.00334	-0.05477			08-8126-4059	10-2993-2407	EcoAnalysts
16			28	15:47	0.1637	0.03286	0.4747			03-3638-8838	12-4289-2851	EcoAnalysts
17			31	16:52	0.1949	0.06408	0.8445			14-3337-1963	13-8011-4764	EcoAnalysts
18			31	16:54	0.06349	-0.06732	-1.531	(-)		01-2022-2925	11-3364-1842	EcoAnalysts
19		Apr	11	16:37	0.1673	0.03653	0.5217			13-1124-3474	18-0348-0749	EcoAnalysts
20			12	15:13	0.1148	-0.01602	-0.2767			18-5662-1396	07-7214-9910	EcoAnalysts
21		May	12	15:35	0.09858	-0.03223	-0.5991			08-2245-0872	03-4589-6060	EcoAnalysts

# CETIS Summary Report

Report Date: 16 May-23 10:06 (p 1 of 1)  
Test Code/ID: P220819.49 / 02-3839-1595

## Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID: 00-1859-9057	Test Type: Development-Survival	Analyst: Marisa Seibert
Start Date: 12 May-23 15:35	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater
Ending Date: 14 May-23 13:45	Species: Mytilus galloprovincialis	Brine: Not Applicable
Test Length: 46h	Taxon: Bivalvia	Source: Taylor Shellfish Age:
Sample ID: 00-5084-2631	Code: P220819.49	Project: Reference Toxicant
Sample Date: 19 Aug-22	Material: Total Ammonia	Source: Reference Toxicant
Receipt Date: 19 Aug-22	CAS (PC):	Station: P220819.49
Sample Age: 266d 16h	Client: Internal Lab	

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
11-9289-6992	Combined Proportion Norma	Dunnett Multiple Comparison Test	3.02	5.93	4.232	28.6%	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
05-0285-3181	Combined Proportion Norma	Linear Interpolation (ICPIN)	EC15	2.559	---	4.332	1
			EC20	3.022	0.9264	4.193	
			EC25	3.227	1.62	4.353	
			EC40	3.907	2.749	4.846	
			EC50	4.42	3.37	5.24	

## Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
11-9289-6992	Combined Proportion Norma	PMSD	0.2863	Lower	Upper	No	Above Criteria

## Combined Proportion Normal Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	3	0.8857	0.6298	1.1420	0.8000	1.0000	0.0595	0.1030	11.63%	0.00%
1.46		3	0.9143	0.5455	1.2830	0.7429	1.0000	0.0857	0.1485	16.24%	-3.23%
3.02		3	0.7238	0.3737	1.0740	0.6286	0.8857	0.0814	0.1409	19.47%	18.28%
5.93		3	0.2286	0.1056	0.3515	0.2000	0.2857	0.0286	0.0495	21.65%	74.19%
11.8		3	0.0095	-0.0315	0.0505	0.0000	0.0286	0.0095	0.0165	173.21%	98.92%
18.3		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

## Combined Proportion Normal Detail

MD5: FEF0B470A8EECD403B4E66036D5A3BBC

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	1.0000	0.8000	0.8571
1.46		1.0000	1.0000	0.7429
3.02		0.6571	0.8857	0.6286
5.93		0.2000	0.2000	0.2857
11.8		0.0000	0.0286	0.0000
18.3		0.0000	0.0000	0.0000

## Combined Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	40/40	28/35	30/35
1.46		39/39	35/35	26/35
3.02		23/35	31/35	22/35
5.93		7/35	7/35	10/35
11.8		0/35	1/35	0/35
18.3		0/35	0/35	0/35



# CETIS Summary Report

Report Date: 16 May-23 10:10 (p 1 of 1)  
Test Code/ID: P220819.49UIA / 08-2245-0872

## Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID: 00-1859-9057	Test Type: Development-Survival	Analyst: Marisa Seibert
Start Date: 12 May-23 15:35	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater
Ending Date: 14 May-23 13:45	Species: Mytilus galloprovincialis	Brine: Not Applicable
Test Length: 46h	Taxon: Bivalvia	Source: Taylor Shellfish Age:
Sample ID: 16-7070-3506	Code: P220819.49UIA	Project: Reference Toxicant
Sample Date: 19 Aug-22	Material: Unionized Ammonia	Source: Reference Toxicant
Receipt Date: 19 Aug-22	CAS (PC):	Station: P220819.49UIA
Sample Age: 266d 16h	Client: Internal Lab	

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
00-9013-8386	Combined Proportion Norma	Dunnett Multiple Comparison Test	0.065	0.127	0.09086	28.6%	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
03-4589-6060	Combined Proportion Norma	Linear Interpolation (ICPIN)	EC15	0.05494	---	0.09924	1
			EC20	0.06506	0.0147	0.09647	
			EC25	0.07057	0.02483	0.0995	
			EC40	0.08729	0.05491	0.1092	
			EC50	0.09858	0.0723	0.1168	

## Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
00-9013-8386	Combined Proportion Norma	PMSD	0.2863	Lower	Upper	No	Above Criteria

## Combined Proportion Normal Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	3	0.8857	0.6298	1.1420	0.8000	1.0000	0.0595	0.1030	11.63%	0.00%
0.025		3	0.9143	0.5455	1.2830	0.7429	1.0000	0.0857	0.1485	16.24%	-3.23%
0.065		3	0.7238	0.3737	1.0740	0.6286	0.8857	0.0814	0.1409	19.47%	18.28%
0.127		3	0.2286	0.1056	0.3515	0.2000	0.2857	0.0286	0.0495	21.65%	74.19%
0.253		3	0.0095	-0.0315	0.0505	0.0000	0.0286	0.0095	0.0165	173.21%	98.92%
0.396		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

## Combined Proportion Normal Detail

MD5: 1591C79734DEC8D602FBBEBD63916273

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	1.0000	0.8000	0.8571
0.025		1.0000	1.0000	0.7429
0.065		0.6571	0.8857	0.6286
0.127		0.2000	0.2000	0.2857
0.253		0.0000	0.0286	0.0000
0.396		0.0000	0.0000	0.0000

## Combined Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	40/40	28/35	30/35
0.025		39/39	35/35	26/35
0.065		23/35	31/35	22/35
0.127		7/35	7/35	10/35
0.253		0/35	1/35	0/35
0.396		0/35	0/35	0/35

# CETIS Test Data Worksheet

Report Date: 16 May-23 10:05 (p 1 of 1)  
 Test Code/ID: P220819.49 / 02-3839-1595

Bivalve Larval Survival and Development Test								EcoAnalysts
Start Date: 12 May-23 15:35		Species: Mytilus galloprovincialis		Sample Code: P220819.49				
End Date: 14 May-23 13:45		Protocol: EPA/600/R-95/136 (1995)		Sample Source: Reference Toxicant				
Sample Date: 19 Aug-22		Material: Total Ammonia		Sample Station: P220819.49				

Conc-mg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	9	35	41	41	40	
0	D	2	12	35	29	29	28	
0	D	3	3	35	30	30	30	
1.46		1	15	35	40	40	39	
1.46		2	2	35	35	35	35	
1.46		3	11	35	27	27	26	
3.02		1	10	35	23	23	23	
3.02		2	7	35	32	32	31	
3.02		3	5	35	25	25	22	
5.93		1	13	35	19	19	7	
5.93		2	14	35	26	26	7	
5.93		3	16	35	34	34	10	
11.8		1	17	35	45	45	0	
11.8		2	4	35	36	36	1	
11.8		3	1	35	25	25	0	
18.3		1	6	35	30	30	0	
18.3		2	8	35	27	27	0	
18.3		3	18	35	37	37	0	

# CETIS Test Data Worksheet

Report Date: 16 May-23 10:09 (p 1 of 1)  
 Test Code/ID: P220819.49UIA / 08-2245-0872

Bivalve Larval Survival and Development Test								EcoAnalysts
Start Date: 12 May-23 15:35		Species: Mytilus galloprovincialis		Sample Code: P220819.49UIA				
End Date: 14 May-23 13:45		Protocol: EPA/600/R-95/136 (1995)		Sample Source: Reference Toxicant				
Sample Date: 19 Aug-22		Material: Unionized Ammonia		Sample Station: P220819.49UIA				
Conc-mg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	4	35	41	41	40	
0	D	2	10	35	29	29	28	
0	D	3	17	35	30	30	30	
0.025		1	3	35	40	40	39	
0.025		2	16	35	35	35	35	
0.025		3	15	35	27	27	26	
0.065		1	2	35	23	23	23	
0.065		2	6	35	32	32	31	
0.065		3	7	35	25	25	22	
0.127		1	11	35	19	19	7	
0.127		2	18	35	26	26	7	
0.127		3	12	35	34	34	10	
0.253		1	1	35	45	45	0	
0.253		2	5	35	36	36	1	
0.253		3	14	35	25	25	0	
0.396		1	9	35	30	30	0	
0.396		2	8	35	27	27	0	
0.396		3	13	35	37	37	0	

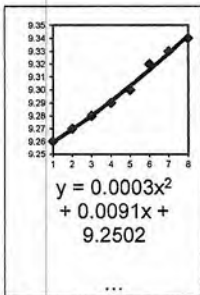
# Un-ionized Ammonia Calculator

CLIENT:	Leon Environmental	Date of Test:	May 12, 2023
PROJECT:	Port of Friday Harbor	Test Type:	M. sp.
COMMENTS: P220819.49			

To convert Total Ammonia (mg/L) to Free (un-ionized) Ammonia (mg/L) enter the corresponding total ammonia, salinity, temperature, and pH.

Sample		Mod	NH3T (mg/L)	salinity (ppt)	pH	temp (C)	temp (K)	pKa <sup>s</sup>	NH <sub>3</sub> U (mg/L)
Target / Sample Name			Actual	Actual	Actual	Actual	Calculated	Calculated	Calculated
Example 3.5			2.000	10.0	7.5	5.0	278.15	9.2520	0.008
1									
2	1.5		1.46	28	7.8	15.6	288.75	9.2555	0.025
3	3		3.02	28	7.9	15.6	288.75	9.2555	0.065
4	6		5.93	28	7.9	15.5	288.65	9.2555	0.127
5	12		11.8	28	7.9	15.5	288.65	9.2555	0.253
6	18		18.3	28	7.9	15.6	288.75	9.2555	0.396
7									
8									
9									
10									
11									
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13									
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16									
17									
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42									
43									
44									
45									

Ionic strength:pKa <sup>s</sup>	
1	9.26
2	9.27
3	9.28
4	9.29
5	9.30
6	9.32
7	9.33
8	9.34



QA ✓  
M22/4

# 48 Hour Bivalve Development Reference Toxicant Test

Test ID: <b>P220819.49</b>	Replicates: 3	Study Director: J. Levengood, M. Seibert	Location: <b>Bath 1</b>
Dilution Water Batch: <b>FSW051223.02</b>	Organism Batch: <b>T5050123.0 B+C</b>	Associated Test(s): Port of Friday Harbor	Organism: M.sp.
Chamber Size/Type: 30 ml shell vial	Exposure Volume: 10 ml		
Toxicant: Ammonium Chloride:	Lot #: <b>22E3156086</b>	Date Prepared: <b>5/12/23</b>	Initials: <b>ML</b>
Target Concentrations:  <b>See spiking worksheet</b>		Quantity of Stock: Target: <b>See spiking worksheet</b>	Quantity of Diluent: Target: <b>200 mL</b>
<b>See spiking worksheet</b>		Actual: <b>See spiking worksheet</b>	Actual: <b>200 mL</b>

SPAWNING DATA						
Initial Spawning Time: <b>1149</b>	Final Spawning Time: <b>1335</b>	Fertilization Time: <b>1335</b>	No. of Females: <b>6</b>	No. of Males: <b>2</b>		
Embryo Density (count/mL):	1. <b>180</b>	2. <b>190</b>	3. <b>201</b>	Mean: <b>0190190</b>		
Stocking Volume Calculation: $2700/19000 = 0.14 \times 40 = 5.68 / 40 - 5.68 = 34.32$ <b>5.68 egg stock</b>						
<b>0 Hours</b> Date: <b>5/12/23</b> WQ Time: <b>1227</b> <b>ML</b> Start Time: <b>1535</b> Initials: <b>NL/MS</b> <b>34.32 mL FSW</b> <div style="text-align: center;"><b>STOCK</b></div>						
	Control	1.5	3	6	12	18
D.O. (%) ( $>4.8$ mg/L)	<b>8.6</b>	<b>8.6</b>	<b>8.7</b>	<b>8.7</b>	<b>8.7</b>	<b>8.7</b>
Temperature ( $16 \pm 1^\circ\text{C}$ )	<b>15.9</b>	<b>15.6</b>	<b>15.6</b>	<b>15.5</b>	<b>15.5</b>	<b>15.6</b>
Salinity ( $30 \pm 2$ ppt) <b>28 <math>\pm</math> 1 ppt</b>	<b>28</b>	<b>28</b>	<b>28</b>	<b>28</b>	<b>28</b>	<b>28</b>
pH (7.5-9)	<b>7.8</b>	<b>7.8</b>	<b>7.9</b>	<b>7.9</b>	<b>7.9</b>	<b>7.9</b>
<b>Day 1</b>	Temperature ( $16 \pm 1^\circ\text{C}$ )	<b>15.6</b>	<b>Tile NL</b>			
<b>Final Day</b>	Date: <b>5/14/23</b>	WQ Time: <b>1012</b>	<b>NL m: 8</b>	End Time: <b>1345</b>	Initials: <b>NL</b>	
Formalin Lot #: <b>220304-50</b>			Rose Bengal Lot #: <b>5135</b>			
STOCK						
	Control	1.5	3	6	12	18
D.O. (%) ( $>4.8$ mg/L)	<b>8.3</b>	<b>8.3</b>	<b>8.2</b>	<b>8.1</b>	<b>8.1</b>	<b>8.3</b>
Temperature ( $16 \pm 1^\circ\text{C}$ )	<b>15.8</b>	<b>15.9</b>	<b>15.9</b>	<b>15.9</b>	<b>15.9</b>	<b>15.9</b>
Salinity ( $30 \pm 2$ ppt) <b>28 <math>\pm</math> 1</b>	<b>28</b>	<b>28</b>	<b>28</b>	<b>28</b>	<b>28</b>	<b>28</b>
pH (7.5-9)	<b>7.9</b>	<b>8.0</b>	<b>8.0</b>	<b>8.0</b>	<b>8.0</b>	<b>8.0</b>

**DIE-NL 5/12**



## 48 Hour Bivalve Development Reference Toxicant Test

Conc.	Rep	Number Normal	Number Abnormal	Date	Initials
Control	1	40	1	5/15/23	MS
	2	28	1	5/15/23	MS
	3	30	0	5/15/23	MS
1.5	1	39	1	5/15/23	MS
	2	35	0	5/15/23	MS
	3	24	1	5/15/23	MS
3	1	23	0	5/15/23	MS
	2	31	1	5/15/23	MS
	3	22	3	5/15/23	MS
6	1	7	12	5/15/23	MS
	2	7	19	5/15/23	MS
	3	10	24	5/15/23	MS
12	1	0	45	5/15/23	MS
	2	1	35	5/15/23	MS
	3	0	25	5/15/23	MS
18	1	0	30	5/15/23	MS
	2	0	27	5/15/23	MS
	3	0	37	5/15/23	MS

Stocking Density		
Rep	Count	Init.
1	37	MS
2	23	MS
3	45	MS
<b>Mean:</b>	35	

<b>QA Count Checks:</b> #1 conc/rep <u>0-2</u> # normal <u>27</u> # abnormal <u>1</u>	#2 conc/rep <u>1.5-3</u> # normal <u>25</u> # abnormal <u>1</u>	#3 conc/rep <u>6-3</u> # normal <u>10</u> # abnormal <u>24</u>	#4 conc/rep <u>12-1</u> # normal <u>0</u> # abnormal <u>45</u>
Tech. Init. <u>52</u>	Tech. Init. <u>52</u>	Tech. Init. <u>52</u>	Tech. Init. <u>52</u>
Calc. $\left( \frac{28}{28+1} - \frac{27}{27+1} \right) \times 100 = 0.12\%$	$\left( \frac{26}{26+1} - \frac{25}{25+1} \right) \times 100 = 0.14\%$	Same count = 0%	Same count = 0%

QA Check Acceptability: ☒ <5% difference in means of QA & orig. counts

# Ammonia Reference Toxicant Spiking Worksheet

Reference Toxicant ID: P220819.49  
 Date Prepared: 5/12/23  
 Technician Initials: ML

## Biv / Echino NH<sub>3</sub> RT

Assumptions in Model  
 Stock ammonia concentration is 9,000 mg/L = 9 mg/mL

Date: 5/11/2023  
 Measurement: 8340

Test Solutions			Volume of stock to reach desired concentration	
Measured Concentration	Desired Concentration	Volume		
mg/L	mg/L	mL	mL stock to increase	
				SALT WATER
1.46	1.5	200		0.054
3.02	3	200		0.108
5.93	6	200		0.216
① 11.2 11.8	12	200		0.432
① 16.5 18.3	18	200		0.647

Remeasured. ML 5/12.

## **APPENDIX B. CHAIN-OF-CUSTODY FORMS, LOGS, AND PRE-TEST DOCUMENTS**



# SUBCONTRACT SAMPLE CHAIN OF CUSTODY

Send Report To Peter Leon

Company Leon Environmental

Address 8047 Burke Ave N

City, State, ZIP Seattle, WA 98103

Phone: (206) 948-5366

SUBCONTRACTER  
Eco Analysts

PROJECT NAME/NO.

PO #

Port of Friday Harbor (PofH)  
Jensen's Marina

REMARKS

FBI SDG 303373 and 303388

Page # 1 of 1

TURNAROUND TIME

☒ Archive

RUSH

Rush charges authorized by:

SAMPLE DISPOSAL

Dispose after 30 days

Return samples

Will call with instructions

Sample ID	Lab ID	Date Sampled	Time Sampled	Matrix	# of jars	ANALYSES REQUESTED										Cooler	Temp (T <sub>24</sub> )	Notes
SED-07G:0-10		3/20/2023	1002	sediment	1											2	0.3	<del>P230324.01</del> Bioassay Hold
SED-11G:0-10		3/20/2023	924	sediment	1											2	0.3	P230324.02
SED-14G:0-10		3/20/2023	1011	sediment	1											2	0.3	P230324.03
SED-15G:0-10		3/20/2023	1042	sediment	1											4	0.2	P230324.04
SED-03G:0-10		3/20/2023	1315	sediment	1											1	0.3	P230324.05
SED-01G:0-10		3/20/2023	1445	sediment	1											4	0.2	P230324.06
SED-05G:0-10		3/21/2023	1310	sediment	1											1	0.3	P230324.07
SED-27G:0-10		3/23/2023	842	sediment	1											3	0.5	P230324.08

Friedman & Bruya, Inc.  
3012 16th Avenue West

Seattle, WA 98119-2029

Ph. (206) 285-8282

Fax (206) 283-5044

SIGNATURE

PRINT NAME

COMPANY

DATE

TIME

Relinquished by:

Michael Erdahl

Friedman & Bruya

3/24/23

10:50

Received by:

Régina Edwards

EcoA

3/24/23

10:50

Relinquished by:

Régina Edwards

EcoA

3/24/23

13:00

Received by:

Sarah Zischke

EcoA

3/24/23

13:00

# CHAIN OF CUSTODY



LIFE IN WATER

EcoAnalysts, Inc.  
4770 NE View Dr., Port Gamble, WA. 9836  
Tel: (360) 297-604

Destination: <b>EcoAnalysts</b>	Sample Originator (Organization): <b>EcoAnalysts</b>	Report Results To:	Phone:
Destination Contact:	PERSON WHO COLLECTED SAMPLE: <b>Sarah Zischke</b>	Contact Name:	Fax:
Date: <b>5/10/23</b>	Address:	Address:	Email:
Turn-Around-Time:	Phone:	Invoicing To:	
Project Name: <b>CARR INLET Reference collection</b>	Fax:	Comments or Special Instructions:	
Contract/PO:	E-mail:		

No.	Sample ID	Matrix	Volume & Type of Container	Date & Time	Analysis:	Preservation	Sample Temp Upon Receipt	LAB ID
1	CARR18-23-REF	Sed	15L	5/10/23 1400		①	①	P230510.02
2	CARR40-23-REF		15L	5/10/23 1130		①	①	P230510.03
3	CARR62-23-REF		15L	5/10/23 1452		①	①	P230510.04
4	CARR76-23-REF		15L	5/10/23 1015		①	①	P230510.05
5	CARR-REF		10L	5/10/23 1330		①	①	P230510.06
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

Relinquished by:	Received by:	Relinquished by:	Received by:	Matrix Codes
Print Name: <b>Sarah Zischke</b>	Print Name: <b>Sarah Zischke</b>	Print Name:	Print Name:	FW = Fresh Water
Signature: <i>[Signature]</i>	Signature: <i>[Signature]</i>	Signature:	Signature:	SB = Salt & Brackish Water
Affiliation: <b>EcoAnalysts</b>	Affiliation: <b>EcoAnalysts</b>	Affiliation:	Affiliation:	SS = Soil & Sediment
Date/Time: <b>5/10/23 1730</b>	Date/Time: <b>5/10/23 1730</b>	Date/Time:	Date/Time:	

① Received sample in lab < 8 hrs from collection - sz 5/10/23

Project: REF-SED Collection

Recorder: Sarah Zischke

[illegible]

Q 1E-52 5/10/23

### Wet-Sieve Procedure for Determining Percent Fines (<63 µm) of Sediment

DATE:	5/10/23	CLIENT:	Various	PROJECT:	Various
-------	---------	---------	---------	----------	---------

**Procedure:**

1. Collect 50 mL of sediment to be analyzed
2. Transfer sediment to a #230 (63  $\mu$ m) testing sieve
3. Rinse sieve thoroughly with a stream of water until water flowing through the sieve is clear
4. Transfer all retained material to a 100mL graduated cylinder using a small funnel and DI squirt bottle
5. Allow sediment to settle. Record the volume of sediment retained below.

[illegible]

①/E-S2 5/10/23

### Wet-Sieve Procedure for Determining Percent Fines (<63 μm) of Sediment

DATE: 5/5/2023	CLIENT: Leon Environmental	PROJECT: Port of Friday Harbor 2023
----------------	----------------------------	--

### Procedure:

1. Collect 50 mL of sediment to be analyzed
2. Transfer sediment to a #230 (63  $\mu\text{m}$ ) testing sieve
3. Rinse sieve thoroughly with a stream of water until water flowing through the sieve is clear
4. Transfer all retained material to a 100mL graduated cylinder using a small funnel and DI squirt bottle
5. Allow sediment to settle. Record the volume of sediment retained below.

[illegible]

CLIENT			PROJECT										SPECIES												
Leon Environmental			Port of Friday Harbor										Various												
Calibration Standards Temperature: 21.3			Date: 5/5/2023			Tech: NL																			
NH <sub>3</sub> sample temperature should be within +/- 1°C of standard temp at time and date of analysis																									
Measured														Calculated											
		Overlying/ Porewater	Total NH <sub>3</sub> (mg/L)	NH <sub>3</sub> sample temp	WQ/Temp Meter	Total Dissolved Sulfide (µg/L as S)	Sulfide Sample Volume (ml)	Sulfide Multiplier	Total Dissolved Sulfide (µg/L as S)	Porewater salinity (ppt)	Porewater pH	Projected test temp (C)	Temp (K)	Unionized NH <sub>3</sub> (mg/L)	Undissociated Sulfide (µg/L as H <sub>2</sub> S)	Undissociated Sulfide (mg/L as H <sub>2</sub> S)									
Sample	Material		Record	Record	Record	Record	Record	Record	Record	Record	Record	Record	Record	Record	Record	Record	Record	Notes							
SED-01G-10	Bulk	PW	7.72	20.3	M8/T17	ND	10	1.0	ND	31	7.5	16.0	289.15	9.26	0.069	6.66	ND								
SED-03G-10	Bulk	PW	3.39	20.3	M8/T17	ND	10	1.0	ND	31	7.5	16.0	289.15	9.26	0.030	6.66	ND								
SED-05G-10	Bulk	PW	5.99	20.4	M8/T17	7	10	1.0	7	31	7.4	16.0	289.15	9.26	0.043	6.66	1.421	0.0014							
SED-07G-10	Bulk	PW	29.1	20.4	M8/T17	26	10	1.0	26	31	7.8	16.0	289.15	9.26	0.517	6.66	2.394	0.0024							
SED-11G-10	Bulk	PW	23.6	20.4	M8/T17	ND	10	1.0	ND	30	7.7	16.0	289.15	9.26	0.334	6.66	ND								
SED-14G-10	Bulk	PW	9.94	20.4	M8/T17	ND	10	1.0	ND	31	7.5	16.0	289.15	9.26	0.089	6.66	ND								
SED-15G-10	Bulk	PW	6.46	20.4	M8/T17	18	10	1.0	18	31	7.4	16.0	289.15	9.26	0.046	6.66	3.654	0.0037							
SED-27G-10	Bulk	PW	12.4	20.3	M8/T17	25	10	1.0	25	31	7.6	16.0	289.15	9.26	0.140	6.66	3.461	0.0035							

CLIENT				PROJECT							SPECIES											
Leon Environmental				Port of Friday Harbor							N.a.											
Calibration Standards Temperature: 20.7				Date: 5/9/2023	Tech: MS																	
NH <sub>3</sub> sample temperature should be within +/- 1°C of standard temp at time and date of analysis																						
measured																	Calculated					
		Total		Sulfide				Total				Projected		Projected		Unionized		Undissociated		Undissociated		
		Dissolved		Sample				Dissolved		Actual		Test		Test		NH <sub>3</sub>		Sulfide		Sulfide		
		Sulfide (µg/L as S)		Volume (ml)				Sulfide (µg/L as S)		Salinity (ppt)		Salinity (ppt)		pH		(mg/L)		(µg/L as H <sub>2</sub> S)		(mg/L as H <sub>2</sub> S)		
Sample		Material		Overlying/ Porewater		Total NH <sub>3</sub> (mg/L)		NH <sub>3</sub> Sample Temp		WQ/Temp Meter		Sulfide Multiplier		Actual pH		Temp (K)		NH <sub>3</sub> pKa <sup>s</sup>		H <sub>2</sub> S pKa <sup>*</sup>		
Record				Record		Record		Record		Record		Record		Record		Record		Record		Record		
SED-07G: 0-10		Mock		OV		1.29		20.8		M9/T17				28		28		8.0		20.0		
SED-07G: 0-10		Mock		PW		7.17		21.4		M9/T17				29		28		7.4		20.0		
														293.15		9.26		0.048				
														293.15		9.26		0.069				



CLIENT			PROJECT										SPECIES											
Calibration Standards Temperature: 20.9			Date: 5/8/2023			Tech: DM																		
NH <sub>3</sub> sample temperature should be within +/- 1°C of standard temp at time and date of analysis																								
Sample	Material	Overlying/ Porewater	Total NH <sub>3</sub>	NH <sub>3</sub> Sample	WQ/Temp	Total	Sulfide	Sulfide	Total	Actual	Projected	Actual	Projected	Temp (K)	NH <sub>3</sub> pKa <sup>1</sup>	Unionized	H <sub>2</sub> S pKa <sup>*</sup>	Undissociated	Undissociated					
			(mg/L)	Temp	Meter	Dissolved	Sample		Dissolved												Salinity	Test Salinity	Test Temp	NH <sub>3</sub>
Record			Record	Record	Record	Record	Record	Record	Calculated	Record	Record	Record	Record	Record	Calculated	Calculated	Calculated	Calculated	Calculated	Notes				
SED-01G-10	Mock	Overlying	0.00	20.1	M8/T17		10	1.0		28	28	7.9	16.0	289.15	9.26	0.000								
SED-03G-10	Mock	Overlying	0.00	19.9	M8/T17		10	1.0		28	28	7.9	16.0	289.15	9.26	0.000								
SED-05G-10	Mock	Overlying	0.00	20.4	M8/T17		10	1.0		28	28	7.8	16.0	289.15	9.26	0.000								
SED-07G-10	Mock	Overlying	0.108	20.0	M8/T17		10	1.0		28	28	7.9	16.0	289.15	9.26	0.002								
SED-11G-10	Mock	Overlying	0.185	20.4	M8/T17		10	1.0		28	28	7.9	16.0	289.15	9.26	0.004								
SED-14G-10	Mock	Overlying	0.00	20.0	M8/T17		10	1.0		28	28	7.9	16.0	289.15	9.26	0.000								
SED-15G-10	Mock	Overlying	0.00	20.5	M8/T17	13	10	1	13	28	28	7.9	16.0	289.15	9.26	0.000	6.66	0.981	0.0010					
SED-27G-10	Mock	Overlying	0.098	20.0	M8/T17	ND	10.0	1.0	ND	28	28	7.8	16.0	289.15	9.26	0.002	6.66	ND	ND					



## Pretest Analysis

Sample ID	Sample Type	Grain Size (% fine s)	Total Ammonia <sup>1,3</sup> (mg/L)	Unionized Ammonia <sup>1,3</sup> (mg/L)	Total Sulfide (µg/L as S)	Hydrogen Sulfide <sup>2</sup> (mg/L as H <sub>2</sub> S)	Salinity	pH
SED-01G: 0-10	Bulk Sediment	34	7.72	<b>0.069 - 0.093</b>	ND	ND	31	7.5
SED-03G: 0-10		50	3.39	<b>0.030 - 0.041</b>	ND	ND	31	7.5
SED-05G: 0-10		64	5.99	<b>0.043 - 0.058</b>	7	0.001	31	7.4
SED-07G: 0-10		12	29.1	<b>0.517 - 0.692</b>	26	0.002	31	7.8
SED-11G: 0-10		26	23.6	<b>0.334 - 0.448</b>	ND	ND	30	7.7
SED-14G: 0-10		58	9.94	<b>0.089 - 0.120</b>	ND	ND	31	7.5
SED-15G: 0-10		50	6.46	<b>0.046 - 0.062</b>	18	<b>0.003 – 0.004</b>	31	7.4
SED-27G: 0-10		44	12.4	<b>0.140 - 0.188</b>	25	<b>0.003</b>	31	7.6
SED-07G: 0-10	Mock Solid Phase - Porewater		7.17	0.069			29	7.4
SED-01G: 0-10	Mock Larval		0.00	0.00			28	7.9
SED-03G: 0-10			0.00	0.00			28	7.9
SED-05G: 0-10			0.00	0.00			28	7.8
SED-07G: 0-10			0.108	0.002			28	7.9
SED-11G: 0-10			0.185	0.004			28	7.9
SED-14G: 0-10			0.00	0.00			28	7.9
SED-15G: 0-10			0.00	0.00	13	0.001	28	7.9
SED-27G: 0-10			0.098	0.002	ND	ND	28	7.8

<sup>1</sup> Unionized Ammonia Purging triggers: >0.04 mg/L for larval, >0.8 mg/L *Eoh* and >0.46 mg/L *Neanthes* (Inouye et al. 2015)

<sup>2</sup> Hydrogen Sulfide Purging triggers: >0.0025 mg/L larval, >0.122 mg/L *Eoh* and >3.4 mg/L *Neanthes* (Inouye et al. 2015)

<sup>3</sup> Estimated values at test temperatures of 16 and 20°C

ND= not detected

**Bold** values exceed purging triggers for Bivalve test

**Bold and Italicized** values exceed purging triggers for Bivalve and *Neanthes* test

**From:** [Peter Leon](#)  
**To:** [Jay Word](#)  
**Cc:** [Marisa Seibert](#); [Julia Levengood](#); [John Malek](#)  
**Subject:** PoFH: 10-day Amphipod Test Call Summary  
**Date:** Wednesday, May 24, 2023 3:28:53 PM

---

Thanks Jay! I agree with your summary, although I recommend that in your report you consider elaborating on your hypothesis that the results suggest that the test species you received were not fully healthy (including Pete's concurrence).

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**From:** Jay Word <jword@ecoanalysts.com>  
**Sent:** Wednesday, May 24, 2023 2:03 PM  
**To:** Peter Leon <peter@leon-environmental.com>  
**Cc:** Marisa Seibert <mseibert@ecoanalysts.com>; Julia Levengood <jlevengood@ecoanalysts.com>  
**Subject:** Summary of the call

Hello Peter,

I wanted to summarize the call in an email so that we are all on the same page. Let me know if you think I missed anything.

Attendees of the meeting (5.24.23) were Peter Leon (Leon Environmental), Todd Nicholson (Port of Friday Harbor), Bonnie Brooks (WDOE), and Pete Adolphson (WDOE). The call was organized to discuss the poor control performance (84% survival) from the Eoh test. We talked through the summary results that even though the controls did not pass the control performance criterion, all three references passed the reference performance criterion. All sediment treatments, when compared to appropriate reference sediment would pass the SCO and CSL criteria, except for SED-03G:0-10. Sample SED-03G:0-10 had >25% mortality (28%) and was statistically different than CARR-40-23-REF which means it would fail SCO criteria but not CSL. We discussed that the reference toxicant test showed 90% survival (barely passing) but was within the +/- 2 SD of the control chart. In addition to the summary Eoh data we showed the preliminary results from the larval test which showed no SCO or CSL failures.

Pete Adolphson wanted to look at the replicate data from the Eoh test. He thought that, based on all of the evidence at this time, all samples including SED-03G:0-10 should be considered to pass all SCO and CSL criteria. No bioassays need to be rerun but we will need to discuss this and qualify the Eoh result for SED-03G:0-10 in the report.

### **Jay Word**

EcoAnalysts, Inc.  
Office: 4729 NE View Drive, PO Box 216, Port Gamble, WA 98364  
C: 206.779.9500  
[linkedin.com/in/jack-jay-word-871ba57](https://www.linkedin.com/in/jack-jay-word-871ba57)

Headquarters: 1420 S. Blaine St., Suite 14 | Moscow, ID 83843  
O: 208.882.2588

## ORGANISM RECEIPT LOG

Date: 5/10/23		Time: 1225		Batch No. NWA051023.01			
Organism: Eohaustorius estuarius							
Source / Supplier: Northwest Amphipod, LLC							
No. Ordered: 1050 + 10%		No. Received: 1815 +		Source Batch: Collection date, hatch date, etc.): collected: 5/8/23			
Condition of Organisms: ①			Approximate Size or Age: (Days from hatch, life stage, size class, etc.): size: 3-5mm				
Shipper: FedEx			B of L (Tracking No.) 8165 6A17 4170				
Condition of Container: Good			Received By: DM/LG				
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
①	①	7A	①	- ①	①	①	DM/LG
*If >10% contact lab manager							
Notes:							

① Received on/in moist sand - SM-5/10/23  
7/27/15

**Northwest Amphipod, LLC**

3101 SE Ferry Slip Rd #803, P.O. Box 542, Newport, OR 97365

Tel: 541-867-7225, [nwamphipod@gmail.com](mailto:nwamphipod@gmail.com)**SUBJECT: Animal Collection Data Sheet (shipping)****SOLD TO:**EcoAnalysts  
4770 NE View Dr.  
P.O. Box 216  
Port Gamble WA 98364Marisa Seibert, Brian Hester/Collin Ray/Hillary Eichler,  
Michelle Knowlen, Lauren Brandkamp, Julia Levengood  
360.297.6040  
Julia Levengood  
360.509.4141

FedEx# 1817-5747-7

P.O. # 2676

**DATE OF SHIPMENT: 5-9-23****ANIMAL HISTORY**

Species	Age/Size	Number Shipped
<i>Eohaustorius estuarius</i>	3-5mm	1650 + 10%

**WATER QUALITY AT TIME OF SHIPMENT**

Temperature (°C): 13.9	pH: 8.0	Salinity (ppt): 30.0	D.O. (mg/L): 8.5
Other:			

**PACKAGED BY:** GS**DATE:** 5-9-23**FIELD COLLECTION/CULTURE NOTES**

Collected 5-8-23 from Yaquina Bay, OR

Interstitial WQ: Temp 6.5°C, Salinity 28.0ppt; salinity adjusted ~5 ppt up or down as needed.

Held at ~15°C in aerated water.

**ADDITIONAL COMMENTS**

4-liters of 0.5 mm sieved home sediment included.

**PLEASE RETURN ALL SHIPPING MATERIALS**

If you have any questions, please call Yves Nakahama, Gary Buhler or Gerald Irissarri at (541) 867-7225.

Thank You.

ORGANISM: E. coli  
LOCATION: Booth 3

LOCATION: Bath 3

Batch Number: NWA051023.01

Date Received: 5/10/23

Initial # of Organisms: 1815

10% mortality = 187

[illegible]

\*For all containers and all days for a given batch; if >10% notify lab manager

<sup>1</sup> Cumulative # Mort is the running total of the **current day's total mortality** + **previous cumulative culture mortality** since acquired in lab

## ORGANISM RECEIPT LOG

Date: 5/11/23		Time: 0834		Batch No. AT5051123.01			
Organism: Neanthus arenaceodentata							
Source / Supplier: Aquatic Toxicology Support							
No. Ordered: 560		No. Received: 560 + 10% (616)		Source Batch: Emerged: 4/24/23			
Condition of Organisms: Good			Approximate Size or Age: 17 days post emerged				
Shipper: Courier			B of L (Tracking No.): NA				
Condition of Container: Good			Received By: DM				
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal (Include Units) PPT	pH (Units)	# Dead	% Dead*	Tech. (Initials)
COMP	16.2	19.1	30	~7.5	0	—	DM
*If >10% contact lab manager							
Notes:							



Aquatic Toxicology Support  
1849 Charleston Beach Road West  
Bremerton, Washington 98312  
(360) 813-1202

Order Summary

Species: <i>Neanthes arenaceodentata</i> *	Emerge Date:
Number Ordered: 560	Number Shipped: 24 April '23
Date Shipped: 11 May '23	Salinity (ppt): 560 + 10‰
pH: 7.8	Temperature (°C): 31
	20.3

\*Smith 1964. CSU Long Beach strain. Feed upon arrival.



# ORGANISM RECEIPT LOG

<b>Date:</b> 5/17/23		<b>Time:</b> 0830		<b>Batch No.</b> ATS051723.01			
<b>Organism:</b> Neanthes arenaceodentata							
<b>Source / Supplier:</b> Aquatic Toxicology Support							
<b>No. Ordered:</b> 180		<b>No. Received:</b> 180 + 10%		<b>Source Batch:</b> Collection date, hatch date, etc.): Emergence 4/24/23			
<b>Condition of Organisms:</b> Good				<b>Approximate Size or Age:</b> (Days from hatch, life stage, size class, etc.): 23 days post emergence			
<b>Shipper:</b> Conner				<b>B of L (Tracking No.):</b> NA			
<b>Condition of Container:</b> Good				<b>Received By:</b> SZ			
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
Comp	10.6	20.3	30	-7.4	0	—	SZ/DM

\*if >10% contact lab manager

**Notes:**





Aquatic Toxicology Support  
1849 Charleston Beach Road West  
Bremerton, Washington 98312  
(360) 813-1202

Order Summary

Species: <i>Neanthes arenaceodentata</i> *	Emerge Date: 24 April '23
Number Ordered: 180	Number Shipped: 180 + 10%
Date Shipped: 17 May '23	Salinity (ppt): 30
pH: 7.8	Temperature (°C): 20.5

\*Smith 1964. CSU Long Beach strain. Feed upon arrival.

## LOCATION:

Bath 2

6116

10% mortality = 102

① IE-Dm-5/11/23

# ORGANISM RECEIPT LOG

<b>Date:</b> 5/1/23		<b>Time:</b> 1615		<b>Batch No.</b> TS050123			
<b>Organism:</b> Mytilus sp.							
<b>Source / Supplier:</b> Taylor Shellfish							
<b>No. Ordered:</b> 1 batch		<b>No. Received:</b> 10 lbs.		<b>Source Batch:</b> Collection date, hatch date, etc.): Harvest:			
<b>Condition of Organisms:</b> Good				<b>Approximate Size or Age:</b> (Days from hatch, life stage, size class, etc.): mixed age adults			
<b>Shipper:</b> courier				<b>B of L (Tracking No.)</b> NA			
<b>Condition of Container:</b> Good				<b>Received By:</b> M. Seibert / S. Zischke			
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	①	9.1°C	①	- ①	①	①	

\*If >10% contact lab manager

**Notes:**  
① rec'd dry - MS 5/1

# TAYLOR SHELLFISH FARMS

SE 130 LYNCH RD, SHELTON WA 98584  
PHONE # : (360) 426-6178  
WASHINGTON STATE CERT # : WA46SP

HARVEST DATE:

HARVEST  
AREA:

HARVEST  
ITEM:

Dept ID

FARM CODE

QUANTITY:

☐ Dozens  
☒ Pounds

☐ Tubs  
☐ Sacks

All Shellstock containers in this lot have the same harvest data and area of harvest.

Harvest  
Hour

Harvest  
Minute

Refer  
Date

Refer  
Hour

Refer  
Minute

8

05

5/1/23

M127

Taylor

# MAINTENANCE LOG FOR CULTURES

ORGANISM: M. sp

LOCATION: Bath 10

Batch Number: <u>T5050123.01</u>	Date Received: <u>5/1/23</u>	Initial # of Organisms: <u>10 lbs</u>	10% mortality =
----------------------------------	------------------------------	---------------------------------------	-----------------

Date	Feed AM/PM	Tub No.	D.O.	Temp (°C)	Cond/ Sal ppt	pH	H <sub>2</sub> O Change	Organisms appear healthy (Y/N)	# Mort (per tub)	<sup>1</sup> Cumulative # Mort*	Init.	Comments
5/2/23	—	A	7.7	14.5	30	7.7	FT	Y			MS	
5/2/23	—	B	7.6	14.5	30	7.7	FT	Y			MS	
5/2/23	—	C	7.0	14.6	30	7.6	FT	Y			MS	
5/3/23	—	A	7.7	13.9	30	7.5	FT	Y	0	—	DM	
5/3/23	—	B	7.7	13.7	30	7.5	FT	Y	0	—	DM	
5/3/23	—	C	7.0	14.3	30	7.4	FT	Y	0	—	DM	
5/7	- ✓	A	8.5	13.8	29	7.9	FT	Y	0	—	UG	
5/7	- ✓	B	8.4	13.0	29	7.9	FT	Y	0	—	UG	
5/7	- ✓	C	8.0	13.3	29	7.8	FT	Y	0	—	UG	
5/9	- ✓	A	8.5	13.6	30	7.9	FT	Y	0	—	UG	
5/9	- ✓	B	8.2	13.5	30	7.8	FT	Y	0	—	UG	
5/9	- ✓	C	8.1	13.5	30	7.8	FT	Y	0	—	UG	
5/11	—	A	8.3	14.2	30	7.9	FT	Y	0	—	UG	
5/11	—	B	8.3	13.8	30	7.8	FT	Y	0	—	UG	
5/11	—	C	8.2	13.7	30	7.8	FT	Y	0	—	UG	
5/12	—	A	8.8	12.9	30	7.9	FT	Y	0	—	MS	
5/12	—	B	8.7	12.5	30	7.9	FT	Y	0	—	MS	
5/12	—	C	8.5	12.9	30	7.9	FT	Y	0	—	MS	

FT = Flow-through

\*For all containers and all days for a given batch; if >10% notify lab manager

<sup>1</sup> Cumulative # Mort is the running total of the current day's total mortality + previous cumulative culture mortality since acquired in lab