TOXICITY TESTING RESULTS

LOTT CLEAN WATER ALLIANCE
OLYMPIA, WASHINGTON

WHOLE EFFLUENT TOXICITY TESTING: AUGUST 2025

Prepared for
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NPDES Permit No.: WA0037061

Report ID: PG2162.01

Submittal Date: September 12, 2025

Accredited in accordance with NELAP, ORELAP ID 4165

All testing reported herein was performed consistent with our laboratory's quality assurance program. All results are intended to be considered in their entirety, and EcoAnalysts is not responsible for use of less than the complete report. The test results summarized in this report apply only to the sample(s) evaluated. This document is uncontrolled when printed or accessed from electronic distribution.

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ACRONYMS AND ABBREVIATIONS

ABS	Aquatic BioSystems, Inc.
ACEC	Acute Critical Effluent Concentration
CCEC	Chronic Critical Effluent Concentration
EPA	Environmental Protection Agency
EC_{25}/IC_{25}	Effect/Inhibition Concentration to 25% of test population
LOEL	Lowest Observed Effect Level
mg/L	Milligrams per Liter
NOEL	No Observed Effect Level
NPDES	National Pollutant Discharge Elimination System
QM	Quality Manual
SOP	Standard Operating Practices
WDOE	Washington Department of Ecology
WET	Whole Effluent Toxicity
WWTP	Wastewater Treatment Plant

1. EXECUTIVE SUMMARY

EcoAnalysts conducted Whole Effluent Toxicity (WET) testing on effluent samples collected by LOTT Clean Water Alliance personnel as part of the effluent characterization. The objective of this program was to assess the potential toxicity of primary discharge water to selected aquatic organisms following procedures defined under the facility's National Pollutant Discharge Elimination System (NPDES) permit. The results of the biological testing are contained in this report.

Statistically significant biological response of the test organisms was not detected at or below the acute critical effluent concentration (ACEC) of 2.8% or the chronic critical effluent concentration (CCEC) of 2.0% for the chronic test endpoints. The chronic endpoints tested are summarized in Table 1-1. The effluent samples do not exceed the defined permit requirements for the chronic test endpoints. (Table 1-2).

Table 1-1. Toxicity Test Results Summary.

Test		NOEL (%)	LOEL (%)	EC_{25}/IC_{25} (%)
Chronic	*Atherinops affinis* 7-Day Survival	100	>100	>100
	Atherinops affinis 7-Day Biomass	100	>100	>100
	Americamysis bahia 7-Day Survival	100	>100	>100
	Americamysis bahia 7-Day Biomass	100	>100	>100

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

EC_{25}/IC_{25} = Effect/ Inhibition Concentration to 25% of test population

Table 1-2. Permit Compliance Results.

Permit Requirement	*The Permittee must:* *Conduct chronic toxicity testing on the final effluent annually. Conduct chronic toxicity testing during effluent characterization on a series of at least five concentrations of effluent and a control. This series of dilutions must include the acute critical effluent concentration (ACEC). The ACEC equals 2.8 percent effluent. The series of dilutions should also contain the CCEC of 2.0 percent effluent. Compare the ACEC to the control using hypothesis testing at the 0.05 level of significance.*
Result	No statistically significant biological effects were detected at or below the acute critical effluent concentration (ACEC) of 2.8% effluent or the chronic critical effluent concentration (CCEC) of 2.0% effluent for both chronic tests.

2. METHODS

The samples were analyzed for toxicity using criteria outlined in the Washington Department of Ecology's (WDOE) Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria (WDOE WQ-R-95-80). These criteria are further defined through the Environmental Protection Agency's (EPA) most recently promulgated effluent guidance documents outlined in Section 4.

2.1 Bioassay Testing

Bioassay testing for this project consisted of two chronic bioassays. The tests conducted in support of this project are summarized in Table 2-1.

Table 2-1. Biological Testing Performed

Test Type	Test Descriptor	Species	Method
Chronic	7-Day Survival and Growth	*Atherinops affinis* (Topsmelt)	WDOE WQ-R-95-80; EPA 600/R-95/136; Test Method 1006.0; SOP TOX002.10
Chronic	7-Day Survival and Growth	*Americamysis bahia* (Opossum Shrimp)	WDOE WQ-R-95-80; EPA-821-R-02-014; Test Method 1007.0; SOP TOX014.13

2.2 Sample Collection and Storage

LOTT Clean Water Alliance personnel collected composite samples on August 7, 11, and 12, 2025. The samples were delivered by overnight courier and received at the EcoAnalysts Port Gamble laboratory on the day following collection. Temperatures upon receipt were within the recommended temperature range of 0 – 6°C. Additional sample conditions are summarized in Table 2-2. The samples were held in a walk-in cold room at 4 ± 2 °C in the dark until utilized for testing.

Table 2-2. Sample Conditions upon Receipt

Sample	Final Effluent #2, Final Effluent #3, Final Effluent #4		
Laboratory ID	P250808.02	P250812.49	P250813.15
Date/Time sampled	08/07/25; 0700	08/11/25; 0700	08/12/25; 0630
Date/Time received	08/08/25; 1230	08/12/25; 1154	08/13/25; 1230
Dissolved Oxygen (mg/L) Recommended: >4.0 mg/L	9.5	8.4	9.8
Temperature (°C) Recommended: 0 – 6°C	0.0	0.6	0.3
pH (units) Recommended: 6 – 9	7.4	7.8	7.5
Salinity (ppt)	0.252	0.250	0.273
Total Chlorine (mg/L)	0.04	0.04	<0.02
Total Ammonia (mg/L)	0.00	0.00	0.00

2.3 Water for Bioassay Testing

Seawater diluent used in this study came from the northern Hood Canal at Port Gamble, Washington. Extensive testing on a variety of test species has shown that there is no significant potential for toxicity or bioaccumulation of contaminants from this water supply. Chemical analysis of this water source is conducted and reviewed on an annual basis.

2.4 Sample Adjustment

Salinity adjustments were necessary to bring the samples within the recommended test salinity for each marine test species. The effluent discharge samples arrived at a salinity of 0.250 – 0.273 ppt. The salinity of the LOTT effluent samples was adjusted to the desired test salinity with Crystal Sea® MarineMix bioassay grade artificial salt. Table 2-3 summarizes the salinity adjustments performed on the project samples to create a salinity range with the tolerance limits of test species.

An artificial salt control sample was created to evaluate any potential negative impacts to the test organisms from the salinity adjustment alone. This sample was designated "Salt Control", and the results are discussed in Section 3.

Table 2-3. Salinity Adjustment of Project Samples

Sample ID: Final Effluent	Sample Salinity Upon Receipt (ppt)	Sample Salinity Adjustment (ppt)
Sample 1: Collected 08/07/25	0.252	30 ± 2
Sample 2: Collected 08/11/25	0.250	30 ± 2
Sample 3: Collected 08/12/25	0.273	30 ± 2

2.5 Data Management and Analysis

Endpoint data was calculated for each replicate, and the mean value and standard deviation were determined for each sample concentration. 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 was reviewed for errors. Review counts were conducted on any apparent outliers.

Statistical comparisons were made according to the EPA guidance (EPA 2002). Statistical comparisons were performed using CETIS™ software.

2.6 Quality Assurance/Quality Control

The quality assurance objectives for toxicity testing conducted by the testing laboratory are detailed in the method specific guidance documents and the laboratory's quality manual (QM). These objectives for accuracy and precision involve all aspects of the testing process, including the following:

- Source and Condition of Test Organisms
- Condition of Equipment
- Test Conditions
- Instrument Calibration
- Use of Reference Toxicants
- Record Keeping
- Data Evaluation

Each batch of test organisms obtained were evaluated in a reference toxicant test that was run within a month of the test period to establish the sensitivity of the test organisms. The reference toxicant LC_{50} or

EC_{50} should fall within two standard deviations of the historical laboratory mean. Water quality measurements were monitored to ensure that they fell within prescribed limits.

The methods employed in every phase of the toxicity testing program are detailed in the EcoAnalysts Standard Operating Practices (SOP). All EcoAnalysts staff members receive regular, documented training in all SOPs and test methods. Finally, all data collected and produced as a result of these analyses were recorded on approved data sheets. If an aspect of a test deviated from protocol, the test was evaluated to determine whether it was valid according to the regulatory agencies responsible for approval of the proposed permitting action.

3. RESULTS

The results of the effluent testing are presented in this section. Statistical comparisons and laboratory documents are provided in Appendix A. Chain-of-custody and sample receipt logs are provided in Appendix B.

3.1 Topsmelt (*Atherinops affinis*) Chronic Test Results

The chronic toxicity test with *A. affinis* was initiated on August 8, 2025. The test met the test acceptability criteria listed in Table 3-3. Mean survival and growth endpoints are summarized in Table 3-1. The statistical results are summarized in Table 3-2 and the test conditions are summarized in Table 3-3.

Water quality parameters were within the acceptable limits throughout the duration of the 7-day static-renewal test.

No significant differences were observed between the laboratory control and the salt control for any of the biological endpoints tested. This indicates that artificial salts should not have contributed significantly to any negative biological effects, if observed.

The reference toxicant test results were within two standard deviations of the laboratory mean for both endpoints (Table 3-3). This indicates that the organisms obtained from this supplier were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

Table 3-1. Endpoint Summary for the *Atherinops affinis* Chronic Test

Conc. (%)	Final Effluent #2, Final Effluent #3, Final Effluent #4		
	Mean Survival (%)	Mean Dry Weight (mg) [1]	Mean Biomass (mg) [2]
Control (0)	96	1.437	1.388
Salt Control	96	1.613	1.527
2.0[3]	100	1.428	1.428
2.8[4]	92	1.517	1.372
10	100	1.370	1.370
30	100	1.414	1.414
100	100	1.525	1.525

[1] Average weight (mg) per survivor.
[2] Average weight (mg) per original number of animals stocked (Biomass).
[3] Chronic Critical Effluent Concentration (CCEC).
[4] Acute Critical Effluent Concentration (ACEC).

Table 3-2. Statistical Results Summary for *Atherinops affinis* Chronic Test

Endpoint	Final Effluent #2, Final Effluent #3, Final Effluent #4		
	Survival	Dry Weight	Biomass
NOEL (%)	100	100	100
LOEL (%)	>100	>100	>100
EC_{25}/ IC_{25} (%)	>100	>100	>100
95% Confidence Limits	NA	NA	NA

NA = Not Applicable

3.1.1 Topsmelt (*Atherinops affinis*) Permit Compliance

From the NPDES permit: Compare the ACEC to the control using hypothesis testing at the 0.05 level of significance. The ACEC equals 2.8%.

- No statistically significant difference between the lab control and 2.8% was shown for the chronic endpoints, which meets permit requirements.

Table 3-3. Test Condition Summary for *Atherinops affinis* Chronic Test

Test Duration / Type		7-Day / Static-Renewal
Species		*Atherinops affinis*
Supplier		Aquatic Bio Systems, Inc.
Date acquired		08/05/25
Test Dates		08/08/25 – 08/15/25
Age at test initiation (Recommended: 9-15 days)		14 Days
Samples used:		P250808.02, P250812.49, P250813.15
Sample Holding Time at Initiation: Recommended: <36 hours		34 hours
Test Procedures		WDOE WQ-R-95-80; EPA 600/R-95/136; Test Method 1006.0; SOP TOX002.10
Test location		EcoAnalysts Port Gamble, WA
Control water / Diluent		0.45 µm-filtered, North Hood Canal seawater
Test Lighting		16-hour light / 8-hour dark
Test Chamber		20 oz. cup
Exposure volume		250 mL
Replicates/treatment		5
Concentration/treatment		2.0, 2.8, 10, 30 and 100%
Organisms/replicate		5
Feeding		250 Artemia nauplii am / 500 pm, except Day 7
Test solution renewal		Daily (Days 1-6)
Test Dissolved Oxygen Recommended: > 4.0 mg/L		5.6 – 8.7 mg/L
Test Temperature Recommended: 20 ± 1°C		18.5 – 21.4 °C
Test Salinity Recommended: 30 ± 2 ppt		28 – 32 ppt
Test pH Recommended: 6 - 9 units		7.7 – 9.0
Quality Assurance		
Control performance standards Survival Recommended: ≥ 80% Dry Weight Recommended: ≥ 0.850 mg Power Standard: ≤39% (Growth)		96%; meets acceptability criterion
		1.437 mg, meets acceptability criterion
		1%; meets criterion
Reference Toxicant		
Reference Toxicant Date		08/07/25
Survival	Reference Toxicant LC_{50} (must be < 205 µg/L)	112.2 µg Cu/L
	Laboratory Mean $LC_{50;}$ Range LC_{50} (±2 SD)	95.4 µg Cu/L (51.1 – 178 µg Cu/L)
	PMSD (must be <25%)	15.9%
Biomass	Reference Toxicant LC_{50}	108.4 µg Cu/L
	Laboratory Mean $LC_{50;}$ Range LC_{50} (±2 SD)	93.3 µg Cu/L (48.7 – 179 µg Cu/L)
	PMSD (must be <50%)	24.9%
Deviations from Test Protocol		None

3.2 Mysid (*Americamysis bahia*) Chronic Test Results

The chronic toxicity test with *A. bahia* was initiated on August 8, 2025. The test met the test acceptability criteria listed in Table 3-6. Mean survival and growth endpoints are summarized in Table 3-4. The statistical results are summarized in Table 3-5 and the test conditions are summarized in Table 3-6.

On Day 6, the test temperature fell below the range of 26 ± 1°C. The temperature decrease was likely caused by the power outage that day. The temperature remained within the range for the remainder of the test. Other than noted, water quality parameters were within the acceptable limits throughout the duration of the 7-day static-renewal test.

The reference toxicant test results were within two standard deviations of the laboratory mean for survival and mean dry biomass (Table 3-6). This indicates that the organisms obtained from this supplier were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

Table 3-4. Endpoint Summary for the *Americamysis bahia* Chronic Test

Conc. (%)	Final Effluent #2, Final Effluent #3, Final Effluent #4		
	Mean Survival (%)	Mean Dry Weight (mg) [1]	Mean Biomass (mg) [2]
Control (0)	92.5	0.338	0.313
Salt Control	95.0	0.330	0.313
2.0[3]	97.5	0.324	0.317
2.8[4]	95.0	0.318	0.302
10	95.0	0.304	0.289
30	95.0	0.339	0.324
100	97.5	0.348	0.342

[1] Average weight (mg) per survivor.
[2] Average weight (mg) per original number of animals stocked (Biomass).
[3] Chronic Critical Effluent Concentration (CCEC).
[4] Acute Critical Effluent Concentration (ACEC).

Table 3-5. Statistical Results Summary for *Americamysis bahia* Chronic Tests

Endpoint	Final Effluent #2, Final Effluent #3, Final Effluent #4		
	Survival	Dry Weight	Biomass
NOEL (%)	100	100	100
LOEL (%)	>100	>100	>100
EC_{25} /IC_{25} (%)	>100	>100	>100
95% Confidence Limits	NA	NA	NA

NOEL = No Observed Effect Level
LOEL = Lowest Observed Effect Level
EC_{25}/IC_{25} = Effect/ Inhibition Concentration to 25% of test population
NA = Not Applicable

3.2.1 Mysid (*Americamysis bahia*) Chronic Permit Compliance

From the NPDES permit: Compare the ACEC to the control using hypothesis testing at the 0.05 level of significance. The ACEC equals 2.8%.

- No statistically significant difference between the lab control and 2.8% was shown for the chronic endpoints, which meets permit requirements.

Table 3-6. Test Condition Summary for *Americamysis bahia* Chronic Test

Test Duration / Type		7-Day / Static-Renewal
Species		*Americamysis bahia*
Supplier		Aquatic Bio Systems, Inc.
Date acquired		08/08/25
Test Dates		08/08/25 – 08/15/25
Age at test initiation (Recommended: 7 days)		7 Days
Samples used:		P250808.02, P250812.49, P250813.15
Sample Holding Time at Initiation: Recommended: <36 hours		34 hours
Test Procedures		WDOE WQ-R-95-80; EPA-821-R-02-014; Test Method 1007.0; SOP TOX014.12
Test location		EcoAnalysts; Port Gamble, WA
Control water / Diluent		0.45 µm-filtered, North Hood Canal seawater
Test Lighting		16-hour light / 8-hour dark
Test Chamber		20 oz. cup
Exposure volume		250 mL
Replicates/treatment		8
Concentration/treatment		2.0, 2.8, 10, 30 and 100%
Organisms/replicate		5
Feeding		375 *Artemia* nauplii twice daily, except Day 7
Test solution renewal		Daily (Days 1-6)
Test Water Quality		
Test Dissolved Oxygen Recommended: > 4.0 mg/L		4.2 – 8.6 mg/L
Test Temperature Recommended: 26 ± 1°C		24.0 – 27.4 °C
Test Salinity Recommended: 30 ± 2 ppt		29 – 31 ppt
Test pH Recommended: 6 - 9 units		7.6 – 9.0
Quality Assurance		
Control performance standards **Survival Recommended: ≥ 80%** **Dry Weight Recommended: ≥ 0.200 mg** **Power Standard: ≤39% (Dry weight)**		92.5%; meets acceptability criterion 0.338 mg, meets acceptability criterion 4%; meets criterion
Reference Toxicant		
Reference Toxicant Test Start Date		08/07/25
Survival	Reference Toxicant LC_{50}	158.4 µg Cu/L
	Laboratory Mean $LC_{50;}$ Range LC_{50} (±2 SD)	232.5 µg Cu/L (152 – 357 µg Cu/L)
	PMSD (must be <25%)	11.6%
Biomass	Reference Toxicant EC_{50}	161 µg Cu/L
	Laboratory Mean $EC_{50;}$ Range LC_{50} (±2 SD)	184.6 µg Cu/L (116 – 293 µg Cu/L)
	PMSD (must be <50%)	21.6%
Deviations from Test Protocol		Test temperature

4. REFERENCES

CETIS. 2022. CETIS™ Comprehensive Environmental Toxicity Information System User's Guide. Tidepool Scientific Software. McKinleyville, CA.

USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, First Edition. EPA/600/R-95-136.

USEPA. 2002. Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine Organisms, Third Edition. EPA-821-R-02-014.

WDOE. 2016. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Washington State Department of Ecology. Water Quality Program. Publication number: WQ-R-95-80, Revised June 2016.

APPENDIX A

LABORATORY DOCUMENTS

ERROR CODES FOR DATASHEETS

CA	Called away; task completed by another tech
DC	Test solution too turbid or too dark to count organisms
FB	Found body – animal found that was previously noted as missing
IE	Incorrect Entry
IW	Illegible writing
MC	Miscount
MR	Meter reading changed; Meter no ready
NB	No body (no organism found)
SM	Stray Mark
WC	Wrong Cell (incorrect data box used)
WD	Wrong Date (incorrect date entered)
WN	Wrong number (incorrect number entered)
WP	Wrong page (incorrect data sheet)
WT	Wrong Time (incorrect time entered)

APPENDIX A1.1

ATHERINOPS AFFINIS (TOPSMELT) 7-DAY SURVIVAL AND GROWTH TEST

STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS

Pacific Topsmelt 7-d Survival and Growth Test **EcoAnalysts**

Batch ID:	19-7078-2411	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Michelle Bennett		
Start Date:	08 Aug-25 17:22	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater		
Ending Date:	15 Aug-25 08:24	**Species:**	Atherinops affinis	**Brine:**	Crystal Sea Marine Mix		
Test Length:	6d 15h	**Taxon:**	Actinopterygii	**Source:**	Aquatic Biosystems, CO	**Age:**	14D
Sample ID:	17-2784-2730	**Code:**	P250808.02	**Project:**	2025 NPDES		
Sample Date:	07 Aug-25 07:00	**Material:**	Effluent Sample	**Source:**	LOTT Clean Water Alliance (WA00370		
Receipt Date:	08 Aug-25 12:30	**CAS (PC):**		**Station:**	Final Effluent #2		
Sample Age:	34h (0 °C)	**Client:**	LOTT				

SIngle Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
01-2479-1104	7d Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	0.7778	Salt Control passed 7d proportion survived	1
02-3605-9295	Mean Dry Biomass-mg	Equal Variance t Two-Sample Test	0.8537	Salt Control passed mean dry biomass-mg	1
02-7059-6777	Mean Dry Weight-mg	Equal Variance t Two-Sample Test	0.8603	Salt Control passed mean dry weight-mg	1

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
00-9799-8621	7d Proportion Survived	Steel Many-One Rank Sum Test		100	>100	---	9.87%	1	1
14-5198-1677	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test		100	>100	---	18.5%	1	1
03-2577-9113	Mean Dry Weight-mg	Steel Many-One Rank Sum Test		100	>100	---	19.9%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
05-7263-1355	7d Proportion Survived	Linear Interpolation (ICPIN)	✓	EC25	>100	---	---	<1	1
05-8602-8843	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	✓	IC25	>100	---	---	<1	1
15-7285-0667	Mean Dry Weight-mg	Linear Interpolation (ICPIN)	✓	IC25	>100	---	---	<1	1

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
00-9799-8621	7d Proportion Survived	Control Resp	0.96	0.8	<<	Yes	Passes Criteria
01-2479-1104	7d Proportion Survived	Control Resp	0.96	0.8	<<	Yes	Passes Criteria
	7d Proportion Survived	Control Resp	0.96	0.8	<<	Yes	Passes Criteria
05-7263-1355	7d Proportion Survived	Control Resp	0.96	0.8	<<	Yes	Passes Criteria
02-3605-9295	Mean Dry Biomass-mg	Control Resp	1.527	0.85	<<	Yes	Passes Criteria
	Mean Dry Biomass-mg	Control Resp	1.388	0.85	<<	Yes	Passes Criteria
05-8602-8843	Mean Dry Biomass-mg	Control Resp	1.388	0.85	<<	Yes	Passes Criteria
14-5198-1677	Mean Dry Biomass-mg	Control Resp	1.388	0.85	<<	Yes	Passes Criteria
00-9799-8621	7d Proportion Survived	PMSD	0.09866	<<	0.25	Yes	Passes Criteria
01-2479-1104	7d Proportion Survived	PMSD	0.1151	<<	0.25	Yes	Passes Criteria
02-3605-9295	Mean Dry Biomass-mg	PMSD	0.165	<<	0.5	Yes	Passes Criteria
14-5198-1677	Mean Dry Biomass-mg	PMSD	0.185	<<	0.5	Yes	Passes Criteria

Pacific Topsmelt 7-d Survival and Growth Test **EcoAnalysts**

7d Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	0.9600	0.8489	1.0710	0.8000	1.0000	0.0400	0.0894	9.32%	0.00%
0	SC	5	0.9600	0.8489	1.0710	0.8000	1.0000	0.0400	0.0894	9.32%	0.00%
2		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-4.17%
2.8		5	0.9200	0.7840	1.0560	0.8000	1.0000	0.0490	0.1095	11.91%	4.17%
10		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-4.17%
30		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-4.17%
100		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-4.17%

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.388	1.09	1.687	0.976	1.56	0.1074	0.2402	17.30%	0.00%
0	SC	5	1.527	1.36	1.695	1.41	1.716	0.06036	0.135	8.84%	-10.00%
2		5	1.428	1.323	1.534	1.296	1.51	0.03788	0.0847	5.93%	-2.88%
2.8		5	1.372	1.079	1.665	1.194	1.69	0.1055	0.236	17.19%	1.15%
10		5	1.37	1.196	1.544	1.148	1.51	0.06254	0.1398	10.21%	1.33%
30		5	1.414	1.288	1.54	1.254	1.534	0.04556	0.1019	7.20%	-1.84%
100		5	1.525	1.321	1.729	1.24	1.662	0.07338	0.1641	10.76%	-9.85%

Mean Dry Weight-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.437	1.265	1.61	1.22	1.56	0.06213	0.1389	9.67%	0.00%
0	SC	5	1.613	1.229	1.997	1.41	2.145	0.1382	0.3091	19.16%	-12.23%
2		5	1.428	1.323	1.534	1.296	1.51	0.03788	0.0847	5.93%	0.61%
2.8		5	1.517	1.057	1.976	1.208	2.113	0.1656	0.3702	24.41%	-5.52%
10		5	1.37	1.196	1.544	1.148	1.51	0.06254	0.1398	10.21%	4.68%
30		5	1.414	1.288	1.54	1.254	1.534	0.04556	0.1019	7.20%	1.61%
100		5	1.525	1.321	1.729	1.24	1.662	0.07338	0.1641	10.76%	-6.12%

Pacific Topsmelt 7-d Survival and Growth Test **EcoAnalysts**

7d Proportion Survived Detail MD5: E9E290AB785C332D655FCB4AF4E8616B

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	0.8000	1.0000	1.0000	1.0000
0	SC	1.0000	1.0000	1.0000	1.0000	0.8000
2		1.0000	1.0000	1.0000	1.0000	1.0000
2.8		1.0000	0.8000	0.8000	1.0000	1.0000
10		1.0000	1.0000	1.0000	1.0000	1.0000
30		1.0000	1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000	1.0000

Mean Dry Biomass-mg Detail MD5: 7E37765768F5F3CD8535E930383BB6A9

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.422	0.976	1.56	1.558	1.426
0	SC	1.484	1.616	1.41	1.41	1.716
2		1.296	1.494	1.428	1.51	1.414
2.8		1.208	1.194	1.69	1.562	1.208
10		1.466	1.51	1.148	1.366	1.36
30		1.43	1.448	1.404	1.254	1.534
100		1.662	1.562	1.24	1.576	1.586

Mean Dry Weight-mg Detail MD5: DB8946E488266BF6D2BFCE8C96C5EB3E

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.422	1.22	1.56	1.558	1.426
0	SC	1.484	1.616	1.41	1.41	2.145
2		1.296	1.494	1.428	1.51	1.414
2.8		1.208	1.493	2.113	1.562	1.208
10		1.466	1.51	1.148	1.366	1.36
30		1.43	1.448	1.404	1.254	1.534
100		1.662	1.562	1.24	1.576	1.586

7d Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	5/5	4/5	5/5	5/5	5/5
0	SC	5/5	5/5	5/5	5/5	4/5
2		5/5	5/5	5/5	5/5	5/5
2.8		5/5	4/5	4/5	5/5	5/5
10		5/5	5/5	5/5	5/5	5/5
30		5/5	5/5	5/5	5/5	5/5
100		5/5	5/5	5/5	5/5	5/5

Pacific Topsmelt 7-d Survival and Growth Test **EcoAnalysts**

Start Date: 08 Aug-25 17:22 **Species:** Atherinops affinis **Sample Code:** P250808.02
End Date: 15 Aug-25 08:24 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** LOTT Clean Water Alliance
Sample Date: 07 Aug-25 07:00 **Material:** Effluent Sample **Sample Station:** Final Effluent #2

Conc-%	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Total Weight-mg	Tare Weight-mg	Pan Count	Notes
0	D	1	19	5							5	66.06	58.95	5	
0	D	2	13	5							4	60.69	55.81	4	
0	D	3	10	5							5	66.22	58.42	5	
0	D	4	11	5							5	65.31	57.52	5	
0	D	5	4	5							5	64.67	57.54	5	
0	SC	1	6	5							5	66.15	58.73	5	
0	SC	2	24	5							5	64.94	56.86	5	
0	SC	3	7	5							5	65.05	58	5	
0	SC	4	22	5							5	65.9	58.85	5	
0	SC	5	23	5							4	67.65	59.07	4	
2		1	31	5							5	66.55	60.07	5	
2		2	30	5							5	66.66	59.19	5	
2		3	20	5							5	66.97	59.83	5	
2		4	29	5							5	65.48	57.93	5	
2		5	18	5							5	66.1	59.03	5	
2.8		1	35	5							5	64.23	58.19	5	
2.8		2	25	5							4	64.54	58.57	4	
2.8		3	1	5							4	65.69	57.24	4	
2.8		4	17	5							5	65.71	57.9	5	
2.8		5	28	5							5	65.25	59.21	5	
10		1	27	5							5	66.85	59.52	5	
10		2	9	5							5	66.32	58.77	5	
10		3	8	5							5	65.36	59.62	5	
10		4	15	5							5	65.61	58.78	5	
10		5	14	5							5	60.84	54.04	5	
30		1	34	5							5	63.71	56.56	5	
30		2	21	5							5	62.32	55.08	5	
30		3	5	5							5	69.53	62.51	5	

Conc-%	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Total Weight-mg	Tare Weight-mg	Pan Count	Notes
30		4	33	5							5	64.18	57.91	5	
30		5	3	5							5	64.12	56.45	5	
100		1	32	5							5	64.24	55.93	5	
100		2	2	5							5	63.88	56.07	5	
100		3	12	5							5	60.85	54.65	5	
100		4	16	5							5	65.37	57.49	5	
100		5	26	5							5	65.45	57.52	5	

Version V.5

GENERAL

Client	LOTT Clean Water Alliance
Project	2025 NPDES
Project Number	PG2162
Project Manager	M. Bennett
Date Sample Received	8/8/2025
Test type	7 Day Chronic Toxicity with Topsmelt
Matrix	Liquid
Test Acceptability	≥ 80% average survival in control Average dry weight is > 0.85 mg per surviving fish
Test Start Date	08/08/25
Test Species	*Atherinops affinis*
Organism Batch	ABS080525.03
Organism Acquired	8/5/2025
Organism Acclimation	3
Organism Age	14 days
Test Protocol	TOX 002
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 0
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	5
Test Chamber Size	20 oz.
Exposure Volume	250 mL
Feeding Information	250 nauplii/chamber am 500 nauplii/chamber pm
Test Dissolved Oxygen	> 4
Test Temperature	20 ± 1
Test Salinity	30 ± 2
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Food Batch ID
311823

CSMM Batch #
082024

Test Parameters		
	Min	**Max**
DO	4.0	
Temp	19	21
Salinity	28	32
pH	6	9

TEST START TIME/INIT:	**1722 NL (MM)**
TEST END TIME/INIT:	**824 MM**

CLIENT SAMPLE ID	**LAB ID**
Final Effluent #2	P250808.02
Final Effluent #3	P250812.49
Final Effluent #4	P250813.15

Concentrations

1	Control
2	Salt Control
3	2%
4	2.8%
5	10%
6	30%
7	100%
8	.
9	.

Copy and Past VALUES fr

Treatment	Rep	Chamber
Control	1	6
Control	2	27
Control	3	19
Control	4	18
Control	5	14
Salt Control	1	7
Salt Control	2	23
Salt Control	3	4
Salt Control	4	9
Salt Control	5	33
2.00%	1	1
2.00%	2	28
2.00%	3	24
2.00%	4	8
2.00%	5	20
2.80%	1	29
2.80%	2	17
2.80%	3	16
2.80%	4	30
2.80%	5	22
10.00%	1	11
10.00%	2	21
10.00%	3	5
10.00%	4	3
10.00%	5	15
30.00%	1	2
30.00%	2	31
30.00%	3	34
30.00%	4	32
30.00%	5	10
100.00%	1	12
100.00%	2	13
100.00%	3	26
100.00%	4	25
100.00%	5	35

ECO ANALYSTS, INC.

7 Day Chronic WET Test

V.5

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 002
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Atherinops affinis*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

Test Parameters	
Salinity of Sample	0.252
Test Salinity	30

CSMM Batch Number
82024

Lab Sample ID
P250808.02

Salinity Adjustment Multiplier	29.748

Coarse salinity adjustment	
mLs. Sample*	19000.0
Grams CSMM	565.2

* Adjust volume so that it equals total volume of sample needed for all dilutions

Fine Salinity Adjustment	
Salinity of coarse-adjusted Sample	28.62
Test Salinity	30
Ratio	1.05
Grams additional CSMM needed to reach tartget salinity	27.48427673

Final salinity	30

Salinity Adjustment Date / Initials		Meter #
8/8/2025	MM	7

Comments

ECO ANALYSTS, INC.

7 Day Chronic WET Test

V.5

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/12/25	**PROTOCOL**	TOX 002
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #3	**TEST END DATE**	8/15/25	**SPECIES**	*Atherinops affinis*
LAB SAMPLE ID	P250812.49	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

Test Parameters	
Salinity of Sample	0.258
Test Salinity	30

CSMM Batch Number
82024

Lab Sample ID
P250812.49

Salinity Adjustment Multiplier	29.742

Coarse salinity adjustment	
mLs. Sample*	19000.0
Grams CSMM	565.1

* Adjust volume so that it equals total volume of sample needed for all dilutions

Fine Salinity Adjustment	
Salinity of coarse-adjusted Sample	27.33
Test Salinity	30
Ratio	1.10
Grams additional CSMM needed to reach tartget salinity	55.68605928

Final salinity	29.61

Salinity Adjustment Date	Initials	Meter #
8/12/2025	EM	8

Comments

ECO ANALYSTS, INC.

7 Day Chronic WET Test

V.5						
	CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**		**PROTOCOL**	TOX 002
	PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
	CLIENT SAMPLE ID	Final Effluent #4	**TEST END DATE**	8/15/25	**SPECIES**	*Atherinops affinis*
	LAB SAMPLE ID	P250813.15	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

Test Parameters	
Salinity of Sample	0.247
Test Salinity	30

CSMM Batch Number
82024

Lab Sample ID
P250813.15

Salinity Adjustment Multiplier	29.753

Coarse salinity adjustment	
mLs. Sample*	19000.0
Grams CSMM	565.3

* Adjust volume so that it equals total volume of sample needed for all dilutions

Fine Salinity Adjustment	
Salinity of coarse-adjusted Sample	26
Test Salinity	30
Ratio	1.15
Grams additional CSMM needed to reach tartget salinity	87.69230769

Final salinity	30.04

Salinity Adjustment Date	Initials	Meter #
8/13/2025	KS	10

Comments

ECO ANALYSTS, INC.

V.5

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 002
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Atherinops affinis*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	FSW
0	0%	0	1250.0	1250		
	Salt Control			1250		
	2%	25	1225.0	1250		
	2.8%	35	1215.0	1250		
	10%	125	1125.0	1250		
	30%	375	875.0	1250		
	100%	1250	0.0	1250		

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
1 - 6	0%	0	1000.0	1000
	Salt Control			1000
	2%	20	980.0	1000
	2.8%	28	972.0	1000
	10%	100	900.0	1000
	30%	300	700.0	1000
	100%	1000	0.0	1000

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
8/8/2025	7	P250808.02	FSW080525.01	MM
8/9/2025	7	P250808.02	FSW080425.01	NL
8/10/2025	7	P250808.02	FSW080525.01	NL
8/11/2025	7	P250808.02	FSW081025.01	TVL
8/12/2025	7	P250812.49	FSW081025.01	KS
8/13/2025	7	P250813.15	FSW081225.01	CS
8/14/2025	7	P250813.15	FSW081225.01	KS

7 Day Chronic WET Test

v.5						
CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 002
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Atherinops affinis*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

			DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	
		Concentration (%)	> 4	19 - 21	28 - 32	6 - 9	**Comment**
Day 0		Control	7.5	20.2	30	8.0	
Stock		Salt Control	7.7	18.5	29	9.0	
Date	08/08/25	2%	7.5	20.0	30	8.1	
Time	1620	2.8%	7.5	20.0	30	8.1	
Tech	MM	10%	7.5	19.7	30	8.2	
Meter #	8	30%	7.7	18.7	30	8.2	
Feed	MM	100%	8.6	19.8	30	8.4	
Day 1		Control	6.4	20.5	30	7.7	
Rep 1		Salt Control	6.3	20.5	29	8.5	
Date	08/09/25	2%	6.4	20.5	30	8.0	
Time	1316	2.8%	6.3	20.8	31	7.9	
Tech	EM	10%	6.2	20.6	30	8.0	
Meter #	8	30%	6.3	20.5	30	8.0	
		100%	5.8	20.5	30	8.2	
Day 1		Control	7.3	20.2	31	8.0	
Renewal Stock		Salt Control	7.5	20.4	29	8.1	
Date	08/09/25	2%	7.5	20.1	31	8.1	
Time	1323	2.8%	7.5	20.1	31	8.1	
Tech	EM	10%	7.6	19.8	31	8.1	
Meter #	10	30%	7.7	19.6	30	8.2	
		100%	8.1	19.7	30	8.4	
Day 2		Control	6.2	20.8	32	7.9	
Rep 2		Salt Control	6.6	20.3	30	8.0	
Date	08/10/25	2%	6.4	20.6	32	7.9	
Time	1410	2.8%	6.4	20.8	32	7.9	
Tech	DM	10%	6.4	20.9	32	7.9	
Meter #	9	30%	6.4	21.0	32	8.0	
		100%	6.5	21.0	31	8.2	
Day 2		Control	7.3	20.6	31	8.0	
Renewal Stock		Salt Control	7.4	20.9	30	8.1	
Date	08/10/25	2%	7.4	20.6	31	8.1	
Time	1357	2.8%	7.4	20.7	32	8.1	
Tech	DM	10%	7.5	20.3	31	8.1	
Meter #	9	30%	7.6	20.0	31	8.2	
		100%	7.8	19.0	30	8.4	

7 Day Chronic WET Test

v.5						
CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 002
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Atherinops affinis*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

			DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	
		Concentration (%)	> 4	19 - 21	28 - 32	6 - 9	**Comment**
Day 3		Control	6.3	20.5	30	7.8	
Rep 3		Salt Control	6.5	20.5	28	7.8	
Date	08/11/25	2%	6.1	20.5	30	7.8	
Time	9:30	2.8%	6.1	20.4	30	7.8	
Tech	KS	10%	6.1	20.5	30	7.8	
Meter #	9	30%	6.2	20.4	30	7.9	
		100%	6.1	20.4	29	8.2	
Day 3		Control	7.4	19.7	30	8.2	
Renewal Stock		Salt Control	7.1	19.8	29	8.1	
Date	08/11/25	2%	7.5	19.5	30	8.1	
Time	10:00	2.8%	7.6	19.4	30	8.0	
Tech	KS	10%	7.7	19.1	30	8.1	
Meter #	9	30%	7.9	18.7	29	8.1	
		100%	8.7	19.6	29	8.2	
Day 4		Control	6.3	21.3	31	7.7	
Rep 4		Salt Control	6.2	21.4	30	7.8	
Date	08/12/25	2%	6.4	21.4	31	7.8	
Time	1552	2.8%	5.8	21.4	31	7.8	
Tech	CS	10%	6.2	21.3	31	7.9	
Meter #	8/T42	30%	6.3	21.4	31	8.0	
		100%	6.2	21.4	30	8.2	
Day 4		Control	7.2	20.3	30	8.0	
Renewal Stock		Salt Control	7.3	20.4	30	8.1	
Date	08/12/25	2%	7.4	20.6	31	8.1	
Time	15:54	2.8%	7.4	20.5	31	8.1	
Tech	CS	10%	7.4	20.3	31	8.1	
Meter #	9	30%	7.5	19.8	30	8.2	
		100%	7.8	18.8	30	8.4	
Day 5		Control	6.1	20.4	31	7.7	
Rep 5		Salt Control	6.2	20.5	30	8.0	
Date	08/13/25	2%	6.2	20.5	31	7.8	
Time	814	2.8%	6.2	20.4	31	7.9	
Tech	EM	10%	6.1	20.5	31	7.9	
Meter #	9	30%	6.1	20.5	30	8.0	
		100%	5.6	20.6	30	8.2	

7 Day Chronic WET Test

v.5						
CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 002
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Atherinops affinis*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

			DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	
		Concentration (%)	> 4	19 - 21	28 - 32	6 - 9	**Comment**
Day 5		Control	7.5	19.4	30	8.0	
Renewal Stock		Salt Control	7.4	20.6	30	8.2	
Date	08/13/25	2%	7.5	19.8	30	8.0	
Time	15:23	2.8%	7.7	19.3	31	8.0	
Tech	CS	10%	7.6	19.2	31	8.1	
Meter #	7	30%	7.7	19.1	31	8.2	
		100%	7.9	18.9	31	8.4	
Day 6		Control	6.4	19.5	31	7.8	
Rep 1		Salt Control	6.4	19.7	30	8.1	
Date	08/14/25	2%	6.4	19.6	31	7.9	
Time	9:30	2.8%	6.5	19.9	31	7.9	
Tech	CS	10%	6.5	19.7	31	7.9	
Meter #	9	30%	6.6	19.7	31	8.0	
		100%	6.3	19.7	31	8.2	
Day 6		Control	6.9	19.8	30	8.1	
Renewal Stock		Salt Control	7.4	19.4	30	8.8	
Date	08/14/25	2%	7.1	19.7	30	8.1	
Time	1130	2.8%	7.4	19.6	31	8.2	
Tech	KS	10%	7.5	19.7	31	8.2	
Meter #	7	30%	7.5	19.6	30	8.2	
		100%	7.6	19.8	31	8.4	
Day 7		Control	6.0	20.9	31	7.8	
Rep 2		Salt Control	5.9	20.9	30	8.2	
Date	08/15/25	2%	5.8	21.0	31	7.9	
Time	802	2.8%	6.0	20.9	31	7.9	
Tech	MM	10%	6.2	20.8	31	7.9	
Meter #	8	30%	5.9	20.9	31	8.0	
		100%	6.0	20.9	31	8.2	
		.					
		.					

		DO	TEMP (°C)	SALINITY (ppt)	pH
Water quality Min/Max Values	**Min**	5.6	18.5	28.0	7.7
	Max	8.7	21.4	32.0	9.0

EcoAnalysts, Inc.

7 Day Chronic WET Test

v.5

CLIENT	LOTT Clean Water Alliance
PROJECT	2025 NPDES
CLIENT SAMPLE ID	Final Effluent #2
LAB SAMPLE ID	P250808.02

DATE RECEIVED	8/8/25
TEST START DATE	8/8/25
TEST END DATE	8/15/25
MATRIX	Liquid

PROTOCOL	TOX 002
PROJECT MANAGER	M. Bennett
SPECIES	*Atherinops affinis*

Abbreviation Key:
NB = No Body
FB = Found Body
ST = Stranded

7 Day Chronic Toxicity with Topsmelt

			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7			
			Date	**08/09/25**		**Date**	**08/10/25**		**Date**	**8/11//25**		**Date**	**08/12/25**		**Date**	**08/13/25**		**Date**	**08/14/25**		**Date**	**08/15/25**		
			Time	**1333**		**Time**	**1430**		**Time**	**1129**		**Time**	**1642**		**Time**	**1546**		**Time**	**1226**		**Time**	**824**		
			Tech	**EM**		**Tech**	**DM**		**Tech**	**KS**		**Tech**	**KS**		**Tech**	**KC**		**Tech**	**KS**		**Tech**	**MM**		
Concentration (%)	**REP**	# Initia	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Comment
Control	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		4	1		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
Salt Control	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		4	1		4	0		4	0		4	0		
2%	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
2.8%	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		4	1		4	0		
	3	5	5	0		5	0		5	0		5	0		5	0		4	1		4	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
10%	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
30%	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
100%	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
Feed (Init.)	AM		NL			NL			CS			CS			EM			KS			NONE			
250 nauplii/chamber am 500 nauplii/chamber pm	PM		NL			NL			KS			LG			KS			MM			NONE			

7 Day Chronic WET Test

EcoAnalysts, Inc.

v.5

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 002
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Atherinops affinis*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

Concentration (%)	REP	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count	Comment
Control	1	1	58.95	66.06	5	
	2	2	55.81	60.69	4	
	3	3	58.42	66.22	5	
	4	4	57.52	65.31	5	1 small fish
	5	5	57.54	64.67	5	
Salt Control	1	6	58.73	66.15	5	
	2	7	56.86	64.94	5	1 small fish
	3	8	58	65.05	5	
	4	9	58.85	65.9	5	1 large fish
	5	10	59.07	67.65	4	1 small fish, 1 large fish
2%	1	11	60.07	66.55	5	1 small fish
	2	12	59.19	66.66	5	
	3	13	59.83	66.97	5	
	4	14	57.93	65.48	5	
	5	15	59.03	66.1	5	
2.8%	1	16	58.19	64.23	5	
	2	17	58.57	64.54	4	1 large fish
	3	18	57.24	65.69	4	
	4	19	57.9	65.71	5	1 small fish
	5	20	59.21	65.25	5	
10%	1	21	59.52	66.85	5	
	2	22	58.77	66.32	5	
	3	23	59.62	65.36	5	2 small fish
	4	24	58.78	65.61	5	1 small fish
	5	25	54.04	60.84	5	
30%	1	26	56.56	63.71	5	2 small fish
	2	27	55.08	62.32	5	
	3	28	62.51	69.53	5	
	4	29	57.91	64.18	5	2 small fish
	5	30	56.45	64.12	5	2 small fish
100%	1	31	55.93	64.24	5	1 large fish
	2	32	56.07	63.88	5	1 large fish
	3	33	54.65	60.85	5	
	4	34	57.49	65.37	5	
	5	35	57.52	65.45	5	

	Oven Event 1	Oven Event 2
Oven ID:	Beelzebub	Beelzebub
Date/Time/Initials In Oven:	8/3/25 1640 EM/KS	8/15/25 934 MM
Oven Temp °C:	60.00	100
Date/Time/Initials Out Oven into Dessicator:	8/4/25 1125 DM	8/16/25 822 MM
Date/Time/Initials Weighed:	8/11/2025 15:45	8/16/25 1336 EM
Balance ID:	3	3

ORGANISM RECEIPT LOG

Date: 8/5/25	Time: 1230	Batch No. ABS080525.03
Organism: Atherinops affinis		
Source / Supplier: Aquatic Biosystems		
No. Ordered: 390	**No. Received:** 425	**Source Batch:** Collection date, hatch date, etc.): hatch: 7/25/25
Condition of Organisms: Good	**Approximate Size or Age:** (Days from hatch, life stage, size class, etc.): ① ~~hat~~ 11 days	
Shipper: FedEx	**B of L (Tracking No.)** 435797360652	
Condition of Container: Good	**Received By:** M. Seibert	

Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	9.4	18.6	30	7.4	15	3.5%	JB/LG

*if >10% contact lab manager

Notes:

① IE-MS 8/5

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524

Toll Free: 800/331-5916
Tel: 970/484-5091 Fax:970/484-2514

ORGANISM HISTORY

DATE: 8/4/2025

SPECIES: *Atherinops affinis*

AGE: 10 day

LIFE STAGE: Larvae

HATCH DATE: 7/25/2025

BEGAN FEEDING: Immediately

FOOD: *Artemia* sp.

Water Chemistry Record:	Current	Range
TEMPERATURE:	20°C	16-20°C
SALINITY/CONDUCTIVITY:	32 ppt**	28-32 ppt
TOTAL HARDNESS (as $CaCO_3$):	--	--
TOTAL ALKALINITY (as $CaCO_3$):	150 mg/l	140-175 mg/l
pH:	7.99	7.60-8.20

Comments: ** Acclimated to 30 ppt 8/4/2025.

Facility Supervisor, **Matt Redmond**

Aquatic BioSystems, Inc • Quality Research Organisms

APPENDIX A1.2

ATHERINOPS AFFINIS (TOPSMELT) 7-DAY SURVIVAL AND GROWTH TEST

REFERENCE TOXICANT DATA SHEETS

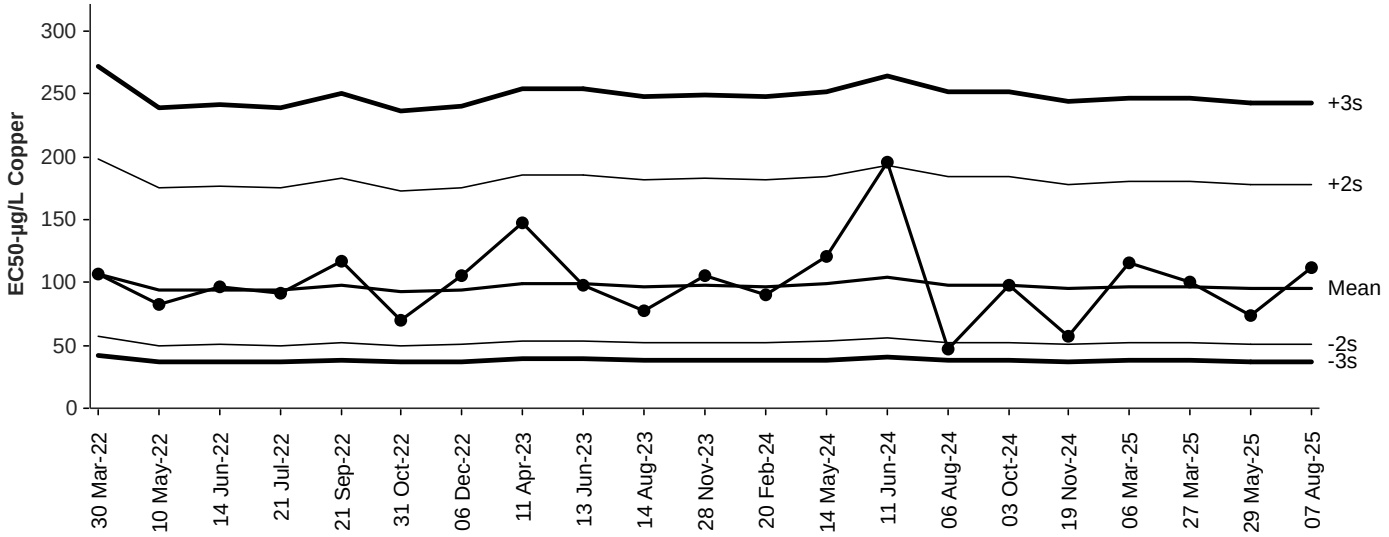
CETIS QC Plot

Report Date: 26 Aug-25 19:30 (1 of 1)

Pacific Topsmelt 7-d Survival and Growth Test **All Matching Labs**

Test Type:	Growth-Survival (7d)	**Organism:**	Atherinops affinis	**Material:**	Copper
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	7d Proportion Survived	**Source:**	Reference Toxicant-REF

Pacific Topsmelt 7-d Survival and Growth Test
7d Proportion Survived Endpoint



Lognormal Cumulative Mean Plot

Mean:	95.39	**Count:**	20	**-2s Warning Limit:**	51.1	**-3s Action Limit:**	37.4
Sigma:	NA	**CV:**	32.00%	**+2s Warning Limit:**	178	**+3s Action Limit:**	243

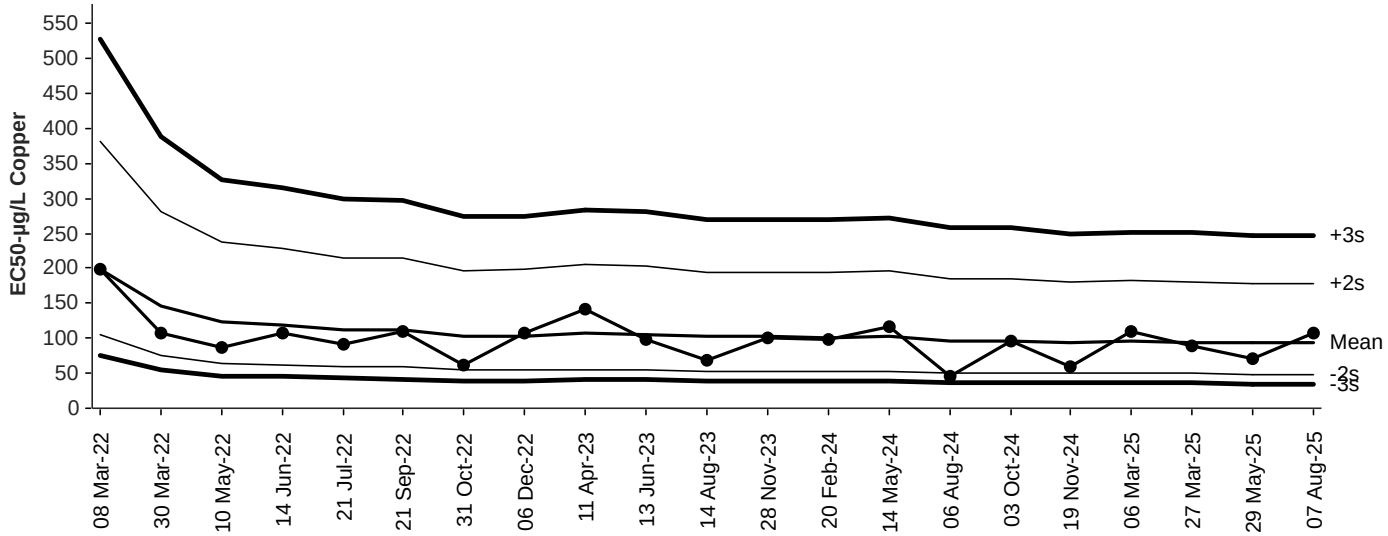
Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	Mar	30	13:20	106.5	11.09	0.3521			17-8194-4154	17-1422-7340	EcoAnalysts
2		May	10	16:20	82.35	-13.04	-0.4707			13-9380-0579	14-9529-7356	EcoAnalysts
3		Jun	14	16:08	96.48	1.09	0.03639			04-7024-7990	02-4116-4567	EcoAnalysts
4		Jul	21	13:33	91.39	-4.001	-0.1372			20-8735-8279	00-5003-0764	EcoAnalysts
5		Sep	21	11:00	116.5	21.12	0.6404			01-2995-1270	08-3404-2648	EcoAnalysts
6		Oct	31	16:27	69.85	-25.54	-0.9977			20-9804-3448	17-4046-8765	EcoAnalysts
7		Dec	6	16:28	105.5	10.07	0.3212			14-7580-4802	07-9899-2754	EcoAnalysts
8	2023	Apr	11	14:46	147.7	52.34	1.401			03-7518-1407	18-0066-2550	EcoAnalysts
9		Jun	13	16:35	97.97	2.577	0.08535			01-8019-5972	12-3754-2314	EcoAnalysts
10		Aug	14	15:25	77.52	-17.87	-0.6644			06-6644-4095	01-7825-3093	EcoAnalysts
11		Nov	28	16:07	106.1	10.75	0.342			00-9729-8180	01-6536-9055	EcoAnalysts
12	2024	Feb	20	15:10	90.08	-5.313	-0.1835			10-1210-8679	00-9207-6252	EcoAnalysts
13		May	14	14:57	120.4	25	0.7453			18-5565-0131	05-5507-4129	EcoAnalysts
14		Jun	11	16:32	195.6	100.2	2.299	(+)		08-3838-5592	18-9323-9443	EcoAnalysts
15		Aug	6	15:25	47.39	-48	-2.24	(-)		00-6133-8911	03-9965-4701	EcoAnalysts
16		Oct	3	16:00	98.54	3.145	0.1039			21-4179-4939	20-7009-6206	EcoAnalysts
17		Nov	19	14:15	57.73	-37.66	-1.608			00-6525-6896	02-0253-9745	EcoAnalysts
18	2025	Mar	6	14:05	115.7	20.28	0.6173			00-9185-6817	04-3708-3157	EcoAnalysts
19			27	13:55	100.6	5.164	0.1688			03-2395-9462	11-5612-9042	EcoAnalysts
20		May	29	14:17	73.39	-22	-0.8396			12-6192-6840	18-6505-2467	EcoAnalysts
21		Aug	7	16:25	112.2	16.8	0.5195			17-3092-0445	13-2942-5413	EcoAnalysts

Pacific Topsmelt 7-d Survival and Growth Test **All Matching Labs**

Test Type:	Growth-Survival (7d)	**Organism:**	Atherinops affinis	**Material:**	Copper
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Mean Dry Biomass-mg	**Source:**	Reference Toxicant-REF

Pacific Topsmelt 7-d Survival and Growth Test
Mean Dry Biomass-mg Endpoint



Lognormal Cumulative Mean Plot

Mean:	93.32	**Count:**	20	**-2s Warning Limit:**	48.7	**-3s Action Limit:**	35.2
Sigma:	NA	**CV:**	33.40%	**+2s Warning Limit:**	179	**+3s Action Limit:**	247

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	Mar	8	14:43	199	105.7	2.331	(+)		07-6546-0140	14-1523-7409	EcoAnalysts
2			30	13:20	107.4	14.11	0.4333			17-8194-4154	16-8645-1277	EcoAnalysts
3		May	10	16:20	87.83	-5.486	-0.1864			13-9380-0579	13-1069-1982	EcoAnalysts
4		Jun	14	16:08	106.3	13.02	0.402			04-7024-7990	12-0762-5164	EcoAnalysts
5		Jul	21	13:33	90.68	-2.638	-0.08823			20-8735-8279	16-3096-3469	EcoAnalysts
6		Sep	21	11:00	108.8	15.44	0.4711			01-2995-1270	08-1104-3649	EcoAnalysts
7		Oct	31	16:27	62.36	-30.96	-1.24			20-9804-3448	15-4107-9735	EcoAnalysts
8		Dec	6	16:28	107.3	14.01	0.4305			14-7580-4802	10-0695-3597	EcoAnalysts
9	2023	Apr	11	14:46	140.8	47.53	1.267			03-7518-1407	21-2232-1584	EcoAnalysts
10		Jun	13	16:35	97.38	4.066	0.1312			01-8019-5972	17-6654-7916	EcoAnalysts
11		Aug	14	15:25	67.74	-25.58	-0.9858			06-6644-4095	05-8575-6137	EcoAnalysts
12		Nov	28	16:07	99.74	6.422	0.2048			00-9729-8180	12-7800-0655	EcoAnalysts
13	2024	Feb	20	15:10	98.05	4.73	0.1522			10-1210-8679	02-9654-5507	EcoAnalysts
14		May	14	14:57	116.5	23.23	0.6839			18-5565-0131	05-7328-0784	EcoAnalysts
15		Aug	6	15:25	44.88	-48.44	-2.252	(-)		00-6133-8911	16-2482-9580	EcoAnalysts
16		Oct	3	16:00	96.8	3.487	0.1129			21-4179-4939	02-6138-6679	EcoAnalysts
17		Nov	19	14:15	58.55	-34.77	-1.434			00-6525-6896	12-7021-0395	EcoAnalysts
18	2025	Mar	6	14:05	110.1	16.79	0.5091			00-9185-6817	20-1342-8821	EcoAnalysts
19			27	13:55	89.24	-4.079	-0.1375			03-2395-9462	09-3332-8237	EcoAnalysts
20		May	29	14:17	71.4	-21.92	-0.8239			12-6192-6840	14-1998-8442	EcoAnalysts
21		Aug	7	16:25	108.4	15.08	0.4609			17-3092-0445	20-6612-8705	EcoAnalysts

Pacific Topsmelt 7-d Survival and Growth Test **EcoAnalysts**

Batch ID:	15-8082-1284	**Test Type:**	Growth-Survival (7d)	**Analyst:**	Michelle Bennett		
Start Date:	07 Aug-25 16:25	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater		
Ending Date:	14 Aug-25 11:30	**Species:**	Atherinops affinis	**Brine:**	Not Applicable		
Test Length:	6d 19h	**Taxon:**	Actinopterygii	**Source:**	Aquatic Biosystems, CO	**Age:**	13D
Sample ID:	00-7817-7393	**Code:**	R240207.113	**Project:**	Reference Toxicant		
Sample Date:	07 Feb-24	**Material:**	Copper	**Source:**	Reference Toxicant		
Receipt Date:	07 Feb-24	**CAS (PC):**		**Station:**	R240207.113		
Sample Age:	547d 16h	**Client:**	Internal Lab				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
13-3835-5067	7d Proportion Survived	Wilcoxon/Bonferroni Adj Test		80	160	113.1	15.9%	1
14-4964-5878	Mean Dry Biomass-mg	Bonferroni Adj t Test	✓	40	80	56.57	24.9%	1
03-7205-7613	Mean Dry Weight-mg	Bonferroni Adj t Test		80	160	113.1	23.2%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
13-2942-5413	7d Proportion Survived	Linear Interpolation (ICPIN)		EC50	112.2	96.09	121.9	1
20-6612-8705	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	✓	IC50	108.4	92.66	120.2	1
14-7520-9008	Mean Dry Weight-mg	Linear Interpolation (ICPIN)		IC50	>160	---	---	1

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
13-2942-5413	7d Proportion Survived	Control Resp	1	0.8	<<	Yes	Passes Criteria
13-3835-5067	7d Proportion Survived	Control Resp	1	0.8	<<	Yes	Passes Criteria
14-4964-5878	Mean Dry Biomass-mg	Control Resp	1.47	0.85	<<	Yes	Passes Criteria
20-6612-8705	Mean Dry Biomass-mg	Control Resp	1.47	0.85	<<	Yes	Passes Criteria
13-3835-5067	7d Proportion Survived	PMSD	0.1587	<<	0.25	Yes	Passes Criteria
14-4964-5878	Mean Dry Biomass-mg	PMSD	0.2488	<<	0.5	Yes	Passes Criteria

7d Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
20		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
40		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
80		5	0.8800	0.6579	1.1020	0.6000	1.0000	0.0800	0.1789	20.33%	12.00%
160		4	0.1000	-0.0837	0.2837	0.0000	0.2000	0.0577	0.1155	115.47%	90.00%
320		5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Mean Dry Biomass-mg Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.47	1.315	1.625	1.27	1.59	0.05571	0.1246	8.47%	0.00%
20		5	1.382	1.171	1.594	1.168	1.584	0.07628	0.1706	12.34%	5.96%
40		4	1.38	1.148	1.613	1.21	1.508	0.07299	0.146	10.57%	6.09%
80		5	1.147	0.8323	1.462	0.892	1.486	0.1134	0.2536	22.11%	21.96%
160		2	0.205	0.03982	0.3702	0.192	0.218	0.013	0.01838	8.97%	86.05%

Mean Dry Weight-mg Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.47	1.315	1.625	1.27	1.59	0.05571	0.1246	8.47%	0.00%
20		5	1.382	1.171	1.594	1.168	1.584	0.07628	0.1706	12.34%	5.96%
40		4	1.38	1.148	1.613	1.21	1.508	0.07299	0.146	10.57%	6.09%
80		5	1.317	1.05	1.584	1.09	1.57	0.09617	0.215	16.32%	10.38%
160		2	1.025	0.1991	1.851	0.96	1.09	0.065	0.09192	8.97%	30.27%

40% replicate #5 removed from statistical analysis due to lab error-DM-9/2/25

Pacific Topsmelt 7-d Survival and Growth Test **EcoAnalysts**

7d Proportion Survived Detail MD5: 4BECBEAB19C33202C70C44885AB006A8

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	1.0000	1.0000	1.0000
20		1.0000	1.0000	1.0000	1.0000	1.0000
40		1.0000	1.0000	1.0000	1.0000	
80		1.0000	0.6000	0.8000	1.0000	1.0000
160		0.0000	0.0000	0.2000	0.2000	
320		0.0000	0.0000	0.0000	0.0000	0.0000

Mean Dry Biomass-mg Detail MD5: EAFE786A35525BF5FB372B894F31E36E

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.27	1.59	1.544	1.506	1.44
20		1.3	1.168	1.526	1.334	1.584
40		1.508	1.21	1.308	1.496	
80		1.09	0.942	0.892	1.326	1.486
160		---	---	0.218	0.192	
320		---	---	---	---	---

Mean Dry Weight-mg Detail MD5: DFE359046CA1664594BA81C25636D78C

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.27	1.59	1.544	1.506	1.44
20		1.3	1.168	1.526	1.334	1.584
40		1.508	1.21	1.308	1.496	
80		1.09	1.57	1.115	1.326	1.486
160		---	---	1.09	0.96	
320		---	---	---	---	---

7d Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	5/5	5/5	5/5	5/5	5/5
20		5/5	5/5	5/5	5/5	5/5
40		5/5	5/5	5/5	5/5	
80		5/5	3/5	4/5	5/5	5/5
160		0/5	0/5	1/5	1/5	
320		0/5	0/5	0/5	0/5	0/5

CETIS Test Data Worksheet

Report Date: 26 Aug-25 19:26 (p 1 of 1)
Test Code/ID: R240207.113 / 17-3092-0445

Pacific Topsmelt 7-d Survival and Growth Test — **EcoAnalysts**

Start Date: 07 Aug-25 16:25 **Species:** Atherinops affinis **Sample Code:** R240207.113
End Date: 14 Aug-25 11:30 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 07 Feb-24 **Material:** Copper **Sample Station:** R240207.113

Conc-µg/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Total Weight-mg	Tare Weight-mg	Pan Count	Notes
0	D	1	25	5							5	64.64	58.29	5	
0	D	2	23	5							5	66.41	58.46	5	
0	D	3	26	5							5	66.99	59.27	5	
0	D	4	5	5							5	66.33	58.8	5	
0	D	5	12	5							5	63.03	55.83	5	
20		1	18	5							5	61.29	54.79	5	
20		2	10	5							5	60.79	54.95	5	
20		3	21	5							5	65.36	57.73	5	
20		4	7	5							5	64.1	57.43	5	
20		5	15	5							5	67.56	59.64	5	
40		1	8	5							5	63.02	55.48	5	
40		2	28	5							5	64.09	58.04	5	
40		3	9	5							5	63.19	56.65	5	
40		4	4	5							5	63.51	56.03	5	
80		1	1	5							5	61.98	56.53	5	
80		2	24	5							3	61.29	56.58	3	
80		3	30	5							4	61.24	56.78	4	
80		4	3	5							5	62.92	56.29	5	
80		5	17	5							5	66.36	58.93	5	
160		1	22	5							0			0	
160		2	13	5							0			0	
160		3	19	5							1	57.98	56.89	1	
160		4	2	5							1	57.38	56.42	1	
320		1	11	5							0			0	
320		2	29	5							0			0	
320		3	20	5							0			0	
320		4	16	5							0			0	
320		5	27	5							0			0	

EcoAnalysts, Inc.

7 Day Chronic WET Test

Version V.1

GENERAL

Client	Internal
Associated Test	Various
Compound	Copper Chloride
Toxicant	Copper
Test Type	Reference Toxicant
Test type	7 Day Chronic Toxicity with Topsmelt
Matrix	Liquid
Test Acceptability	≥ 80% average survival in control Average dry weight is > 0.85 mg per surviving fish. LC50 ≤ 205µg/L; <25% PMSD Survival, <50% PMSD Growth
Test Start Date	08/07/25
Test Species	*Atherinops affinis*
Organism Batch	ABS080525.03
Organism Acquired	8/5/2025
Organism Acclimation	2
Organism Age	13 days old
Test Protocol	TOX 002 / TOX 099
Test Location	Bath 1
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	5
Test Chamber Size	20 oz.
Exposure Volume	250 mL
Feeding Information	250 nauplii/chamber am 500 nauplii/chamber pm
Test Dissolved Oxygen	> 4
Test Temperature	20 ± 1
Test Salinity	30 ± 2
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters	Min	Max
DO	4.0	
Temp	19	21
Salinity	28	32
pH	6	9

TEST START TIME/INIT:	**1625 NL(LG)**
TEST END TIME/INIT:	**1130 KS**

REFERENCE TOXICANT TEST ID	**LOT #**
R240207.113	BCCH9104

Food Batch ID
311823

Concentrations (µg/L)

1	Control
2	20
3	40
4	80
5	160
6	320

Copy and Past VALUES fr

Treatment	Rep	Chamber
Control	1	26
Control	2	2
Control	3	4
Control	4	13
Control	5	11
20	1	3
20	2	28
20	3	17
20	4	27
20	5	7
40	1	18
40	2	24
40	3	19
40	4	8
40	5	21
80	1	30
80	2	15
80	3	23
80	4	6
80	5	12
160	1	14
160	2	20
160	3	5
160	4	22
160	5	1
320	1	25
320	2	29
320	3	10
320	4	9
320	5	16

EcoAnalysts, Inc.

7 Day Chronic WET Test

v.1 **CLIENT**	Internal	**TEST TYPE**	Reference Toxicant	**PROTOCOL**	TOX 002 / TOX 099
ASSOCIATED TEST	Various	**TEST START DATE**	8/7/25	**TOXICANT**	Copper
REF TOX ID	R240207.113	**TEST END DATE**	8/14/25	**SPECIES**	*Atherinops affinis*
LOT #	BCCH9104	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

Dilution Preparation (Serial dilute by 50%)

$CuCl_2*2H_20$ Stock Solution (µg/L Cu)	Target Stock Solution Conc. (µg/L)	Volume of Diluent (mL)	Amt. of Toxicant (mL)
400,000	320	2500	2.0
400,000	160	2500	1.0
400,000	80	2500	0.5

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Highest Concentration Prepared	Comments
8/7/2025	7	FSW080525.01	LG	320	
8/8/2025	7	FSW080525.01	CS	320	
8/9/2025	7	FSW080425.01	NL	320	
8/10/2025	7	FSW080525.01	NL	320	
8/11/2025	7	FSW081025.01	TVL	320	
8/12/2025	7	FSW081025.01	TVL	320	
8/13/2025	7	FSW081025.01	CS	320	

Water Quality

			DO (mg/L)	TEMP (°C)	SALINITY	pH
		Concentration (µg/L)	4	19 - 21	28 - 32	6 - 9
Day 0 (Stock)		Control	7.3	20.7	31	8.0
Date	8/7/2025	20	7.4	20.7	31	8.0
Time	15:49	40	7.4	20.7	31	8.1
Tech	CS	80	7.4	20.8	31	8.1
Meter #	9	160	7.4	20.8	31	8.1
Feed (initials)	NL	320	7.4	20.8	31	8.1

Temperature	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Meter #	T33	T43	T33	T43	T43	T42/ T33
Old	20.8	20.7	20.8	20.0	20.2	20.2
New	20.1	20.0	20.2	19.2	19.4	19.9
Tech	CS	EM	DM	KS	KS	EM/ KC

Day 7		Control	6.0	19.6	31	7.8
Replicate #	4	20	6.3	19.7	31	7.9
Date	8/14/2025	40	6.4	19.7	31	7.9
Time	817	80	6.4	19.6	31	7.9
Tech	EM	160	6.8	19.7	31	8.0
Meter #	9	320				

Comments

EcoAnalysts, Inc.

v.1

CLIENT	Internal	**TEST TYPE**	Reference Toxicant	**PROTOCOL**	TOX 002 / TOX 099
ASSOCIATED TEST	Various	**TEST START DATE**	8/7/25	**TOXICANT**	Copper
REF TOX ID	R240207.113	**TEST END DATE**	8/14/25	**SPECIES**	*Atherinops affinis*
LOT #	BCCH9104	**MATRIX**	Liquid		

Abbreviation Key:
NB = No Body
FB = Found Body
ST = Stranded

7 Day Chronic Toxicity with Topsmelt

			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7			
			Date	08/08/25		Date	08/09/25		Date	08/08/25		Date	08/11/25		Date	08/12/25		Date	08/13/25		Date	08/14/25		
			Time	1400		Time	1517		Time	1534		Time	1015		Time	1010		Time	1443		Time	1130		
			Tech	CS		Tech	MM		Tech	DM		Tech	KS		Tech	KS		Tech	KC		Tech	KS		
Concentration (µg/L)	**REP**	# initiated	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
20	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
40	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
80	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	4	0	1NB	4	0		4	0		4	0		3	1		3	0		3	0		
	3	5	5	0		5	0		5	0		5	0		4	1		4	0		4	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
160	1	5	5	0		4	1		2	2		1	1		0	1								
	2	5	5	0		5	0		4	1		2	2		1	1		0	1					
	3	5	5	0		3	2		2	1		1	1		1	0		1	0		1	0		
	4	5	5	0		4	1		2	2		1	1		1	0		1	0		1	0		
	5	5	5	0																				Cup accidentally knocked over - MM 8/9/25
320	1	5	4	1		3	1		1	2		1	0		1	0		0	1					
	2	5	1	4		1	0		0	1														
	3	5	3	2		3	0		1	2		1	0		0	1								
	4	5	5	0		3	2		1	2		0	1											
	5	5	4	1		2	2		0	2														
Feed (Init.)	AM		MM			NL			NL			CS			CS			EM			NONE			
250 nauplii/chamber am 500 nauplii/chamber pm	PM		NL			NL			NL			KS			LG			KS			NONE			

EcoAnalysts, Inc.

7 Day Chronic WET Test

v.1

CLIENT	Internal	**TEST TYPE**	Reference Toxicant	**PROTOCOL**	TOX 002 / TOX 099
ASSOCIATED TEST	Various	**TEST START DATE**	8/7/25	**TOXICANT**	Copper
REF TOX ID	R240207.113	**TEST END DATE**	8/14/25	**SPECIES**	*Atherinops affinis*
LOT #	BCCH9104	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Topsmelt

Concentration (µg/L)	REP	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count	Comments
Control	1	1	58.29	64.64	5	
	2	2	58.46	66.41	5	
	3	3	59.27	66.99	5	
	4	4	58.8	66.33	5	
	5	5	55.83	63.03	5	
20	1	6	54.79	61.29	5	
	2	7	54.95	60.79	5	
	3	8	57.73	65.36	5	
	4	9	57.43	64.1	5	
	5	10	59.64	67.56	5	
40	1	11	55.48	63.02	5	
	2	12	58.04	64.09	5	
	3	13	56.65	63.19	5	
	4	14	56.03	63.51	5	
	5	15	57.01	62.69	5	Some fish parts stuck to bottom of tray; attempted to recover biomass by scraping off onto boat, but some weight likely was lost - EM 8/22/25
80	1	16	56.53	61.98	5	
	2	17	56.58	61.29	3	
	3	18	56.78	61.24	4	
	4	19	56.29	62.92	5	
	5	20	58.93	66.36	5	
160	1	21	55.52			
	2	22	58.66			
	3	23	56.89	57.98	1	
	4	24	56.42	57.38	1	
	5	25	54.28			
320	1	26	54.52			
	2	27	56.9			
	3	28	56.86			
	4	29	56.4			
	5	30	59.46			

	Oven Event 1	Oven Event 2
Oven ID:	Beelzebub	BEELZEBUB
Date/Time/Initials In Oven:	8/3/25 1640 EM/KS	8/14/25 1130 KS
Oven Temp °C:	60.00	100.00
Date/Time/Initials Out Oven into Dessicator:	8/4/25 1125 DM	8/15/25 934 MM
Date/Time/Initials Weighed:	8/11/25 14:00 KS	8/22/25 1609 EM
Balance ID:	3.00	3.00

APPENDIX A2.1

AMERICAMYSIS BAHIA (MYSID) 7-DAY SURVIVAL AND GROWTH TEST

STATISTICAL COMPARISONS AND LABORATORY DATA SHEETS

Mysidopsis 7-d Survival, Growth and Fecundity Test **EcoAnalysts**

Batch ID:	03-3795-0929	**Test Type:**	Growth-Survival-Fec (7d)	**Analyst:**	Michelle Bennett		
Start Date:	08 Aug-25 17:11	**Protocol:**	EPA/821/R-02-014 (2002)	**Diluent:**	Laboratory Seawater		
Ending Date:	15 Aug-25 08:50	**Species:**	Americamysis bahia	**Brine:**	Crystal Sea Marine Mix		
Test Length:	6d 16h	**Taxon:**	Malacostraca	**Source:**	Aquatic Biosystems, CO	**Age:**	7D
Sample ID:	17-2784-2730	**Code:**	P250808.02	**Project:**	2025 NPDES		
Sample Date:	07 Aug-25 07:00	**Material:**	Effluent Sample	**Source:**	LOTT Clean Water Alliance (WA00370		
Receipt Date:	08 Aug-25 12:30	**CAS (PC):**		**Station:**	Final Effluent #2		
Sample Age:	34h (0 °C)	**Client:**	LOTT				

SIngle Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
02-9241-0821	7d Proportion Survived	Wilcoxon Rank Sum Two-Sample Test	0.8590	Salt Control passed 7d proportion survived	1
04-0372-7135	Mean Dry Biomass-mg	Equal Variance t Two-Sample Test	0.4993	Salt Control passed mean dry biomass-mg	1
08-4811-1529	Mean Dry Weight-mg	Equal Variance t Two-Sample Test	0.3423	Salt Control passed mean dry weight-mg	1

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
13-0610-0086	7d Proportion Survived	Steel Many-One Rank Sum Test		100	>100	---	11.0%	1	1
13-7517-2231	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test		100	>100	---	21.2%	1	1
21-2526-2633	Mean Dry Weight-mg	Dunnett Multiple Comparison Test		100	>100	---	15.6%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
10-9502-9276	7d Proportion Survived	Linear Interpolation (ICPIN)	✓	EC25	>100	---	---	<1	1
21-0291-2870	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	✓	IC25	>100	---	---	<1	1
17-6203-7899	Mean Dry Weight-mg	Linear Interpolation (ICPIN)	✓	IC25	>100	---	---	<1	1

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
02-9241-0821	7d Proportion Survived	Control Resp	0.95	0.8	<<	Yes	Passes Criteria
	7d Proportion Survived	Control Resp	0.925	0.8	<<	Yes	Passes Criteria
10-9502-9276	7d Proportion Survived	Control Resp	0.925	0.8	<<	Yes	Passes Criteria
13-0610-0086	7d Proportion Survived	Control Resp	0.925	0.8	<<	Yes	Passes Criteria
04-0372-7135	Mean Dry Biomass-mg	Control Resp	0.3132	0.2	<<	Yes	Passes Criteria
	Mean Dry Biomass-mg	Control Resp	0.3133	0.2	<<	Yes	Passes Criteria
13-7517-2231	Mean Dry Biomass-mg	Control Resp	0.3133	0.2	<<	Yes	Passes Criteria
21-0291-2870	Mean Dry Biomass-mg	Control Resp	0.3133	0.2	<<	Yes	Passes Criteria
04-0372-7135	Mean Dry Biomass-mg	PMSD	0.1359	0.11	0.37	Yes	Passes Criteria
13-7517-2231	Mean Dry Biomass-mg	PMSD	0.2122	0.11	0.37	Yes	Passes Criteria

Mysidopsis 7-d Survival, Growth and Fecundity Test **EcoAnalysts**

7d Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	8	0.9250	0.8385	1.0120	0.8000	1.0000	0.0366	0.1035	11.19%	0.00%
0	SC	8	0.9500	0.8726	1.0270	0.8000	1.0000	0.0327	0.0926	9.75%	-2.70%
2		8	0.9750	0.9159	1.0340	0.8000	1.0000	0.0250	0.0707	7.25%	-5.41%
2.8		8	0.9500	0.8726	1.0270	0.8000	1.0000	0.0327	0.0926	9.75%	-2.70%
10		8	0.9500	0.8726	1.0270	0.8000	1.0000	0.0327	0.0926	9.75%	-2.70%
30		8	0.9500	0.8726	1.0270	0.8000	1.0000	0.0327	0.0926	9.75%	-2.70%
100		8	0.9750	0.9159	1.0340	0.8000	1.0000	0.0250	0.0707	7.25%	-5.41%

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	8	0.3133	0.2668	0.3598	0.22	0.392	0.01966	0.05561	17.75%	0.00%
0	SC	8	0.3133	0.28	0.3465	0.238	0.378	0.01406	0.03977	12.70%	0.01%
2		8	0.317	0.2604	0.3736	0.232	0.414	0.02392	0.06765	21.34%	-1.18%
2.8		8	0.3021	0.2546	0.3495	0.216	0.364	0.02006	0.05674	18.78%	3.58%
10		8	0.2888	0.2587	0.3188	0.238	0.336	0.01269	0.03589	12.43%	7.83%
30		8	0.3241	0.272	0.3762	0.224	0.428	0.02204	0.06233	19.23%	-3.46%
100		8	0.3418	0.2895	0.394	0.212	0.418	0.02209	0.06247	18.28%	-9.08%

Mean Dry Weight-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	8	0.3376	0.3057	0.3695	0.275	0.392	0.01347	0.03811	11.29%	0.00%
0	SC	8	0.3302	0.3024	0.358	0.296	0.38	0.01175	0.03324	10.07%	2.20%
2		8	0.3242	0.2742	0.3743	0.252	0.414	0.02118	0.0599	18.47%	3.96%
2.8		8	0.3184	0.2754	0.3614	0.216	0.364	0.01819	0.05144	16.16%	5.69%
10		8	0.3042	0.283	0.3254	0.258	0.336	0.008978	0.02539	8.35%	9.90%
30		8	0.3393	0.3027	0.3759	0.28	0.428	0.01548	0.04379	12.91%	-0.51%
100		8	0.3484	0.3084	0.3884	0.265	0.418	0.01691	0.04784	13.73%	-3.19%

Mysidopsis 7-d Survival, Growth and Fecundity Test **EcoAnalysts**

7d Proportion Survived Detail MD5: 8F9622F4E65EFD035CC9485804F5B9C4

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	1.0000	0.8000	1.0000	0.8000	0.8000	1.0000	1.0000	1.0000
0	SC	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8000
2		1.0000	1.0000	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000
2.8		0.8000	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
10		1.0000	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8000
30		0.8000	1.0000	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000
100		1.0000	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Mean Dry Biomass-mg Detail MD5: 7C030C81F4E5BAED90E0979DC6CFC219

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.392	0.262	0.3583	0.296	0.22	0.332	0.298	0.348
0	SC	0.238	0.304	0.324	0.33	0.378	0.296	0.332	0.304
2		0.414	0.252	0.348	0.396	0.34	0.232	0.266	0.288
2.8		0.244	0.278	0.364	0.348	0.3367	0.358	0.216	0.272
10		0.336	0.256	0.284	0.258	0.312	0.328	0.298	0.238
30		0.262	0.428	0.364	0.352	0.33	0.224	0.315	0.318
100		0.368	0.212	0.378	0.34	0.31	0.328	0.418	0.38

Mean Dry Weight-mg Detail MD5: A0E10303AEABB506B3E284258FAAEAD4

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.392	0.3275	0.3583	0.37	0.275	0.332	0.298	0.348
0	SC	0.2975	0.304	0.324	0.33	0.378	0.296	0.332	0.38
2		0.414	0.252	0.348	0.396	0.34	0.29	0.266	0.288
2.8		0.305	0.3475	0.364	0.348	0.3367	0.358	0.216	0.272
10		0.336	0.32	0.284	0.258	0.312	0.328	0.298	0.2975
30		0.3275	0.428	0.364	0.352	0.33	0.28	0.315	0.318
100		0.368	0.265	0.378	0.34	0.31	0.328	0.418	0.38

7d Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	5/5	4/5	6/6	4/5	4/5	5/5	5/5	5/5
0	SC	4/5	5/5	5/5	5/5	5/5	5/5	5/5	4/5
2		5/5	5/5	5/5	5/5	5/5	4/5	5/5	5/5
2.8		4/5	4/5	5/5	5/5	6/6	5/5	5/5	5/5
10		5/5	4/5	5/5	5/5	5/5	5/5	5/5	4/5
30		4/5	5/5	5/5	5/5	5/5	4/5	6/6	5/5
100		5/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5

Mysidopsis 7-d Survival, Growth and Fecundity Test **EcoAnalysts**

Start Date: 08 Aug-25 17:11 **Species:** Americamysis bahia **Sample Code:** P250808.02
End Date: 15 Aug-25 08:50 **Protocol:** EPA/821/R-02-014 (2002) **Sample Source:** LOTT Clean Water Alliance
Sample Date: 07 Aug-25 07:00 **Material:** Effluent Sample **Sample Station:** Final Effluent #2

Conc-%	Code	Rep	Pos	# Exposed	1d Survival	2d Survival 3d Survival	4d Survival	5d Survival 6d Survival	7d Survival	Total Weight-mg	Tare Weight-mg	Pan Count	Total Females	Gravid Females	Notes
0	D	1	27	5					5	14.62	12.66	5			
0	D	2	29	5					4	13.97	12.66	4			
0	D	3	22	6					6	14.69	12.54	6			
0	D	4	21	5					4	13.93	12.45	4			
0	D	5	1	5					4	13.6	12.5	4			
0	D	6	12	5					5	14.28	12.62	5			
0	D	7	58	5					5	13.98	12.49	5			
0	D	8	57	5					5	14.39	12.65	5			
0	SC	1	14	5					4	13.79	12.6	4			
0	SC	2	15	5					5	14.09	12.57	5			
0	SC	3	44	5					5	14.31	12.69	5			
0	SC	4	51	5					5	14.17	12.52	5			
0	SC	5	8	5					5	14.7	12.81	5			
0	SC	6	24	5					5	14.1	12.62	5			
0	SC	7	5	5					5	14.18	12.52	5			
0	SC	8	32	5					4	14.6	13.08	4			
2		1	28	5					5	14.23	12.16	5			
2		2	54	5					5	13.61	12.35	5			
2		3	11	5					5	14.14	12.4	5			
2		4	41	5					5	16.4	14.42	5			
2		5	63	5					5	14.37	12.67	5			
2		6	39	5					4	13.68	12.52	4			
2		7	56	5					5	13.8	12.47	5			
2		8	9	5					5	13.87	12.43	5			
2.8		1	36	5					4	13.77	12.55	4			
2.8		2	53	5					4	13.89	12.5	4			
2.8		3	43	5					5	14.45	12.63	5			
2.8		4	60	5					5	14.42	12.68	5			

Conc-%	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Total Weight-mg	Tare Weight-mg	Pan Count	Total Females	Gravid Females	Notes
2.8		5	38	6							6	14.63	12.61	6			
2.8		6	4	5							5	14.5	12.71	5			
2.8		7	23	5							5	13.62	12.54	5			
2.8		8	34	5							5	14.4	13.04	5			
10		1	47	5							5	14.33	12.65	5			
10		2	30	5							4	13.88	12.6	4			
10		3	13	5							5	14.02	12.6	5			
10		4	2	5							5	13.67	12.38	5			
10		5	25	5							5	14.04	12.48	5			
10		6	42	5							5	14.16	12.52	5			
10		7	61	5							5	14.15	12.66	5			
10		8	3	5							4	13.88	12.69	4			
30		1	17	5							4	13.82	12.51	4			
30		2	37	5							5	14.26	12.12	5			
30		3	49	5							5	14.16	12.34	5			
30		4	55	5							5	14.32	12.56	5			
30		5	46	5							5	26.74	25.09	5			
30		6	20	5							4	13.65	12.53	4			
30		7	62	6							6	14.29	12.4	6			
30		8	18	5							5	14.32	12.73	5			
100		1	64	5							5	14.41	12.57	5			
100		2	35	5							4	13.56	12.5	4			
100		3	7	5							5	14.45	12.56	5			
100		4	45	5							5	14.11	12.41	5			
100		5	19	5							5	14.05	12.5	5			
100		6	52	5							5	14.35	12.71	5			
100		7	48	5							5	14.55	12.46	5			
100		8	40	5							5	14.34	12.44	5			

EcoAnalysts, Inc.

7 Day Chronic WET Test

Version V.4

GENERAL

Client	LOTT Clean Water Alliance
Project	2025 NPDES
Project Number	PG2162
Project Manager	M. Bennett
Date Sample Received	8/8/2025
Test type	7 Day Chronic Toxicity with Mysid
Matrix	Liquid
Test Acceptability	≥ 80% average survival of control mysid Average dry weight is 0.20 mg or greater per surviving control mysids
Test Start Date	08/08/25
Test Species	*Americamysis bahia*
Organism Batch	ABS080825.01
Organism Acquired	8/8/2025
Organism Acclimation	0
Organism Age	7 Days
Test Protocol	TOX 014
Test Location	Temp Control Room
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	5
Test Chamber Size	12 oz.
Exposure Volume	250 mL
Feeding Information	375 nauplii/chamber twice per day except Day 7
Test Dissolved Oxygen	> 4
Test Temperature	26 ± 1
Test Salinity	30 ± 2
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters	Min	Max
DO	4.0	
Temp	25	27
Salinity	28	32
pH	6	9

TEST START TIME/INIT:	**1711 NL (MS)**
TEST END TIME/INIT:	**0850 CS**

Food Batch ID
311823

CSMM Batch #
082024

CLIENT SAMPLE ID	**LAB ID**
Final Effluent #2	P250808.02
Final Effluent #3	P250812.49
Final Effluent #4	P250813.15

Concentrations

1	Control
2	Salt Control
3	2%
4	2.8%
5	10%
6	30%
7	100%
8	.
9	.

Copy and Past VALUES fr

Treatment	Rep	Chamber
Control	1	41
Control	2	19
Control	3	50
Control	4	54
Control	5	8
Control	6	37
Control	7	39
Control	8	20
Salt Control	1	28
Salt Control	2	21
Salt Control	3	12
Salt Control	4	44
Salt Control	5	53
Salt Control	6	4
Salt Control	7	47
Salt Control	8	25
2.0%	1	34
2.0%	2	6
2.0%	3	14
2.0%	4	32
2.0%	5	22
2.0%	6	18
2.0%	7	13
2.0%	8	26
2.8%	1	30
2.8%	2	2
2.8%	3	33
2.8%	4	45
2.8%	5	49
2.8%	6	52
2.8%	7	36
2.8%	8	24
10.0%	1	56
10.0%	2	16
10.0%	3	3
10.0%	4	5
10.0%	5	42
10.0%	6	43
10.0%	7	17
10.0%	8	31
30.0%	1	29
30.0%	2	55
30.0%	3	10
30.0%	4	23
30.0%	5	38
30.0%	6	48
30.0%	7	46
30.0%	8	9
100.0%	1	1
100.0%	2	27
100.0%	3	51
100.0%	4	11
100.0%	5	7
100.0%	6	40
100.0%	7	35
100.0%	8	15

ECO ANALYSTS, INC.

7 Day Chronic WET Test

V.4						
CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 014
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

Test Parameters	
Salinity of Sample	0.252
Test Salinity	30

CSMM Batch Number
82024

Sample Lab ID
P250808.02

Salinity Adjustment Multiplier	29.748

Coarse salinity adjustment	
mLs. Sample*	19000.0
Grams CSMM	565.2

* Adjust volume so that it is at least the total volume of sample needed for all dilutions, see cell D16 on Dilutions Worksheet.

Fine Salinity Adjustment	
Salinity of coarse-adjusted Sample	28.62
Test Salinity	30
Ratio	1.05
Grams additional CSMM needed to reach target salinity	27.48427673

Final salinity	30

Salinity Adjustment Date / Initials		Meter #
8/8/2025	MM	7

Comments

ECO ANALYSTS, INC.

7 Day Chronic WET Test

V.4						
	CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/12/25	**PROTOCOL**	TOX 014
	PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
	CLIENT SAMPLE ID	Final Effluent #3	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
	LAB SAMPLE ID	P250812.49	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

Test Parameters	
Salinity of Sample	0.258
Test Salinity	30

CSMM Batch Number
82024

Sample Lab ID
P250812.49

Salinity Adjustment Multiplier	29.742

Coarse salinity adjustment	
mLs. Sample*	19000.0
Grams CSMM	565.1

* Adjust volume so that it is at least the total volume of sample needed for all dilutions, see cell D28 on Dilutions Worksheet.

Fine Salinity Adjustment	
Salinity of coarse-adjusted Sample	27.33
Test Salinity	30
Ratio	1.10
Grams additional CSMM needed to reach target salinity	55.68605928

Final salinity	29.61

Salinity Adjustment Date / Initials		Meter #
8/12/2025	EM	8

Comments

ECO ANALYSTS, INC.

7 Day Chronic WET Test

V.4						
	CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**		**PROTOCOL**	TOX 014
	PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
	CLIENT SAMPLE ID	Final Effluent #4	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
	LAB SAMPLE ID	P250813.15	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

Test Parameters	
Salinity of Sample	0.247
Test Salinity	30

CSMM Batch Number
82024

Sample Lab ID
P250813.15

Salinity Adjustment Multiplier	29.753

Coarse salinity adjustment	
mLs. Sample*	19000.0
Grams CSMM	565.3

* Adjust volume so that it is at least the total volume of sample needed for all dilutions, see cell D28 on Dilutions Worksheet.

Fine Salinity Adjustment	
Salinity of coarse-adjusted Sample	26
Test Salinity	30
Ratio	1.15
Grams additional CSMM needed to reach target salinity	87.69230769

Final salinity	30.04

Salinity Adjustment Date	Initials	Meter #
8/13/2025	KS	10

Comments

EcoAnalysts, Inc.

7 Day Chronic WET Test

V.4

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 014
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	FSW
0	0%	0	2000.0	2000		
	Salt Control			2000		
	2%	40	1960.0	2000		
	2.8%	56	1944.0	2000		
	10%	200	1800.0	2000		
	30%	600	1400.0	2000		
	100%	2000	0.0	2000		
	Total Volume Needed	2896				

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
1 - 6	0%	0	1600.0	1600
	Salt Control			1600
	2%	32	1568.0	1600
	2.8%	44.8	1555.2	1600
	10%	160	1440.0	1600
	30%	480	1120.0	1600
	100%	1600	0.0	1600
	Total Volume Needed	2316.8		

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials	Comments
8/8/2025	7	P250808.02	FSW080525.01	MM	
8/9/2025	7	P250808.02	FSW080425.01	NL	
8/10/2025	7	P250808.02	FSW080425.01	NL	
8/11/2025	7	P250808.02	FSW081025.01	TVL	
8/12/2025	7	P250812.49	FSW081025.01	KS	
8/13/2025	7	P250813.15	FSW081225.01	CS	
8/14/2025	7	P250813.15	FSW081225.01	KS	

EcoANALYSTS, INC.

v.4

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 014
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

			DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	
		Concentration (%)	> 4	25 - 27	28 - 32	6 - 9	**Comments**
Day 0		Control	7.0	24.9	30	8.0	
Stock		Salt Control	6.8	24.8	29	8.9	
Date	8/8/2025	2%	7.0	24.8	30	8.0	
Time	1622	2.8%	7.1	24.8	30	8.0	
Tech	NL	10%	7.4	24.8	30	8.0	
Meter #	10	30%	7.5	24.8	30	8.1	
Feed	NL	100%	8.2	24.9	30	8.3	
Day 1		Control	6.0	24.9	31	7.6	
Rep 1		Salt Control	6.1	25.0	29	8.3	
Date	8/9/2025	2%	6.1	25.0	31	7.9	
Time	856	2.8%	6.1	25.1	31	7.9	
Tech	EM	10%	6.1	25.1	31	7.9	
Meter #	8	30%	5.8	25.3	30	8.0	
		100%	5.8	25.3	30	8.2	
Day 1		Control	7.2	27.0	31	7.9	
Renewal Stock		Salt Control	7.3	25.1	29	8.0	
Date	8/9/2025	2%	7.2	26.5	31	8.0	
Time	1056	2.8%	7.1	26.7	31	8.0	
Tech	EM	10.0%	7.2	27.1	31	8.0	
Meter #	10	30.0%	7.2	27.1	31	8.1	
		100%	8.2	25.6	30	8.3	
Day 2		Control	6.1	25.2	31	7.8	
Rep 2		Salt Control	6.2	25.3	30	7.8	
Date	8/10/2025	2.0%	6.0	25.4	31	7.8	
Time	1431	2.8%	6.1	25.5	31	7.8	
Tech	NL	10.0%	5.9	25.5	31	7.8	
Meter #	10	30.0%	5.3	25.7	31	7.9	
		100.0%	5.3	25.6	31	8.1	
Day 2		Control	7.3	25.9	31	8.0	
Renewal Stock		Salt Control	7.5	25.7	29	8.0	
Date	8/10/2025	2.0%	7.4	25.7	31	8.0	
Time	1027	2.8%	7.5	25.8	31	8.0	
Tech	NL	10.0%	7.5	26.0	31	8.0	
Meter #	10	30.0%	7.9	26.2	30	8.1	
		100.0%	8.6	26.4	30	8.3	

EcoANALYSTS, INC.

v.4 **CLIENT**	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 014
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

			DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	
		Concentration (%)	> 4	25 - 27	28 - 32	6 - 9	**Comments**
Day 3		Control	5.6	25.0	31	7.7	
Rep 3		Salt Control	6.1	24.9	30	7.8	
Date	8/11/2025	2%	6.1	24.9	31	7.8	
Time	9:40	3%	5.5	25.2	30	7.7	
Tech	JB	10%	5.4	25.3	30	7.8	
Meter #	10	30.0%	5.9	25.1	30	7.9	
		100.0%	5.3	25.2	29	8.1	
Day 3		Control	7.4	25.8	30	7.9	
Renewal Stock		Salt Control	7.4	25.8	29	8.0	
Date	8/11/2025	2%	7.4	26.1	30	7.9	
Time	10:10	2.8%	7.4	25.9	30	7.9	
Tech	JB	10%	7.6	26.2	30	7.9	
Meter #	10/T45	30%	7.7	26.0	30	8.0	
		100%	8.5	24.9	29	8.3	
Day 4		Control	5.7	24.6	31	7.8	
Rep 4		Salt Control	6.2	24.6	30	7.9	
Date	8/12/2025	2%	6.1	24.8	31	7.9	
Time	827	2.8%	5.8	24.9	31	7.8	
Tech	JB	10%	6.2	24.8	31	7.9	
Meter #	8	30%	6.1	24.9	31	8.0	
		100%	5.3	24.9	30	8.1	
Day 4		Control	7.2	26.0	30	8.0	
Renewal Stock		Salt Control	7.2	25.2	29	8.1	
Date	8/12/2025	2%	7.3	26.0	30	8.0	
Time	1523	2.8%	7.3	26.5	30	8.0	
Tech	TVL	10%	7.4	26.4	30	8.0	
Meter #	10	30%	7.5	27.2	30	8.1	
		100%	7.9	26.4	29	8.3	

EcoANALYSTS, INC.

V.4 **CLIENT**	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 014
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

			DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	
		Concentration (%)	> 4	25 - 27	28 - 32	6 - 9	**Comments**
Day 5		Control	5.2	24.9	31	7.7	
Rep 5		Salt Control	5.2	24.9	30	7.7	
Date	8/13/2025	2%	4.9	24.8	31	7.7	
Time	1018	2.8%	4.5	25.3	31	7.6	
Tech	KR	10%	4.5	25.2	31	7.7	
Meter #	10	30%	5.0	25.0	30	7.8	
		100%	4.2	25.1	30	8.0	
Day 5		Control	7.5	26.7	30	7.9	
Renewal Stock		Salt Control	6.5	25.2	29	9.0	
Date	8/13/2025	2%	7.2	27.0	31	7.9	
Time	15:38	2.8%	7.1	27.3	31	7.9	
Tech	CS	10%	7.4	27.1	31	8.0	
Meter #	10	30%	7.2	27.4	31	8.0	
		100%	7.5	27.2	31	8.2	
Day 6		Control	5.8	24.0	31	7.8	Temperatures low due to power outage- MM 8/14/25
Rep 6		Salt Control	5.8	24.0	30	8.1	
Date	8/14/2025	2%	6.0	24.3	31	7.8	
Time	1054	2.8%	5.5	24.5	31	7.7	
Tech	MM	10%	5.5	24.5	31	7.8	
Meter #	10	30%	5.5	24.3	31	7.9	
		100%	5.4	24.6	31	8.1	
Day 6		Control	7.1	26.2	31	7.9	
Renewal Stock		Salt Control	7.1	26.1	30	8.6	
Date	8/14/2025	2%	7.3	26.7	31	7.9	
Time	1146	2.8%	7.3	26.7	31	7.9	
Tech	MM	10%	7.4	26.4	31	8.0	
Meter #	10	30%	7.5	27.2	31	8.1	
		100%	7.8	26.5	31	8.2	
Day 7		Control	5.9	24.7	31	7.8	
Rep 7		Salt Control	5.9	25.1	30	7.9	
Date	8/15/2025	2%	5.9	25.3	31	7.8	
Time	8:33	2.8%	5.8	25.3	31	7.8	
Tech	CS	10%	5.3	25.3	31	7.8	
Meter #	10	30%	5.3	25.3	31	7.9	
		100%	5.5	25.3	31	8.1	

EcoAnalysts, Inc.

v.4

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 014
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

Abbreviation Key:
NB = No Body
FB = Found Body
ST = Stranded

7 Day Chronic Toxicity with Mysid

			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7			
			Date	08/09/25		Date	08/10/25		Date	08/11/25		Date	08/12/25		Date	08/13/25		Date	08/14/25		Date	08/15/25		
			Time	1145		Time	1456		Time	1229		Time	1538		Time	1600		Time	1213		Time	850		
			Tech	NL		Tech	NL		Tech	CS		Tech	EM		Tech	CS		Tech	CS		Tech	CS		
Concentration (%)	**REP**	# Initiated	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	4	1		4	0		4	0		4	0		4	0		4	0		4	0		
	3	5	6	0	1FB	6	0		6	0		6	0		6	0		6	0		6	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		4	0	1NB	
	5	5	5	0		4	0	1NB	4	0		4	0		4	0		4	0		4	0		
	6	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	7	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	8	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
Salt Control	1	5	4	0	1NB	4	0		4	0		4	0		4	0		4	0		4	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	6	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	7	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	8	5	5	0		5	0		5	0		4	0	1NB	4	0		4	0		4	0		
2%	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	6	5	5	0		4	0	1NB	4	0		4	0		4	0		4	0		4	0		
	7	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	8	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
2.8%	1	5	5	0		5	0		5	0		4	0	1NB	4	0		4	0		4	0		
	2	5	5	0		5	0		5	0		4	0	1NB	4	0		4	0		4	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		6	0	1FB	6	0		6	0		6	0		
	6	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	7	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	8	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		

v.4

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 014
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

Abbreviation Key:
NB = No Body
FB = Found Body
ST = Stranded

7 Day Chronic Toxicity with Mysid

			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7			
			Date	08/09/25		Date	08/10/25		Date	08/11/25		Date	08/12/25		Date	08/13/25		Date	08/14/25		Date	08/15/25		
			Time	1145		Time	1456		Time	1229		Time	1538		Time	1600		Time	1213		Time	850		
			Tech	NL		Tech	NL		Tech	CS		Tech	EM		Tech	CS		Tech	CS		Tech	CS		
Concentration (%)	**REP**	# Initiated	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Comments
10%	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		4	0	1NB	4	0		4	0		4	0		4	0		4	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	6	5	5	0		4	0	1NB	5	0	1FB	5	0		5	0		5	0		5	0		
	7	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	8	5	5	0		5	0		4	0	1NB	4	0		4	0		4	0		4	0		
30%	1	5	4	1		4	0		4	0		4	0		4	0		4	0		4	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	6	5	5	0		5	0		4	0	1NB	4	0		4	0		4	0		4	0		
	7	5	5	0		5	0		5	0		6	0	1FB	6	0		6	0		6	0		
	8	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
100%	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		4	0	1NB	4	0		4	0		4	0		4	0		4	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	6	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	7	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	8	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
Feed (Init.)	AM		NL			NL			CS			CS			EM			KS			NONE			
375 nauplii/chamber twice per day except Day 7	PM		NL			NL			KS			KR			KS			MM			NONE			

ECO ANALYSTS, INC.

7 Day Chronic WET Test

v.4

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 014
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

Concentration (%)	REP	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count	Comments
Control	1	1	12.66	14.62	5	
	2	2	12.66	13.97	4	
	3	3	12.54	14.69	6	
	4	4	12.45	13.93	4	
	5	5	12.5	13.6	4	
	6	6	12.62	14.28	5	
	7	7	12.49	13.98	5	
	8	8	12.65	14.39	5	
Salt Control	1	9	12.6	13.79	4	
	2	10	12.57	14.09	5	
	3	11	12.69	14.31	5	
	4	12	12.52	14.17	5	
	5	13	12.81	14.7	5	
	6	14	12.62	14.1	5	
	7	15	12.52	14.18	5	
	8	16	13.08	14.6	4	
2%	1	17	12.16	14.23	5	
	2	18	12.35	13.61	5	
	3	19	12.4	14.14	5	
	4	20	14.42	16.4	5	
	5	21	12.67	14.37	5	
	6	22	12.52	13.68	4	
	7	23	12.47	13.8	5	
	8	24	12.43	13.87	5	
2.8%	1	25	12.55	13.77	4	
	2	26	12.5	13.89	4	
	3	27	12.63	14.45	5	
	4	28	12.68	14.42	5	
	5	29	12.61	14.63	6	
	6	30	12.71	14.5	5	
	7	31	12.54	13.62	5	
	8	32	13.04	14.4	5	

V.4

CLIENT	LOTT Clean Water Alliance	**DATE RECEIVED**	8/8/25	**PROTOCOL**	TOX 014
PROJECT	2025 NPDES	**TEST START DATE**	8/8/25	**PROJECT MANAGER**	M. Bennett
CLIENT SAMPLE ID	Final Effluent #2	**TEST END DATE**	8/15/25	**SPECIES**	*Americamysis bahia*
LAB SAMPLE ID	P250808.02	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

Concentration (%)	REP	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count	Comments
10%	1	33	12.65	14.33	5	
	2	34	12.6	13.88	4	
	3	35	12.6	14.02	5	
	4	36	12.38	13.67	5	
	5	37	12.48	14.04	5	
	6	38	12.52	14.16	5	
	7	39	12.66	14.15	5	
	8	40	12.69	13.88	4	
30%	1	41	12.51	13.82	4	
	2	42	12.12	14.26	5	
	3	43	12.34	14.16	5	
	4	44	12.56	14.32	5	
	5	45	25.09	26.74	5	
	6	46	12.53	13.65	4	
	7	47	12.4	14.29	6	
	8	48	12.73	14.32	5	
100%	1	49	12.57	14.41	5	
	2	50	12.5	13.56	4	
	3	51	12.56	14.45	5	
	4	52	12.41	14.11	5	
	5	53	12.5	14.05	5	
	6	54	12.71	14.35	5	
	7	55	12.46	14.55	5	
	8	56	12.44	14.34	5	

	Oven Event 1	Oven Event 2
Oven ID:	Beelzebub	Beelzebub
Date/Time/Initials In Oven:	8/3/25 1640 EM/KS	8/15/25 1019 CS
Oven Temp °C:	60.00	105.00
Date/Time/Initials Out Oven into Dessicator:	8/4/25 1125 DM	8/16/25 822 MM
Date/Time/Initials Weighed:	8/11/25 1630 KS	8/22/25 1656 EM
Balance ID:	3.00	3.00

ORGANISM RECEIPT LOG

Date:	Time:	Batch No.
8/8/25	1230	ABS080825.01

Organism: Americamysis bahia

Source / Supplier: Aquatic Bio Systems

No. Ordered:	No. Received:	Source Batch: Collection date, hatch date, etc.):
310	340	8/1/25

Condition of Organisms:	Approximate Size or Age: (Days from hatch, life stage, size class, etc.):
Good	~7 days old
Shipper: FedEx	**B of L (Tracking No.)** 4357 9736 1020
Condition of Container: Good	**Received By:** CS

Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	13.2	21.9	33	7.4	0	0	CS

*if >10% contact lab manager

Notes:

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524

Toll Free: 800/331-5916
Tel: 970/484-5091 Fax:970/484-2514

ORGANISM HISTORY

DATE: 8/7/2025

SPECIES: *Americamysis bahia* (formerly Mysidopsis)

AGE: 6 day

LIFE STAGE: Juvenile

HATCH DATE: 8/1/2025

BEGAN FEEDING: Immediately

FOOD: *Artemia* sp.

Water Chemistry Record:	**Current**	**Range**
TEMPERATURE:	26°C	23-26 °C
SALINITY/CONDUCTIVITY:	30 ppt**	21-30 ppt
TOTAL HARDNESS (as $CaCO_3$):	--	--
TOTAL ALKALINITY (as $CaCO_3$):	155 mg/l	140-170 mg/l
pH:	8.12	7.60-8.20

Comments:

Facility Supervisor

APPENDIX A2.2

AMERICAMYSIS BAHIA (MYSID) 7-DAY SURVIVAL AND GROWTH TEST

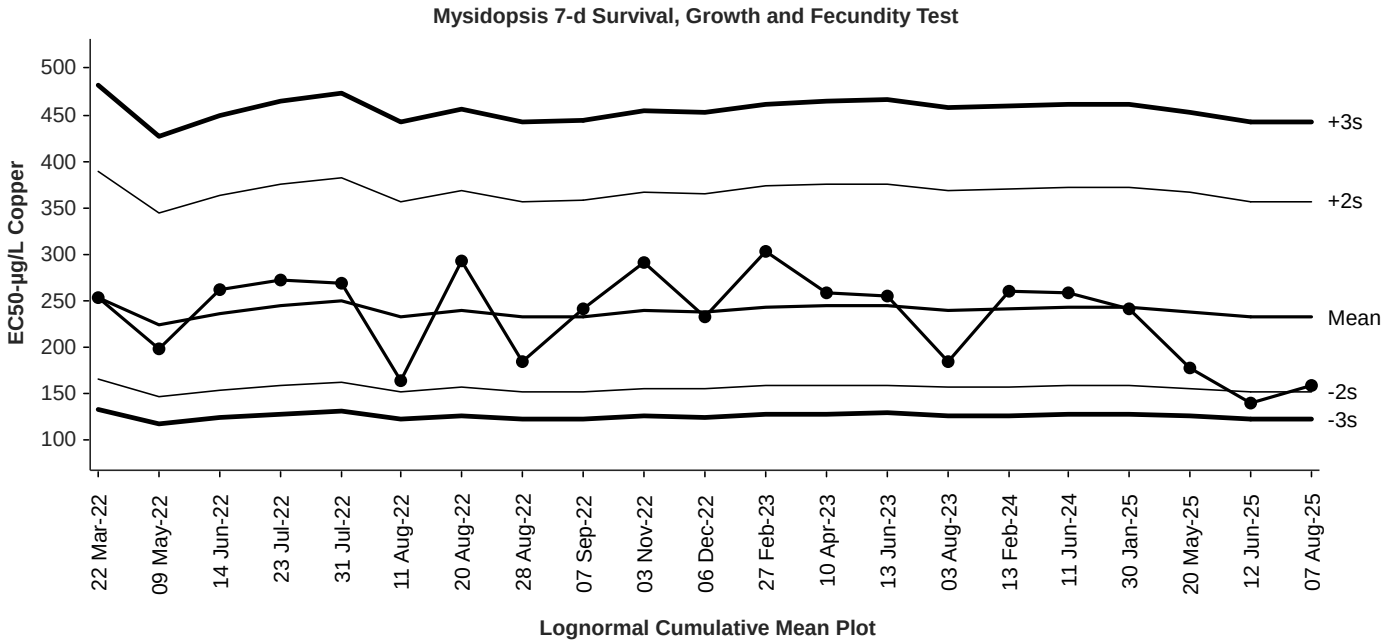
REFERENCE TOXICANT DATA SHEETS

CETIS QC Plot

Report Date: 26 Aug-25 15:51 (1 of 1)

Mysidopsis 7-d Survival, Growth and Fecundity Test — **All Matching Labs**

Test Type:	Growth-Survival-Fec (7d)	**Organism:**	Americamysis bahia	**Material:**	Copper
Protocol:	EPA/821/R-02-014 (2002)	**Endpoint:**	7d Proportion Survived	**Source:**	Reference Toxicant-REF



Lognormal Cumulative Mean Plot

Mean:	232.5	**Count:**	20	**-2s Warning Limit:**	152	**-3s Action Limit:**	122
Sigma:	NA	**CV:**	21.60%	**+2s Warning Limit:**	357	**+3s Action Limit:**	442

Quality Control Data

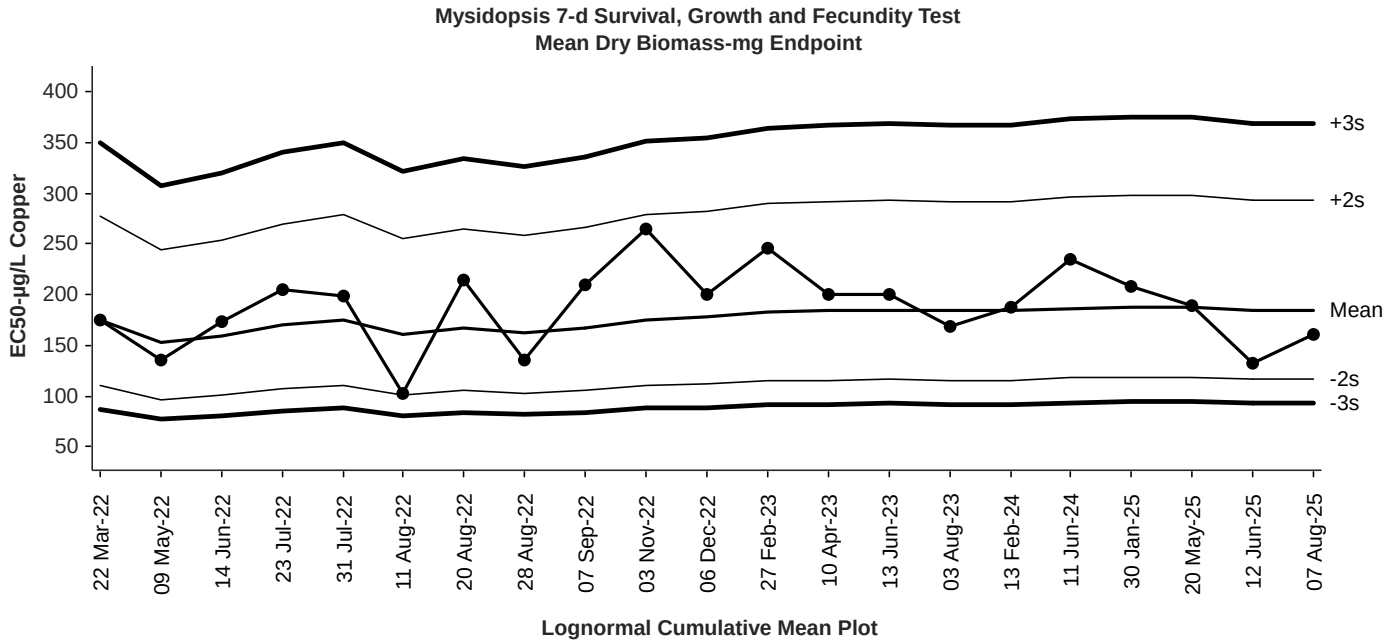
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	Mar	22	14:41	253.6	21.05	0.4053			04-5639-7943	10-6451-7186	EcoAnalysts
2		May	9	16:19	198.5	-34.04	-0.7404			19-7074-6899	02-8778-9883	EcoAnalysts
3		Jun	14	16:43	262.7	30.18	0.5707			18-6733-3888	19-0995-8777	EcoAnalysts
4		Jul	23	15:34	272.6	40.1	0.7442			14-0652-4817	05-1773-8802	EcoAnalysts
5			31	12:16	268.7	36.18	0.6764			10-3271-2251	04-2299-0711	EcoAnalysts
6		Aug	11	13:22	164	-68.52	-1.633			12-4100-9672	17-8127-6600	EcoAnalysts
7			20	10:40	292.7	60.17	1.076			01-4401-6269	16-9819-5130	EcoAnalysts
8			28	13:09	185.4	-47.13	-1.06			04-1946-2277	12-2436-9224	EcoAnalysts
9		Sep	7	13:34	241.5	8.973	0.1771			18-0154-0991	02-5982-4853	EcoAnalysts
10		Nov	3	13:54	292	59.46	1.065			20-7272-6779	07-6122-8250	EcoAnalysts
11		Dec	6	15:51	233.7	1.223	0.02454			08-6777-4627	08-6463-0470	EcoAnalysts
12	2023	Feb	27	15:02	302.5	69.97	1.23			17-9014-8001	16-9229-9852	EcoAnalysts
13		Apr	10	17:30	258.8	26.3	0.5012			04-8759-0090	09-2424-8749	EcoAnalysts
14		Jun	13	18:05	255.1	22.56	0.4332			11-7127-0699	01-4524-1817	EcoAnalysts
15		Aug	3	15:33	184.8	-47.77	-1.076			21-0880-2523	14-9844-3951	EcoAnalysts
16	2024	Feb	13	14:40	260.3	27.81	0.5284			14-4204-2247	03-3460-5424	EcoAnalysts
17		Jun	11	17:13	258.8	26.3	0.5012			06-4568-3295	18-6468-4033	EcoAnalysts
18	2025	Jan	30	13:41	241.5	8.973	0.1771			00-5191-5740	19-0121-5560	EcoAnalysts
19		May	20	16:47	177.6	-54.9	-1.26			12-7933-0764	13-6902-0313	EcoAnalysts
20		Jun	12	14:05	140.3	-92.22	-2.363	(-)		13-9813-6557	01-9464-0633	EcoAnalysts
21		Aug	7	15:20	158.4	-74.16	-1.796			16-3240-2586	15-8097-2790	EcoAnalysts

CETIS QC Plot

Report Date: 26 Aug-25 15:52 (1 of 1)

Mysidopsis 7-d Survival, Growth and Fecundity Test — **All Matching Labs**

Test Type:	Growth-Survival-Fec (7d)	**Organism:**	Americamysis bahia	**Material:**	Copper
Protocol:	EPA/821/R-02-014 (2002)	**Endpoint:**	Mean Dry Biomass-mg	**Source:**	Reference Toxicant-REF



Lognormal Cumulative Mean Plot

Mean:	184.6	**Count:**	20	**-2s Warning Limit:**	116	**-3s Action Limit:**	92.3
Sigma:	NA	**CV:**	23.40%	**+2s Warning Limit:**	293	**+3s Action Limit:**	369

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	Mar	22	14:41	174.8	-9.769	-0.2355			04-5639-7943	19-2364-7596	EcoAnalysts
2		May	9	16:19	134.9	-49.62	-1.356			19-7074-6899	18-1195-6589	EcoAnalysts
3		Jun	14	16:43	172.8	-11.78	-0.2855			18-6733-3888	06-0726-9833	EcoAnalysts
4		Jul	23	15:34	204.5	19.91	0.4437			14-0652-4817	08-4986-5607	EcoAnalysts
5			31	12:16	198	13.4	0.3036			10-3271-2251	19-6237-8378	EcoAnalysts
6		Aug	11	13:22	103.1	-81.51	-2.524	(-)		12-4100-9672	14-2152-6823	EcoAnalysts
7			20	10:40	213.8	29.26	0.6373			01-4401-6269	10-4166-9605	EcoAnalysts
8			28	13:09	136	-48.59	-1.323			04-1946-2277	06-3356-9952	EcoAnalysts
9		Sep	7	13:34	209.5	24.95	0.549			18-0154-0991	06-6736-0182	EcoAnalysts
10		Nov	3	13:54	265.2	80.68	1.571			20-7272-6779	18-6450-7829	EcoAnalysts
11		Dec	6	15:51	199.4	14.83	0.3348			08-6777-4627	17-5306-9671	EcoAnalysts
12	2023	Feb	27	15:02	245.7	61.15	1.239			17-9014-8001	19-6476-3661	EcoAnalysts
13		Apr	10	17:30	199.8	15.2	0.3426			04-8759-0090	06-2698-9039	EcoAnalysts
14		Jun	13	18:05	199.7	15.16	0.3418			11-7127-0699	05-8075-4244	EcoAnalysts
15		Aug	3	15:33	168.2	-16.32	-0.401			21-0880-2523	06-6104-2414	EcoAnalysts
16	2024	Feb	13	14:40	186.9	2.341	0.05458			14-4204-2247	00-0305-3310	EcoAnalysts
17		Jun	11	17:13	235.1	50.56	1.049			06-4568-3295	12-5447-0312	EcoAnalysts
18	2025	Jan	30	13:41	208.2	23.68	0.5229			00-5191-5740	08-1652-3484	EcoAnalysts
19		May	20	16:47	189.7	5.172	0.1197			12-7933-0764	03-3314-6978	EcoAnalysts
20		Jun	12	14:05	133.1	-51.5	-1.417			13-9813-6557	00-5201-4128	EcoAnalysts
21		Aug	7	15:20	161	-23.61	-0.5929			16-3240-2586	12-0558-9580	EcoAnalysts

Mysidopsis 7-d Survival, Growth and Fecundity Test **EcoAnalysts**

Batch ID:	01-3990-2953	**Test Type:**	Growth-Survival-Fec (7d)	**Analyst:**	Michelle Bennett		
Start Date:	07 Aug-25 15:20	**Protocol:**	EPA/821/R-02-014 (2002)	**Diluent:**	Laboratory Seawater		
Ending Date:	14 Aug-25 11:31	**Species:**	Americamysis bahia	**Brine:**	Not Applicable		
Test Length:	6d 20h	**Taxon:**	Malacostraca	**Source:**	EcoAnalysts Florida	**Age:**	7D

Sample ID:	00-7107-0459	**Code:**	R240207.112	**Project:**	Reference Toxicant		
Sample Date:	07 Feb-24	**Material:**	Copper	**Source:**	Reference Toxicant		
Receipt Date:	07 Feb-24	**CAS (PC):**		**Station:**	R240207.112		
Sample Age:	547d 15h	**Client:**	Internal Lab				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
08-7565-3254	7d Proportion Survived	Steel Many-One Rank Sum Test	✓	62.5	125	88.39	11.6%	1
15-3560-1619	Mean Dry Biomass-mg	Bonferroni Adj t Test	✓	62.5	125	88.39	21.6%	1
15-7015-7303	Mean Dry Weight-mg	Wilcoxon/Bonferroni Adj Test		250	>250	---	60.9%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
15-8097-2790	7d Proportion Survived	Linear Interpolation (ICPIN)	✓	EC50	158.4	143.6	168.1	1
12-0558-9580	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)		IC50	161	128.7	194.5	1
08-3017-8824	Mean Dry Weight-mg	Linear Interpolation (ICPIN)		IC50	>250	---	---	1

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
08-7565-3254	7d Proportion Survived	Control Resp	0.975	0.8	<<	Yes	Passes Criteria
15-8097-2790	7d Proportion Survived	Control Resp	0.975	0.8	<<	Yes	Passes Criteria
12-0558-9580	Mean Dry Biomass-mg	Control Resp	0.2295	0.2	<<	Yes	Passes Criteria
15-3560-1619	Mean Dry Biomass-mg	Control Resp	0.2295	0.2	<<	Yes	Passes Criteria
15-3560-1619	Mean Dry Biomass-mg	PMSD	0.2162	0.11	0.37	Yes	Passes Criteria

7d Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	8	0.9750	0.9159	1.0340	0.8000	1.0000	0.0250	0.0707	7.25%	0.00%
62.5		8	0.9750	0.9159	1.0340	0.8000	1.0000	0.0250	0.0707	7.25%	0.00%
125		8	0.6750	0.5506	0.7994	0.4000	0.8000	0.0526	0.1488	22.05%	30.77%
250		8	0.1250	0.0385	0.2115	0.0000	0.2000	0.0366	0.1035	82.81%	87.18%
500		8	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
1000		8	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Mean Dry Biomass-mg Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	8	0.2295	0.2049	0.2541	0.176	0.276	0.01038	0.02937	12.80%	0.00%
62.5		8	0.2495	0.2295	0.2695	0.216	0.282	0.008441	0.02387	9.57%	-8.71%
125		8	0.1593	0.1203	0.1982	0.084	0.208	0.01649	0.04663	29.28%	30.61%
250		5	0.0508	-0.0174	0.119	0.016	0.146	0.02457	0.05493	108.13%	77.86%

Mean Dry Weight-mg Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	8	0.235	0.2176	0.2524	0.208	0.276	0.007348	0.02078	8.84%	0.00%
62.5		8	0.2562	0.2392	0.2733	0.226	0.282	0.007225	0.02044	7.98%	-9.04%
125		8	0.2325	0.2146	0.2504	0.2067	0.26	0.007581	0.02144	9.22%	1.06%
250		5	0.254	-0.08702	0.595	0.08	0.73	0.1228	0.2746	108.13%	-8.09%

Mysidopsis 7-d Survival, Growth and Fecundity Test **EcoAnalysts**

7d Proportion Survived Detail MD5: F5B14E713029AFE86544940DA6C0F173

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	1.0000	0.8000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
62.5		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8000
125		0.8000	0.8000	0.8000	0.8000	0.6000	0.4000	0.6000	0.6000
250		0.0000	0.0000	0.2000	0.2000	0.2000	0.2000	0.2000	0.0000
500		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Mean Dry Biomass-mg Detail MD5: 1748FD91A0F99DCDD8E395DD3B2EA6E1

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.24	0.176	0.232	0.276	0.25	0.228	0.226	0.208
62.5		0.244	0.282	0.236	0.252	0.28	0.26	0.226	0.216
125		0.206	0.208	0.192	0.194	0.126	0.084	0.124	0.14
250		---	---	0.016	0.018	0.146	0.05	0.024	---
500		---	---	---	---	---	---	---	---
1000		---	---	---	---	---	---	---	---

Mean Dry Weight-mg Detail MD5: 2B92409D89412B54C17155514C93E016

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.24	0.22	0.232	0.276	0.25	0.228	0.226	0.208
62.5		0.244	0.282	0.236	0.252	0.28	0.26	0.226	0.27
125		0.2575	0.26	0.24	0.2425	0.21	0.21	0.2067	0.2333
250		---	---	0.08	0.09	0.73	0.25	0.12	---
500		---	---	---	---	---	---	---	---
1000		---	---	---	---	---	---	---	---

7d Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	5/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5
62.5		5/5	5/5	5/5	5/5	5/5	5/5	5/5	4/5
125		4/5	4/5	4/5	4/5	3/5	2/5	3/5	3/5
250		0/5	0/5	1/5	1/5	1/5	1/5	1/5	0/5
500		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
1000		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Mysidopsis 7-d Survival, Growth and Fecundity Test **EcoAnalysts**

Start Date: 07 Aug-25 15:20 **Species:** Americamysis bahia **Sample Code:** R240207.112
End Date: 14 Aug-25 11:31 **Protocol:** EPA/821/R-02-014 (2002) **Sample Source:** Reference Toxicant
Sample Date: 07 Feb-24 **Material:** Copper **Sample Station:** R240207.112

Conc-µg/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Total Weight-mg	Tare Weight-mg	Pan Count	Total Females	Gravid Females	Notes
0	D	1	37	5							5	13.82	12.62	5			
0	D	2	24	5							4	13.18	12.3	4			
0	D	3	17	5							5	13.61	12.45	5			
0	D	4	5	5							5	13.99	12.61	5			
0	D	5	38	5							5	13.85	12.6	5			
0	D	6	21	5							5	13.62	12.48	5			
0	D	7	35	5							5	13.68	12.55	5			
0	D	8	27	5							5	13.55	12.51	5			
62.5		1	46	5							5	13.76	12.54	5			
62.5		2	30	5							5	14.01	12.6	5			
62.5		3	33	5							5	13.73	12.55	5			
62.5		4	13	5							5	13.85	12.59	5			
62.5		5	42	5							5	13.96	12.56	5			
62.5		6	26	5							5	13.86	12.56	5			
62.5		7	44	5							5	13.86	12.73	5			
62.5		8	32	5							4	13.94	12.86	4			
125		1	43	5							4	13.66	12.63	4			
125		2	2	5							4	13.52	12.48	4			
125		3	11	5							4	13.58	12.62	4			
125		4	31	5							4	13.63	12.66	4			
125		5	41	5							3	13.24	12.61	3			
125		6	34	5							2	13	12.58	2			
125		7	18	5							3	13.29	12.67	3			
125		8	9	5							3	13.2	12.5	3			
250		1	23	5							0			0			
250		2	20	5							0			0			
250		3	6	5							1	12.69	12.61	1			
250		4	12	5							1	12.82	12.73	1			

Conc-µg/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Total Weight-mg	Tare Weight-mg	Pan Count	Total Females	Gravid Females	Notes
250		5	10	5							1	12.88	12.15	1			
250		6	22	5							1	12.88	12.63	1			
250		7	40	5							1	12.35	12.23	1			
250		8	45	5							0			0			
500		1	15	5							0			0			
500		2	47	5							0			0			
500		3	4	5							0			0			
500		4	48	5							0			0			
500		5	29	5							0			0			
500		6	14	5							0			0			
500		7	39	5							0			0			
500		8	1	5							0			0			
1000		1	16	5							0			0			
1000		2	8	5							0			0			
1000		3	7	5							0			0			
1000		4	28	5							0			0			
1000		5	19	5							0			0			
1000		6	3	5							0			0			
1000		7	36	5							0			0			
1000		8	25	5							0			0			

EcoAnalysts, Inc.

7 Day Chronic WET Test

Version V.1

GENERAL

Client	Internal
Associated Test	Various
Compound	Copper Chloride
Toxicant	Copper
Test Type	Reference Toxicant
Test type	7 Day Chronic Toxicity with Mysid
Matrix	Liquid
Test Acceptability	≥ 80% average survival of control mysid Average dry weight is 0.20 mg or greater per surviving control mysids
Test Start Date	08/07/25
Test Species	*Americamysis bahia*
Organism Batch	EF080725.02
Organism Acquired	8/7/2025
Organism Acclimation	0
Organism Age	7 days old
Test Protocol	TOX 014 / TOX 099
Test Location	Temp Control Room
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	5
Test Chamber Size	12 oz.
Exposure Volume	250 mL
Feeding Information	375 nauplii/chamber twice per day except Day 7
Test Dissolved Oxygen	> 4
Test Temperature	26 ± 1
Test Salinity	30 ± 2
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters	Min	Max
DO	4	
Temp	25	27
Salinity	28	32
pH	6	9

TEST START TIME/INIT: 1520 LG (CS)

TEST END TIME/INIT: 1131 CS

REFERENCE TOXICANT TEST ID	**LOT #**
R240207.112	BCCH9104

Food Batch ID
311823

CSMM Batch #
82024

Concentrations (µg/L)

1	Control
2	62.5
3	125
4	250
5	500
6	1000
7	.
8	.
9	.

Copy and Past VALUES fr

Treatment	Rep	Chamber
Control	1	29
Control	2	41
Control	3	25
Control	4	24
Control	5	45
Control	6	20
Control	7	3
Control	8	35
62.5	1	7
62.5	2	27
62.5	3	39
62.5	4	26
62.5	5	13
62.5	6	42
62.5	7	14
62.5	8	5
125	1	8
125	2	48
125	3	36
125	4	21
125	5	23
125	6	22
125	7	34
125	8	47
250	1	19
250	2	46
250	3	2
250	4	32
250	5	37
250	6	9
250	7	31
250	8	44
500	1	12
500	2	16
500	3	1
500	4	6
500	5	28
500	6	4
500	7	18
500	8	40
1000	1	15
1000	2	33
1000	3	17
1000	4	43
1000	5	10
1000	6	38
1000	7	30
1000	8	11

EcoANALYSTS, INC.

7 Day Chronic WET Test

v.1 **CLIENT**	Internal	**TEST TYPE**	Reference Toxicant	**PROTOCOL**	TOX 014 / TOX 099
ASSOCIATED TEST	Various	**TEST START DATE**	8/7/25	**TOXICANT**	Copper
REF TOX ID	R240207.112	**TEST END DATE**	8/14/25	**SPECIES**	*Americamysis bahia*
LOT #	BCCH9104	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

Dilution Preparation (Serial dilute by 50%)

$CuCl_2*2H_20$ Stock Solution (µg/L Cu)	Target Stock Solution Conc. (µg/L)	Volume of Diluent (mL)	Amt. of Toxicant (mL)
400,000	1000	4000	10.0
400,000	500	4000	5.0
400,000	250	4000	2.5

Test Dilution Prep

Date	Balance ID	Water Batch ID	Initials	Highest Concentration Prepared	Comments
8/7/2025	7	FSW080525.01	LG	1000	
8/8/2025	7	FSW080525.01	MM	1000	
8/9/2025	7	FSW080425.01	NL	1000	
8/10/2025	7	FSW080525.01	NL	250	
8/11/2025	7	FSW081025.01	TVL	250	
8/12/2025	7	FSW081025.01	TVL	250	
8/13/2025	7	FSW081025.01	CS	250	

Water Quality

			DO (mg/L)	TEMP (°C)	SALINITY	pH
		Concentration (µg/L)	4	25 - 27	30 ± 2	6 - 9
Day 0 (Stock)		Control	7.4	25.1	30	8.0
Date	8/7/2025	62.5	7.4	25.4	30	8.0
Time	1427	125	7.4	25.7	30	8.0
Tech	CS	250	7.4	25.6	30	8.0
Meter #	8	500	7.4	25.8	30	8.0
Feed (initials)	NL	1000	7.4	25.8	30	8.0
Temperature	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Meter #	T42	T42	T42	T42	T42	T43
Old	25.4	25.2	26.3	25.3	25.5	25.8
New	25.6	25.9	25.6	24.9	24.8	25.1
Tech	NL	EM	NL	CS	TVL	KS
Day 7		Control	5.4	24.6	31	7.7
Replicate #	3	62.5	5.8	24.5	31	7.8
Date	8/14/2025	125	6.0	24.5	30	7.8
Time	1042	250	6.4	24.5	30	7.9
Tech	MM	500				
Meter #	10	1000				

Comments

EcoAnalysts, Inc.

v.1

CLIENT	Internal	**TEST TYPE**	Reference Toxicant	**PROTOCOL**	TOX 014 / TOX 099
ASSOCIATED TEST	Various	**TEST START DATE**	8/7/25	**TOXICANT**	Copper
REF TOX ID	R240207.112	**TEST END DATE**	8/14/25	**SPECIES**	*Americamysis bahia*
LOT #	BCCH9104	**MATRIX**	Liquid		

Abbreviation Key:
NB = No Body
FB = Found Body
ST = Stranded

7 Day Chronic Toxicity with Mysid

			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7			
			Date	08/08/25		Date	08/09/25		Date	08/10/25		Date	08/11/25		Date	08/12/25		Date	08/13/25		Date	08/14/25		
			Time	1511		Time	1248		Time	1542		Time	10:20		Time	1450		Time	1259		Time	1131		
			Tech	NL		Tech	NL		Tech	NL		Tech	CS		Tech	TVL/JB		Tech	KS		Tech	CS		
Concentration (µg/L)	**REP**	# Initiated	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Comments
Control	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	4	1		4	0		4	0		4	0		4	0		4	0		4	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	6	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	7	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	8	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
62.5	1	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	2	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	3	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	4	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	5	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	6	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	7	5	5	0		5	0		5	0		5	0		5	0		5	0		5	0		
	8	5	4	0	1NB	4	0		4	0		4	0		4	0		4	0		4	0		
125	1	5	5	0		5	0		4	1		4	0		4	0		4	0		4	0		
	2	5	4	0	1NB	4	0		4	0		4	0		4	0		4	0		4	0		
	3	5	5	0		5	0		4	0	1NB	4	0		4	0		4	0		4	0		
	4	5	4	1		4	0		4	0		4	0		4	0		4	0		4	0		
	5	5	5	0		4	0	1NB	4	0		4	0		3	1		3	0		3	0		
	6	5	4	0	1NB	4	0		3	1		3	0		3	0		2	1		2	0		
	7	5	5	0		5	0		4	0	1NB	3	0	1NB	3	0		3	0		3	0		
	8	5	5	0		5	0		4	1		3	0	1NB	3	0		3	0		3	0		
250	1	5	5	0		4	1		0	4														
	2	5	3	2		1	1	1NB	0	1														
	3	5	2	3		2	0		2	0		1	1		1	0		1	0		1	0		
	4	5	1	4		1	0		1	0		1	0		1	0		1	0		1	0		
	5	5	2	3		1	1		1	0		1	0		1	0		1	0		1	0		
	6	5	3	2		3	0		1	2		1	0		1	0		1	0		1	0		
	7	5	4	1		3	1		1	2		1	0		1	0		1	0		1	0		
	8	5	4	0	1NB	3	1		1	2		0	1											

v.1

CLIENT	Internal	**TEST TYPE**	Reference Toxicant	**PROTOCOL**	TOX 014 / TOX 099
ASSOCIATED TEST	Various	**TEST START DATE**	8/7/25	**TOXICANT**	Copper
REF TOX ID	R240207.112	**TEST END DATE**	8/14/25	**SPECIES**	*Americamysis bahia*
LOT #	BCCH9104	**MATRIX**	Liquid		

Abbreviation Key:
NB = No Body
FB = Found Body
ST = Stranded

7 Day Chronic Toxicity with Mysid

			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7			
			Date	08/08/25		Date	08/09/25		Date	08/10/25		Date	08/11/25		Date	08/12/25		Date	08/13/25		Date	08/14/25		
			Time	1511		Time	1248		Time	1542		Time	10:20		Time	1450		Time	1259		Time	1131		
			Tech	NL		Tech	NL		Tech	NL		Tech	CS		Tech	TVL/JB		Tech	KS		Tech	CS		
Concentration (µg/L)	**REP**	# Initiated	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Alive	Dead	Obs	Comments
500	1	5	1	4		0	1																	
	2	5	0	5																				
	3	5	1	4		0	1																	
	4	5	0	5																				
	5	5	0	5																				
	6	5	2	3		0	2																	
	7	5	0	4	1NB																			
	8	5	0	5																				
1000	1	5	0	5																				
	2	5	0	5																				
	3	5	1	4		0	1																	
	4	5	0	5																				
	5	5	0	5																				
	6	5	0	5																				
	7	5	0	5																				
	8	5	1	4		0	1																	
Feed (Init.)	AM		MM			NL			NL			CS			CS			EM			NONE			
375 nauplii/chamber twice per day except Day 7	PM		NL			NL			NL			KS			KR			KS			NONE			

ECO ANALYSTS, INC.

v.1

CLIENT	Internal	**DATE RECEIVED**	Reference Toxicant	**PROTOCOL**	TOX 014 / TOX 099
ASSOCIATED TEST	Various	**TEST START DATE**	8/7/25	**TOXICANT**	Copper
REF TOX ID	R240207.112	**TEST END DATE**	8/14/25	**SPECIES**	*Americamysis bahia*
LOT #	BCCH9104	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

Concentration (µg/L)	REP	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count	Comments
Control	1	1	12.62	13.82	5	
	2	2	12.3	13.18	4	
	3	3	12.45	13.61	5	
	4	4	12.61	13.99	5	
	5	5	12.6	13.85	5	
	6	6	12.48	13.62	5	
	7	7	12.55	13.68	5	
	8	8	12.51	13.55	5	
62.5	1	9	12.54	13.76	5	
	2	10	12.6	14.01	5	
	3	11	12.55	13.73	5	
	4	12	12.59	13.85	5	
	5	13	12.56	13.96	5	
	6	14	12.56	13.86	5	
	7	15	12.73	13.86	5	
	8	16	12.86	13.94	4	
125	1	17	12.63	13.66	4	
	2	18	12.48	13.52	4	
	3	19	12.62	13.58	4	
	4	20	12.66	13.63	4	
	5	21	12.61	13.24	3	
	6	22	12.58	13	2	
	7	23	12.67	13.29	3	
	8	24	12.5	13.2	3	
250	1	25	12.67			
	2	26	12.43			
	3	27	12.61	12.69	1	
	4	28	12.73	12.82	1	
	5	29	12.15	12.88	1	
	6	30	12.63	12.88	1	
	7	31	12.23	12.35	1	
	8	32	12.58			

ECO ANALYSTS, INC.

7 Day Chronic WET Test

V.1

CLIENT	Internal	**DATE RECEIVED**	Reference Toxicant	**PROTOCOL**	TOX 014 / TOX 099
ASSOCIATED TEST	Various	**TEST START DATE**	8/7/25	**TOXICANT**	Copper
REF TOX ID	R240207.112	**TEST END DATE**	8/14/25	**SPECIES**	*Americamysis bahia*
LOT #	BCCH9104	**MATRIX**	Liquid		

7 Day Chronic Toxicity with Mysid

Concentration (µg/L)	REP	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count	Comments
500	1	33	12.65			
	2	34	12.62			
	3	35	12.15			
	4	36	12.57			
	5	37	12.51			
	6	38	12.73			
	7	39	12.72			
	8	40	13.17			
1000	1	41	12.61			
	2	42	13.06			
	3	43	12.55			
	4	44	13.59			
	5	45	12.52			
	6	46	12.67			
	7	47	12.55			
	8	48	12.69			

	Oven Event 1	Oven Event 2
Oven ID:	Beelzebub	Beelzebub
Date/Time/Initials In Oven:	8/3/25 1640 EM/KS	8/14/25 1206 CS
Oven Temp °C:	60.00	100.00
Date/Time/Initials Out Oven into Dessicator:	8/4/25 1125 DM	8/15/25 934 MM
Date/Time/Initials Weighed:	8/11/25 16:00 KS	8/22/25 1636 EM
Balance ID:	3.00	3.00

ORGANISM RECEIPT LOG

Date:	Time:	Batch No.
8/7/25	1050	EF080725.02

Organism: Americamysis bahia

Source / Supplier: EcoAnalysts Florida

No. Ordered:	No. Received:	Source Batch: Collection date, hatch date etc.):
630+	630+	7/31/25

Condition of Organisms:	Approximate Size or Age: (Days from hatch, life stage, size class, etc.):
Good	N7 days old
Shipper: FedEx	**B of L (Tracking No.)** 883356655610
Condition of Container: Good	**Received By:** CS

Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	6.4	23.9	31	7.2	0	0	CS

*if >10% contact lab manager

Notes:

ECOANALYSTS, INC.
11842 Research Circle
Alachua, FL 32615
386.462.7889

MS

Organism Information

Species	*Americamysis bahia (formerly Mysidopsis bahia)*		
Release Date	**7/31/2025**	Life Stage	**Juvenile**
Quantity	**630+**	Lot Number	**MS·7669**
	Aquatic Indicators, St. Augustine, FL		
Source of Species	☑ Laboratory Cultured, ☐ Hatchery Reared, ☐ Field Collected, ☐ Other		

Culture / Holding Water Quality

Conductance	NA	Salinity (‰)	**30 ppt**
Alkalinity	50-70 mg $CaCO_3$/L	Hardness	NA
Temp (°C)	25±1°C	Other: _____	
Feeding	*Artemia* nauplii	Photoperiod	16L:8D

Shipment Information

Sold to	PGB
Carrier	Fedex
Tracking Number	883356655610
Shipment Date	8/6/25

Laboratory receipt "Stamp" goes here

APPENDIX B

CHAIN-OF-CUSTODY AND SAMPLE RECEIPT FORMS

SAMPLE RECEIPT

Client:	Client ID:	Lab ID:	Renewals:	
Lost Clean Water Alliance	Final Effluent #2	P250808.02	P250812.49 ①	
Project:	Final Effluent #3		P250812.49	
2025 NPDES	Final Effluent #4			P250813.15
Date/Time Received:		8/8/25 1230	8/12/25 1154	8/13/25 1230
Airbill #:		8834 0064 9371	8834 7101 7729	8835 0627 4821
Shipper Tracking Information Kept for Records: (Y/N/NA)		N	N	N
Collection Date/Time:		8/7/25 0700	8/11/25 0700	8/12/25 0630
Sample Holding Time (must be ≤36 hours at test initiation)		✓	✓	✓
Condition of Shipping Container:		Good	Good	Good
Type and Capacity of Sample Container:		20L cubi	20L cubi	20L cubi
Total Sample Volume (L):		~19L	~20L	~20L
Condition of Sampling Container:		Good	Good	Good
Sample Container Appropriate: (Y/N)		Y	Y	Y
Custody Seals Intact: (Intact/Broken/Not Present)		Intact	Intact	Intact
Frozen Wet or Blue Ice Present During Shipment/Transport: (Y/N)		Ice/water mix	Ice/water mix	Ice/water mix
Sampler's Name Present on COC Form: (Print Name/Not Present)		Meghan Feuk	Meghan Feuk	Meghan Feuk
Color:		yellow	yellow	yellow

TAKE THE FOLLOWING MEASUREMENTS UPON ARRIVAL

LAB ID	Meter #	Temp. (°C) (0-6°C) *	Meter #	Dissolved Oxygen (mg/L)	Meter #	pH	Meter #	Cond. (µS/cm)	Meter #	Sal. (ppt)	Hardness (mg CaCO3/L)	Alkalinity (mg CaCO3/L)	Total Chlorine (mg/L)	Total NH3 (mg/L)	Tech
P250808.02	T33	0.0	8	9.5	8	7.4	8	557	8	0.252	—	—	0.04	0.0	DM
P250812.49	T33	0.6	7	8.4	7	7.8	7	567	7	0.250	126	106	0.04	④ 0.0	KS/EM
P250813.15	T33	0.3	9	9.8	9	7.5	9	560	9	0.273	—	—	0.01	② ④ 0.0	CS

*Notify project manager or study director of temperatures above 6°C or ≥36 hours holding time. Client must be notified ASAP.

If there are sample receipt problems, complete the following:	
Reason for unacceptability:	
Name of Client Contact:	Contacted by:
Client Response and/or Action to be Taken:	Date Action Taken:

① WC-EM 8/12/25 ② Preserved NH3 ④ NH3 run 8/14/25-TH 8/14/25

NH3 preserved for later analysis-CS 8/13/25

V.1

CHAIN OF CUSTODY

EcoAnalysts, Inc.

Destination: EcoAnalysts	Sample Originator (Organization): LOTT CLEAN WATER ALLIANCE	Report Results To: MEGHAN FEUK
Destination Contact: Michelle Bennett	Samplers Name: MEGHAN FEUK	Contact Name:
Date: 8/6/25	Address: 500 ADAMS ST NE OLYMPIA, WA 98501	Address: 500 ADAMS ST NE OLYMPIA, WA 98501
Project Name:	Phone: 360 528 5760	Phone: 360 528 5760
Contract/PO #:	Email: meghanfeuk@lottcleanwater.org	Email: meghanfeuk@lottcleanwater.org

Ship Samples to:

EcoAnalysts, Inc.
4770 NE View Drive
Port Gamble, WA 98364

No.	Sample ID	Volume	Grab Sample		Composite Sample				Preservation	Analyses: Chronic Mysid	Analyses: Chronic Topsmelt	Analyses	Analyses	Analyses	Analyses	Analyses	Invoicing To: / Comments or Special Instructions: Sample Temp Upon Receipt	Lab ID
			Date	Time	Start Date	Start Time	End Date	End Time										
1	FINAL EFFLUENT #1	20L	—	—	8/5/25	0700	8/6/25	0600	ice (wet)	X	X						0	P2508607.02
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		

Relinquished by:	Received by:	Relinquished by:	Received by:
Print Name: MEGHAN FEUK	Print Name: Kaylie Smith	Print Name:	Print Name:
Signature:	Signature:	Signature:	Signature:
Affiliation: LOTT	Affiliation: EcoAnalysts	Affiliation:	Affiliation:
Date/Time: 8/6/25 0830	Date/Time: 080725 1120	Date/Time:	Date/Time:

Small hole in cubie found upon checkin
-KS/MS
8/07/25

Sample not used for testing. MB 8/26/25

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CHAIN OF CUSTODY

ECO ANALYSTS, INC.

Destination: EcoAnalysts	Sample Originator (Organization): LOTT CLEAN WATER ALLIANCE	Report Results To: MEGHAN FEUK
Destination Contact: Michelle Bennett	Samplers Name: MEGHAN FEUK	Contact Name:
Date: 8/7/25	Address: 500 ADAMS ST NE OLYMPIA, WA 98501	Address: 500 ADAMS ST NE OLYMPIA, WA 98501
Project Name:	Phone: 360 528 5760	Phone: 360 528 5760
Contract/PO #:	Email: meghanfeuk@lottcleanwater.org	Email: meghanfeuk@lottcleanwater.org

Ship Samples to:

EcoAnalysts, Inc.
4770 NE View Drive
Port Gamble, WA 98364

No.	Sample ID	Volume	Grab Sample Date	Grab Sample Time	Composite Sample Start Date	Composite Sample Start Time	Composite Sample End Date	Composite Sample End Time	Preservation	Analyses: Chronic Mysid	Analyses: Chronic Topsmelt	Analyses	Analyses	Analyses	Analyses	Analyses	Analyses	Sample Temp Upon Receipt	Lab ID
1	FINAL EFFLUENT #2 20L		—	—	8/6/25	0800	8/7/25	0700	ice	X	X							0.0C	P250800.02
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			

Invoicing To:

Comments or Special Instructions:

Relinquished by:	Received by:	Relinquished by:	Received by:
Print Name: MEGHAN FEUK	Print Name: Danielle Mulligan	Print Name:	Print Name:
Signature:	Signature: Danielle Mulligan	Signature:	Signature:
Affiliation: LOTT	Affiliation: EcoAnalysts	Affiliation:	Affiliation:
Date/Time: 8/7/25 1230	Date/Time: 8/8/25 1230	Date/Time:	Date/Time:

Version 1.1, Effective 6/29/17

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CHAIN OF CUSTODY

ECO ANALYSTS, INC.

Destination: EcoAnalysts	Sample Originator (Organization): LOTT CLEAN WATER	Report Results To: MEGHAN FEUK
Destination Contact: Michelle Bennett	Samplers Name: MEGHAN FEUK	Contact Name: MEGHAN FEUK
Date: 8/11/25	Address: 500 ADAMS ST NE OLYMPIA, WA 98501	Address: 500 ADAMS ST NE OLYMPIA, WA 98501
Project Name:	Phone: 360 528 5760	Phone: 360 528 5760
Contract/PO #:	Email: meghanfeuk@lottcleanwater.org	Email: meghanfeuk@lottcleanwater.org

Ship Samples to:

EcoAnalysts, Inc.
4770 NE View Drive
Port Gamble, WA 98364

Invoicing To: MARK PETRIE

Comments or Special Instructions:

No.	Sample ID	Volume	Grab Sample Date	Grab Sample Time	Composite Sample Start Date	Composite Sample Start Time	Composite Sample End Date	Composite Sample End Time	Preservation	Analyses: Chronic Toxicity	Analyses: Chronic Mysid	Sample Temp Upon Receipt	Lab ID
1	FINAL EFFLUENT #3	20L	~~8/10/25~~		8/10/25	0800	8/11/25	0700	notice	X	X	0.6°C	P250812.49
2													
3													
4													
5													
6													
7													
8													
9													
10													

Relinquished by:	Received by:	Relinquished by:	Received by:
Print Name: MEGHAN FEUK	Print Name: Elyse Misola	Print Name:	Print Name:
Signature:	Signature:	Signature:	Signature:
Affiliation: LOTT	Affiliation: EcoAnalysts	Affiliation:	Affiliation:
Date/Time: 8/11/25 1000	Date/Time: 8/12/25 1154	Date/Time:	Date/Time:

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CHAIN OF CUSTODY

ECO ANALYSTS, INC.

Destination: EcoAnalysts	Sample Originator (Organization): LOTT	Report Results To:
Destination Contact: Michelle Bennett	Samplers Name: MEGHAN FEUK	Contact Name: same
Date:	Address: 500 ADAMS ST NE	Address:
Project Name:	Phone: 360 528 5760	Phone:
Contract/PO #:	Email:	Email:

Ship Samples to:

EcoAnalysts, Inc.
4770 NE View Drive
Port Gamble, WA 98364

Invoicing To:

Comments or Special Instructions:

No.	Sample ID	Volume	Grab Sample Date	Grab Sample Time	Composite Sample Start Date	Composite Sample Start Time	Composite Sample End Date	Composite Sample End Time	Preservation	Analyses: Chronic Mysid	Analyses: Chronic Topsmelt	Sample Temp Upon Receipt	Lab ID
1	FINAL EFFLUENT #4 2a				8/11/25	0730	8/12/25	0630	metrice	X	X	0.3°C	P250813.15
2													
3													
4													
5													
6													
7													
8													
9													
10													

Relinquished by:	Received by:	Relinquished by:	Received by:
Print Name: Meghan Feuk	Print Name: Elyse Misola	Print Name:	Print Name:
Signature:	Signature:	Signature:	Signature:
Affiliation: LOTT	Affiliation: EcoAnalysts	Affiliation:	Affiliation:
Date/Time: 8/12/25 0900	Date/Time: 8/13/25 1230	Date/Time:	Date/Time:

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