



# Bio-Aquatic Testing, Inc.



TCEQ TNi Accredited

**Natural Energy Laboratory of Hawaii Authority  
SSW-28 Sump**

**Chronic Biomonitoring Report**

**97321**

*Americamysis bahia*  
*Menidia beryllina*

**May 02, 2025**

Approved by: *Johnny Reed*  
Lab director

*Bio-Aquatic Testing, Inc. ♦ 2501 Mayes Rd. Ste. 100 ♦ Carrollton, Texas ♦ 75006*

## TABLE OF CONTENTS

TOXICITY TEST REPORT	3
SURVIVAL TEST SUMMARY	6
STATISTICAL & CHEMICAL ANALYSIS	Appendix A
REFERENCE TOXICANTS	Appendix B
LITERATURE REFERENCES	Appendix C
CHAIN-OF-CUSTODY SHEETS	Appendix D

**Unless otherwise noted in the body of the report, all data reported in this document are in compliance with TNI standards and apply only to the samples referenced within. This report document may not be edited or reproduced in part or in full by any other entity, unless Bio-Aquatic Testing, Inc. issues written approval.**

**\*HAND-WRITTEN RAW DATA TABLES ARE AVAILABLE UPON REQUEST**

**BIO-AQUATIC TESTING, INC.**

2501 Mayes Road, Suite 100

Carrollton, Texas 75006

Tel: (972) 242-7750

Fax: (972) 242-7749

**TOXICITY TEST REPORT - Chronic**

---

Client: Natural Energy Laboratory of Hawaii Authority

Facility: Hawaii Ocean Science Technology Park

Permit No. N/A

Sample:

SSW-28 Sump

Laboratory Number:

97321

Date:

May 02, 2025

---

**SAMPLE COLLECTION:**

A grab sample from the Natural Energy Laboratory of Hawaii Authority, SSW-28 Sump, was transported to Bio-Aquatic Testing on May 02, 2025. The sample was collected from the 28" surface seawater sump at the northern pump station by facility personnel.

The sample was analyzed for total residual chlorine using the Hanna Ion Specific Meter #711 and contained <0.10 mg/L. The sample and laboratory dilution water pH, temperature, salinity, and dissolved oxygen data were collected daily.

**TEST PROCEDURES:**

*Americamysis bahia*

**EPA METHOD: 1007**

The seven-day Chronic *Americamysis bahia* survival and growth test was initiated at 14:30 hours on May 02, 2025. One concentration of 100% was prepared utilizing synthetic water as dilution water. The test was set up with 266mL plastic cups containing 200mL of test solution or control dilution water. Each concentration consisted of five replicate cups with five organisms each, giving a total of 25 (twenty-five) per treatment. The control was run concurrently with the test. Test organisms were 7-day old laboratory cultured juveniles. Juveniles were randomly introduced into test solutions and controls. The number of surviving organisms, and water quality parameters in the old test solutions, were recorded after each 24-hour period. Water quality parameters were again measured after the test was renewed with fresh solutions. Surviving organisms in each test chamber were fed freshly hatched brine shrimp two times per day. The test proceeded for seven days.

At the end of the test, all organisms were sacrificed, dried, and weighed. The test ended at 12:40 hours on May 09, 2025. Survival and growth (weight) data were statistically ( $p=0.05$ ) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

## SURVIVAL:

*Americamysis bahia*

The Equal and Unequal variance t-test performed on survival test data demonstrated no statistically significant differences between the control and the sample concentration tested.

**LOEC: Not Calculable (Q)**

**NOEC: 100%**

## GROWTH:

*Americamysis bahia*

The *Americamysis bahia* growth data were normally distributed at the alpha level of 0.01 (13.277) using the Chi-square test for normality. Using the Equal and Unequal variance t-test on *Americamysis bahia* growth data demonstrated no statistically significant differences between the control and the sample concentration tested.

**LOEC: Not Calculable (Q)**

**NOEC: 100%**

## TEST PROCEDURES:

*Menidia beryllina*

EPA METHOD: 1006

The seven-day Chronic *Menidia beryllina* survival and growth test was initiated at 14:24 hours on May 02, 2025. One concentration of 100% was prepared utilizing synthetic water as dilution water. The test was set up with 650mL plastic cups containing 600mL of test solution as test chambers. The test organisms were initiated in synthetic lab water 24 hours before the test began. Each concentration consisted of three replicate chambers containing eight laboratory-cultured larvae each, giving a total of 24 (twenty-four) per treatment. The control was run concurrently with the test. Test organisms were laboratory cultured *Menidia beryllina* between seven and eleven days old. Juveniles were randomly introduced into test solutions and controls. The number of surviving juveniles, and water quality parameters in the old test solutions were recorded after each 24-hour period. Water quality parameters were again measured after the test was renewed with fresh solutions. Surviving organisms in each test chamber were fed freshly hatched brine shrimp two times per day. The test proceeded for seven days.

At the end of the test, all organisms were sacrificed, dried, and weighed. The test ended at 13:15 hours on May 09, 2025. Survival and growth (weight) were statistically ( $p=0.05$ ) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

#### SURVIVAL:

*Menidia beryllina*

The Equal and Unequal variance t-test performed on survival test data demonstrated no statistically significant differences between the control and the sample concentration tested.

**LOEC: Not Calculable (Q)**

**NOEC: 100%**

#### GROWTH:

*Menidia beryllina*

The *Menidia beryllina* growth data were normally distributed at the alpha level of 0.01 (0.900) using Shapiro Wilk's test for normality. Using the Equal and Unequal variance t-test on *Americamysis bahia* growth data demonstrated no statistically significant differences between the control and the sample concentration tested.

**LOEC: Not Calculable (Q)**

**NOEC: 100%**

**BIO-AQUATIC TESTING, INC.**  
**TOXICITY TEST**

**Chronic**     *Americamysis bahia*

**Client:** Natural Energy Laboratory of Hawaii Hawaii Ocean Science and Technology Park

**Lab ID:** 97321

**Permit Number:** N/A

**Test Temperature (oC):** 25 ± 1

**Sample Type:** Grab

**Outfall Name:** SSW-28 Sump

**Photo Period:** 16 Hours Light  
8 Hours Dark

**Receiving Water Name:**

**Begin Date:** 5/2/2025

Test Start Time: 14:30

Test End Time: 12:40

**End Date:** 5/9/2025

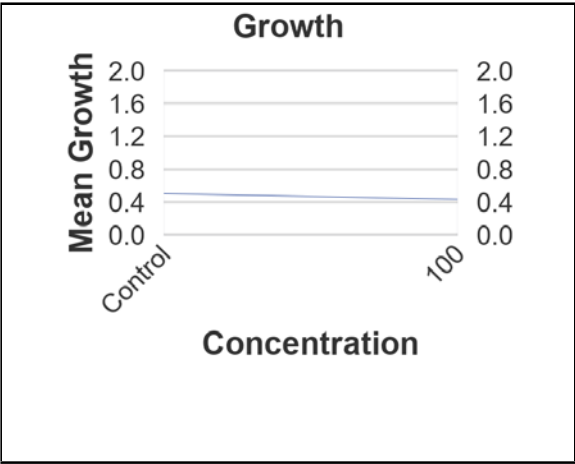
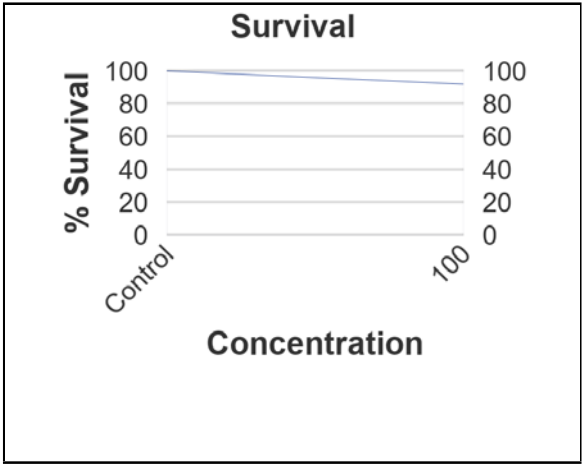
**SURVIVAL**

Effluent Con. %		Number of Alive								Avg% Surv.
		5/2	5/3	5/4	5/5	5/6	5/7	5/8	5/9	
Control	A	5	5	5	5	5	5	5	5	100.0%
	B	5	5	5	5	5	5	5	5	
	C	5	5	5	5	5	5	5	5	
	D	5	5	5	5	5	5	5	5	
	E	5	5	5	5	5	5	5	5	
100	A	5	5	5	5	5	5	5	5	92.0%
	B	5	5	4	4	4	4	4	4	
	C	5	5	5	5	5	4	4	4	
	D	5	5	5	5	5	5	5	5	
	E	5	5	5	5	5	5	5	5	
	A									
	B									
	C									
	D									
	E									
	A									
	B									
	C									
	D									
	E									

BIO-AQUATIC TESTING, INC.

Effluent Con. %	Number Of Alive								Avg% Surv.
	5/2	5/3	5/4	5/5	5/6	5/7	5/8	5/9	
A									
B									
C									
D									
E									

Concentration Response Relationships



# BIO-AQUATIC TESTING, INC.

Chronic

Americamysis bahia SURVIVAL

Lab ID: 97321

Client: Natural Energy Laboratory of Hawaii Facility SSW-28 Sump

Outfall:  
Sample Type Grab

TEST INSTRUCTIONS: Mysid test is Abbreviated Reps (only need 5 NOT 8)

5 of 5

Culture No. : ARO-25-115

Photo Period: 16hr light, 8hr dark

RANDOMIZATION: SC-5

1

Dilution: Control

100

	DATE/TIME/ TECHNICIAN	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr	5-2-25 AB 1430	5	—	—	—	—	8/5	—	—	—	—										
24Hr	5-3-25 1031 M	5	—	—	—	—	5	—	—	—	—										
48Hr	5-4-25 SG 0940	5	—	—	—	—	5	4	5	—	—										
72Hr	5-5-25 1222 AM	5	—	—	—	—	5	4	5	—	—										
96Hr	5-6-25 AB 1139	5	—	—	—	—	5	4	5	—	—										
5 days	5-7-25 OG 0813	5	—	—	—	—	5	4	4	5	5										
6 days	5-8-25 1320	5	—	—	—	—	5	4	4	5	5										
7 days	5-9-25 5 1240	5	—	—	—	—	5	4	4	5	5										

Dilution:

	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr																				
24Hr																				
48Hr																				
72Hr																				
96Hr																				
5 days																				
6 days																				
7 days																				



# BIO-AQUATIC TESTING, INC.

Chronic

Americamysis bahia SURVIVAL

Lab ID: 97321

Client: Natural Energy Laboratory of Hawaii Facility SSW-28 Sump

Outfall:

Sample Type Grab

TEST INSTRUCTIONS: Mysid test is Abbreviated Reps (only need 5 NOT 8)

## Test Temperatures

	0Hr	24Hr	48Hr	72Hr	96Hr	5 days	6 days	7 days
	new	old / new	old / new	old / new	old / new	old / new	old / new	old
Control	<del>24.8</del>	<del>25.5</del> <del>25.1</del>	<del>25.3</del> <del>25.3</del>	<del>25.8</del> <del>25.8</del>	<del>25.4</del> <del>25.4</del>	<del>25.5</del> <del>25.0</del>	<del>25.6</del> <del>25.2</del>	<del>25.2</del>
100	<del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del>
	<del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del>
	<del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del>
	<del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del>
	<del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del>
	<del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del>
	<del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del>
	<del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del>
	<del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del> <del>1</del>	<del>1</del>
TIME/DATE TECH	5-2-25 AB 1430	5-3-25 1033 m	5-4-25 SG 0940	5-5-25 1222 AL	5-6-25 AB 1139	5-7-25 M 0845	5-8-25 1330	5-9-25 5-124
IR GUN ID #	013	013	013	013	013	017	013	013

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

*Chronic Americamysis bahia*Client: Natural Energy Laboratory of Hawaii Ocean Science and Technology Park

Lab ID: 97321

Permit Number: N/A

Sample Type: Grab

Outfall Name: SSW-28 Sump

Receiving Water Name:

## Synthetic

100

	ON	SN	Wt.	Avg.	SN Avg.
A	5	5	2.590	0.518	0.518
B	5	5	2.680	0.536	0.536
C	5	5	2.590	0.518	0.518
D	5	5	2.350	0.470	0.470
E	5	5	2.400	0.480	0.480

Mean	C.V. %
0.504	5.56

SN Mean	SN C.V. %
0.504	5.6

	ON	Wt.	Avg.
A	5	2.430	0.486
B	5	1.920	0.384
C	5	1.660	0.332
D	5	2.900	0.580
E	5	2.010	0.402

Mean	C.V. %
0.437	22.29

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

\* = spilled cup

Note: ON stands for original number per replicate, while SN refers to the number surviving after test completion.

# BIO-AQUATIC TESTING, INC. TOXICITY TEST

**Chronic**

**Americamysis bahia**

Lab ID: **97321**

Client: Natural Energy Laboratory of Hawaii - SSW-28 Sump

Balance: Radwag BAL-007

Begin Date: 5/2/2025

End Date: 5/9/2025

Organism: Americamysis bahia

Analyst: JG

Date/Time placed in Oven: 05/09/2025 | 1400

Weigh Date: 05/12/2025

Date/Time removed from Oven: 05/10/2025 | 1400

**Control**

**100 %**

	Qty.	Wt.
A	5	2.590
B	1	2.180
C		2.590
D		2.350
E		2.400
F		
G		
H		

	Qty.	Wt.
A	5	2.430
B	4	1.720
C	4	1.660
D	5	2.900
E	5	2.010
F		
G		
H		

	Qty.	Wt.
A		
B		
C		
D		
E		
F		
G		
H		

	Qty.	Wt.
A		
B		
C		
D		
E		
F		
G		
H		

	Qty.	Wt.
A		
B		
C		
D		
E		
F		
G		
H		

	Qty.	Wt.
A		
B		
C		
D		
E		
F		
G		
H		

	Qty.	Wt.
A		
B		
C		
D		
E		
F		
G		
H		

	Qty.	Wt.
A		
B		
C		
D		
E		
F		
G		
H		

	Qty.	Wt.
A		
B		
C		
D		
E		
F		
G		
H		

**Chronic** *Menidia beryllina*Client: Natural Energy Laboratory of Hawaii Hawaii Ocean Science and Technology

Lab ID: 97321

Permit Number: N/A

Test Temperature (oC): 25 ± 1

Outfall Name: SSW-28 Sump

Sample Type: Grab

Photo Period: 16 Hours Light  
8 Hours Dark

Receiving Water Name:

Test Start Time: 14:24

Test End Time: 13:15

Begin Date: 5/2/2025

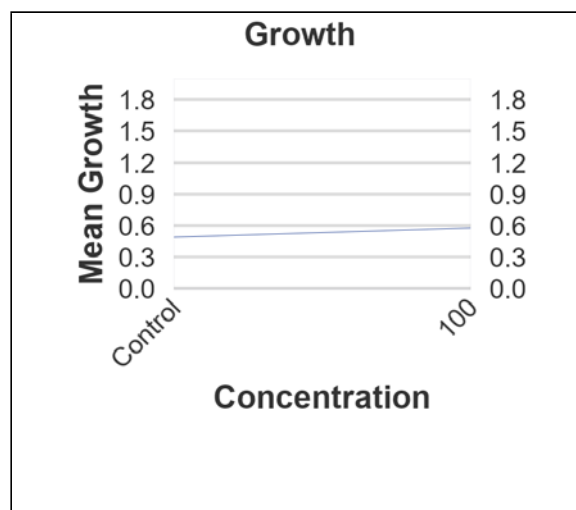
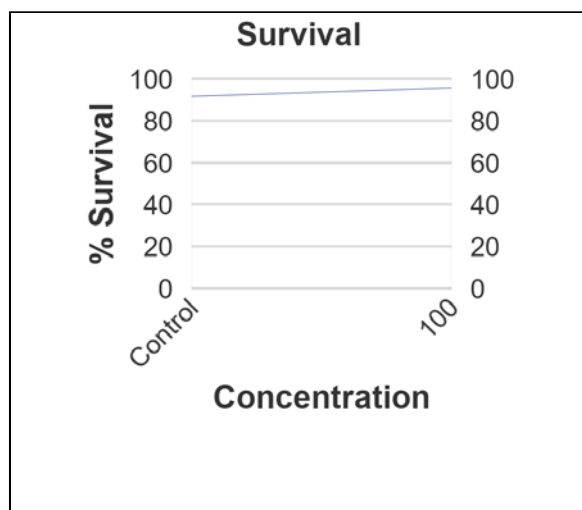
End Date: 5/9/2025

**SURVIVAL**

Effluent Concentration	Number Of Alive								Avg% Surv.
	5/2	5/3	5/4	5/5	5/6	5/7	5/8	5/9	
Control	A	8	8	7	7	7	7	7	91.7%
	B	8	8	8	8	8	8	8	
	C	8	8	8	8	7	7	7	
	D								
	E								
100	A	8	8	8	8	8	8	8	95.8%
	B	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	7	
	D								
	E								
	A								
	B								
	C								
	D								
	E								
	A								
	B								
	C								
	D								
	E								

Effluent Concentration	Number Of Alive								Avg% Surv.
	5/2	5/3	5/4	5/5	5/6	5/7	5/8	5/9	
A									
B									
C									
D									
E									

### Concentration Response Relationships



# BIO-AQUATIC TESTING, INC.

Chronic

Menidia beryllina SURVIVAL

Lab ID: **97321**

Client: Natural Energy Laboratory of Hawaii Facility: SSW-28 Sump

Outfall:  
Sample Type: Grab

TEST INSTRUCTIONS: Mysid test is Abbreviated Reps (only need 5 NOT 8)

3 of 8

Culture No. : MN-25-108

Photo Period: 16hr light, 8hr dark

RANDOMIZATION:

Dilution:		Control					100														
	DATE/TIME/TECHNICIAN	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr	1424 AB	8	—	—	—	—	8	—	—	—	—										
	5-2-25	8	—	—	—	—	8	—	—	—	—										
24Hr	5-3-25	8	—	—	—	—	8	—	—	—	—										
	1035 M	8	—	—	—	—	8	—	—	—	—										
48Hr	5-4-25	7	8	8	—	—	8	—	—	—	—										
	86 0945	7	8	8	—	—	8	—	—	—	—										
72Hr	5-5-25	7	8	8	—	—	8	—	—	—	—										
	1219 ALK	7	8	8	—	—	8	—	—	—	—										
96Hr	5-6-25	7	8	7	—	—	8	—	—	—	—										
	1142 AB	7	8	7	—	—	8	—	—	—	—										
5 days	5-7-25	7	8	7	—	—	8	—	—	—	—										
	5-0946	7	8	7	—	—	8	—	—	—	—										
6 days	5-8-25	7	8	7	—	—	8	—	—	—	—										
	09 1320	7	8	7	—	—	8	—	—	—	—										
7 days	5-9-25	8*	8	7	—	—	8	8	7	—	—										
	15-1315	8*	8	7	—	—	8	8	7	—	—										

Dilution: \* counted 3

Dilution:																					
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr																					
24Hr																					
48Hr																					
72Hr																					
96Hr																					
5 days																					
6 days																					
7 days																					

# BIO-AQUATIC TESTING, INC.

Chronic Menidia beryllina SURVIVAL

Lab ID: 97321

Client: Natural Energy Laboratory of Hawaii Facility SSW-28 Sump

Outfall:  
Sample Type: Grab

TEST INSTRUCTIONS: Mysid test is Abbreviated Reps (only need 5 NOT 8)

## Test Temperatures

	0Hr	24Hr	48Hr	72Hr	96Hr	5 days	6 days	7 days
	new	old / new	old / new	old / new	old / new	old / new	old / new	old
Control	24.5	25.3	25.0	25.9	24.8	25.5	25.0	25.2
100	1	1	1	1	1	1	1	1
TIME/DATE TECH	5-2-25 081425	5-3-25 1041	5-4-25 080945	5-5-25 1219	5-6-25 08142	5-7-25 0846	5-8-25 1326	5-9-25 131
IR GUN ID #	013	013	013	013	013	013	013	013

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

Chronic *Menidia beryllina*Client: Natural Energy Laboratory of Hawaii Ocean Science and Technology

Lab ID: 97321

Permit Number: N/A

Sample Type: Grab

Outfall Name: SSW-28 Sump

Receiving Water Name:

## Synthetic

100

	ON	SN	Wt.	Avg.	SN Avg.
A	8	7	3.840	0.480	0.549
B	8	8	3.980	0.498	0.498
C	8	7	3.960	0.495	0.566
D					
E					

Mean C.V. %

0.491	1.9
-------	-----

SN Mean SN C.V. %

0.537	6.6
-------	-----

	ON	Wt.	Avg.
A	8	4.740	0.593
B	8	4.690	0.586
C	8	4.500	0.563
D			
E			

Mean C.V. %

0.580	2.7
-------	-----

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean C.V. %

--	--

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean C.V. %

--	--

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean C.V. %

--	--

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean C.V. %

--	--

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean C.V. %

--	--

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean C.V. %

--	--

Note: ON stands for original number per replicate, while SN refers to the number surviving after test completion.



# BIO-AQUATIC TESTING, INC. TOXICITY TEST

**Chronic**

**Menidia beryllina**

Lab ID: **97321**

Client: Natural Energy Laboratory of Hawaii - SSW-28 Sump

Balance: Radwag BAL-007

Begin Date: 5/2/2025

End Date: 5/9/2025

Organism: Menidia beryllina

Analyst: SG  
Weigh Date: 05/12/2025

Date/Time placed in Oven: 05/09/2025 | 1400  
Date/Time removed from Oven: 05/10/2025 | 1400

## Control

	Qty.	Wt.
A	7	3.840
B	8	3.900
C	7	3.960
D		
E		

## 100 %

	Qty.	Wt.
A	8	4.740
B	8	4.090
C	7	4.500
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

## APPENDIX A

### STATISTICS SUMMARY

Both the lethal and sub-lethal endpoints were statistically calculated according to their respective EPA guidelines. The Chronic Freshwater organisms were calculated according to EPA-821-R-02-013, October 2002 Fourth Edition. The Chronic Marine and Estuarine organisms were calculated according to EPA-821-R-02-014, October 2002 Third Edition. The Acute Freshwater and Marine organisms were calculated according to EPA-821-R-02-012, October 2002 Fifth Edition. The fertilization organisms were calculated according to EPA-600-R-95-136 or EPA-600-R-12-022, dependent upon the species. Listed below are the basic principles of these guidelines. If you would like a copy of the raw statistical calculations for your test then please contact us.

The chronic and acute *Pimephales promelas* and *Menidia beryllina* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts (parametric). If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test (non-parametric) is used. The chronic *Pimephales promelas* and *Menidia beryllina* growth data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Mysidopsis bahia* survival data is analyzed using Chi-square test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test or Bartlett's Test then Steels Many One Test is used. *Mysidopsis bahia* growth data is analyzed using Chi-square test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Mysidopsis bahia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Ceriodaphnia dubia* survival data are analyzed using the Fisher's Exact Test. The chronic *Ceriodaphnia dubia* reproduction and are analyzed using the Chi-square test and Bartlett Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Daphnia pulex* and *Ceriodaphnia dubia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The fertilization data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation or TST methodology may also be used.

mysid growth

File: 97321.myg

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.670	2.420	3.820	2.420	0.670
OBSERVED	0	4	3	3	0

Calculated Chi-Square goodness of fit test statistic = 2.6866

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

mysid growth

File: 97321.myg

Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.001	
2	100	0.009	12.045

Critical F = 23.20 (P=0.01, 4, 4)

Since  $F \leq \text{Critical } F$ , FAIL TO REJECT  $H_0$ : Equal Variances.

mysid growth

File: 97321.myg

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.011	0.011	2.226
Within (Error)	8	0.041	0.005	
Total	9	0.052		

Critical F value = 5.32 (0.05,1,8)  
 Since F < Critical F FAIL TO REJECT Ho: All equal

mysid growth  
 File: 97321.myg Transform: NO TRANSFORMATION

EQUAL VARIANCE t-TEST		TABLE 1 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.504	0.504		
2	100	0.437	0.437	1.492	
2 Sample t table value = 1.86 (1 Tailed Value, P=0.05, df=8,1)					

UNEQUAL VARIANCE t-TEST		Ho:Control<Treatment			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.504	0.504		
2	100	0.437	0.437	1.492	
2 Sample t table value = 2.13 (1 Tailed Value, P=0.05, df=4,1)					

mysid growth  
 File: 97321.myg Transform: NO TRANSFORMATION

EQUAL VARIANCE t-TEST		TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	con	5			
2	100	5	0.084	16.7	0.068

UNEQUAL VARIANCE t-TEST		Ho:Control<Treatment			
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	con	5			
2	100	5	0.097	19.1	0.068

-----

menidia growth  
File: 97321.meg            Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

-----

D =     0.001

W =     0.882

Critical W (P = 0.05) (n = 6) = 0.788

Critical W (P = 0.01) (n = 6) = 0.713

-----

Data PASS normality test at P=0.01 level. Continue analysis.

menidia growth  
File: 97321.meg            Transform: NO TRANSFORMATION

F-Test for equality of two variances

-----

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.000	
2	100	0.000	2.649

-----

Critical F = 199.00    (P=0.01, 2, 2)

Since F <= Critical F, FAIL TO REJECT    Ho: Equal Variances.

menidia growth  
File: 97321.meg            Transform: NO TRANSFORMATION

ANOVA TABLE

-----

SOURCE	DF	SS	MS	F
Between	1	0.012	0.012	71.082
Within (Error)	4	0.001	0.000	

-----

Total 5 0.013

Critical F value = 7.71 (0.05,1,4)  
Since  $F > \text{Critical } F$  REJECT  $H_0$ : All equal

menidia growth

File: 97321.meg Transform: NO TRANSFORMATION

EQUAL VARIANCE t-TEST - TABLE 1 OF 2  $H_0$ :Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.491	0.491		
2	100	0.581	0.581	-8.431	

2 Sample t table value = 2.13 (1 Tailed Value,  $P=0.05$ ,  $df=4,1$ )

UNEQUAL VARIANCE t-TEST  $H_0$ :Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.491	0.491		
2	100	0.581	0.581	-8.431	

2 Sample t table value = 2.35 (1 Tailed Value,  $P=0.05$ ,  $df=3,1$ )

menidia growth

File: 97321.meg Transform: NO TRANSFORMATION

EQUAL VARIANCE t-TEST - TABLE 2 OF 2  $H_0$ :Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	con	3			
2	100	3	0.023	4.6	-0.090

UNEQUAL VARIANCE t-TEST  $H_0$ :Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
-------	----------------	----------------	--------------------------------------	-----------------	----------------------------

1	con	3			
2	100	3	0.025	5.1	-0.090

---

# Bio-Aquatic Testing, Inc.

## SALT WATER TEST SETUP FORM

Client: Natural Energy Laboratory of HawaiiPermit N/AFacility: Hawaii Ocean Science and TechnologyLab Number 97321Outfall Name: SSW-28 SumpNumber of samples 1Dilution Water: Synthetic Lab

Receiving Water Name: \_\_\_\_\_

Dechlorinate Sample: \_\_\_\_\_

Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
			Begin Date	End Date	Start	End
1	05/02/25	10:39	04/29/25	04/29/25	12:20	12:20

### Type of Test(s)

*Americamysis bahia*

Chronic

*Menidia beryllina*

Chronic

Start Sx # 1 Date: 5/2/2025Renew Sx # 1 Date: 5/3/2025Renew Sx # 1 Date: 5/4/2025Renew Sx # 1 Date: 5/5/2025Renew Sx # 1 Date: 5/6/2025Renew Sx # 1 Date: 5/7/2025Renew Sx # 1 Date: 5/8/2025

Test Start Date: \_\_\_\_\_ Test End Date: \_\_\_\_\_

5/2/2025 5/9/2025Controls: Synthetic

pH Match: \_\_\_\_\_

Hardness Match: \_\_\_\_\_

Americamysis Test Set Up: 5 Reps & 5 Organisms per RepMenidia beryllina Test Set Up: 3 Reps & 8 Organism per RepConcentrations: 100 %Test Chemistry on these dilutions: 100

Samples received by:

☐ Express Delivery☐ UPS Next Day☐ via Air Cargo☐ DHL☒ Federal Express☐ the Client☐ Bio-Aquatic personnel

Other: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_



# BIO-AQUATIC TESTING, INC.

Hardness, Alkalinity, Residual Chlorine, Specific Conductivity, and Salinity Analysis Data

**Client:** Natural Energy Laboratory of

**Lab ID:** 97321

**Facility:** Hawaii Ocean Science and

**Dilution Water(s):** Synthetic Lab

**Outfall:** SSW-28 Sump

**Test Date:** May 2, 2025

## EFFLUENT PARAMETERS

Effluent Sample #	Received		Residual Cl <sub>2</sub> (mg/L)	DeChlor (ml/L) <sup>1</sup>	Ammonia (mg/L)	Analyst Initials	Temp. Received
	Date	Time					
1	5/2/25	10:39	<0.10	N/A	<0.25	JR	2.7

**<sup>1</sup>Dechlorination Reagent:** 0.025 N Sodium Thiosulfate

Effluent Sample #	pH	DO (mg/L)	Init. Salinity (ppt)	Adjusted Salinity	Analyst Initials
1	8.2	7.3	32.2	N/A	JR

**Analysis Methods:** Chlorine: Hanna Colorimeter #HI711, Ammonia: Hanna Colorimeter #HI733, Hardness: Hanna Photometer #HI97735, Alkalinity: Hanna Colorimeter #HI775, pH, DO, Conductivity: Thermo Versa Star Benchtop Meter

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen, Salinity

ChronicAmericamysis bahia

Client: Natural Energy Laboratory of HawaiiLab Number: 97321

Facility: Hawaii Ocean Science andDilution Water(s): Synthetic Lab  
Outfall: SSW-28 SumpTest Begin Date: May 2, 2025

						Concentration							
ANALYST	DATE	TIME	SX#	UNIT	%	Control	100						
GJ	5/2	Start	1	pH		7.9	8.0						
				DO (mg/L)		7.2	6.5						
		25 ± 1		Salinity (ppt)		20.5	36.5						
GJ	5/3	24 Hr	1	pH		8.1	8.0						
				DO (mg/L)		7.4	6.5						
		25 ± 1		Salinity (ppt)		20.8	37.1						
		Renew	1	pH		8.0	8.0						
				DO (mg/L)		7.3	6.6						
				Salinity (ppt)		20.7	36.0						
SG	5/4	48 Hr	1	pH		7.8	7.9						
				DO (mg/L)		6.6	6.3						
		25 ± 1		Salinity (ppt)		21.9	38.1						
		Renew	1	pH		8.2	8.1						
				DO (mg/L)		7.4	6.8						
				Salinity (ppt)		19.1	33.7						
AK	5/5	72 Hr	1	pH		7.9	7.9						
				DO (mg/L)		6.5	6.6						
		25 ± 1		Salinity (ppt)		21.2	34.6						
		Renew	1	pH		8.1	8.2						
				DO (mg/L)		7.5	7.0						
				Salinity (ppt)		22.2	31.7						
TT	5/6	96 Hr	1	pH		7.7	7.8						
				DO (mg/L)		6.1	5.9						
		25 ± 1		Salinity (ppt)		23.9	32.1						
		Renew	1	pH		7.8	8.0						
				DO (mg/L)		7.0	6.6						
				Salinity (ppt)		19.9	35.5						
GJ	5/7	120 Hr	1	pH		7.8	7.8						
				DO (mg/L)		6.8	6.3						
		25 ± 1		Salinity (ppt)		22.8	34.9						
		Renew	1	pH		8.0	8.1						
				DO (mg/L)		7.1	6.9						
				Salinity (ppt)		20.3	35.3						
GJ	5/8	144 Hr	1	pH		7.9	7.9						
				DO (mg/L)		6.8	6.1						
		25 ± 1		Salinity (ppt)		23.6	34.5						
		Renew	1	pH		8.1	8.0						
				DO (mg/L)		7.1	6.8						
				Salinity (ppt)		20.5	35.2						
CAP	5/9	168 Hr	1	pH		7.9	7.9						
				DO (mg/L)		7.4	6.5						
		25 ± 1		Salinity (ppt)		20.2	32.4						

# BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen, Salinity

Chronic

*Menidia beryllina*

Client: Natural Energy Laboratory of

Lab Number: 97321

Facility: Hawaii Ocean Science and

Dilution Water(s): Synthetic Lab

Outfall: SSW-28 Sump

Test Begin Date: May 2, 2025

ANALYST	DATE	TIME	SX#	UNIT	%	Concentration							
						Control	100						
GJ	5/2	Start	1		pH	7.9	8.0						
					DO (mg/L)	7.2	6.5						
		25 ± 1			Salinity (ppt)	20.5	36.5						
GJ	5/3	24 Hr	1		pH	8.0	7.8						
					DO (mg/L)	7.3	6.8						
		25 ± 1			Salinity (ppt)	19.7	32.0						
		Renew	1		pH	8.0	8.0						
					DO (mg/L)	7.3	6.6						
					Salinity (ppt)	20.7	36.0						
SG	5/4	48 Hr	1		pH	7.8	7.9						
					DO (mg/L)	7.1	6.5						
		25 ± 1			Salinity (ppt)	21.4	33.9						
		Renew	1		pH	8.2	8.1						
					DO (mg/L)	7.4	6.8						
					Salinity (ppt)	19.1	33.7						
AK	5/5	72 Hr	1		pH	7.8	7.9						
					DO (mg/L)	7.2	6.8						
		25 ± 1			Salinity (ppt)	21.2	36.4						
		Renew	1		pH	8.1	8.2						
					DO (mg/L)	7.5	7.0						
					Salinity (ppt)	22.2	31.7						
TT	5/6	96 Hr	1		pH	7.9	7.8						
					DO (mg/L)	7.1	6.4						
		25 ± 1			Salinity (ppt)	19.9	37.6						
		Renew	1		pH	7.8	8.0						
					DO (mg/L)	7.0	6.6						
					Salinity (ppt)	19.9	35.5						
GJ	5/7	120 Hr	1		pH	8.0	8.0						
					DO (mg/L)	7.1	6.6						
		25 ± 1			Salinity (ppt)	20.1	38.3						
		Renew	1		pH	8.0	8.1						
					DO (mg/L)	7.1	6.9						
					Salinity (ppt)	20.3	35.3						
GJ	5/8	144 Hr	1		pH	7.9	7.9						
					DO (mg/L)	7.2	6.3						
		25 ± 1			Salinity (ppt)	21.3	37.1						
		Renew	1		pH	8.1	8.0						
					DO (mg/L)	7.1	6.8						
					Salinity (ppt)	20.5	35.2						
CAP	5/9	168 Hr	1		pH	8.0	7.9						
					DO (mg/L)	8.4	8.3						
		25 ± 1			Salinity (ppt)	22.1	38.5						

# Appendix B

*Americamysis bahia*

## BIO-AQUATIC TESTING, INC.

Carrollton, TX

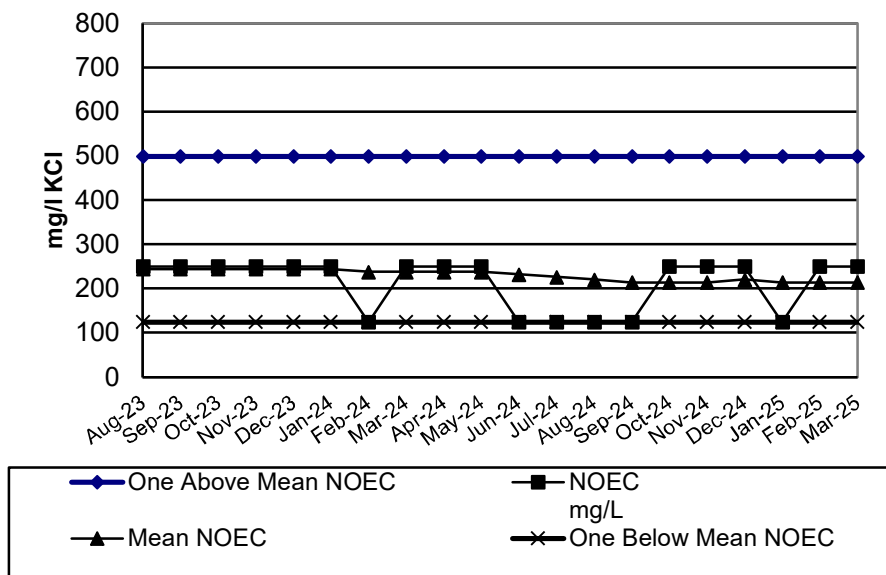
### REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, copper chloride, potassium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

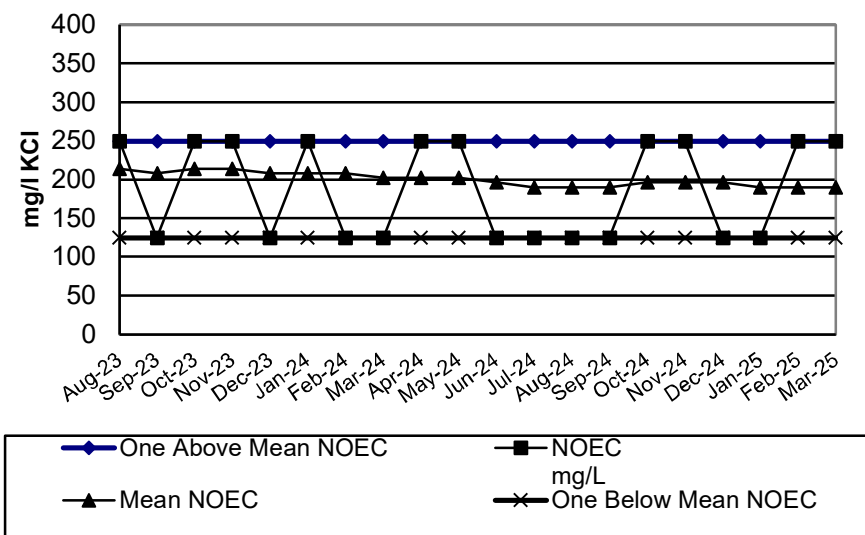
### CHRONIC REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Saltwater						
CHEMICAL:	Potassium Chloride						
DURATION:	7 Days						
TEST NUMBER:	165						
PROJECT NUMBER:	97618						
START DATE:	3/25/2025						
START TIME:	15:26						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	25	50	125	250	500	1000
NUMBER DEAD PER CONCENTRATION:	2	3	1	0	1	40	40
TEST METHODS:	As listed in EPA-821-R-02-014						
STATISTICAL METHODS:	SURVIVAL: Steel's Many-One Rank Test GROWTH: ANOVA w/Dunnett's Test FECUNDITY: Not Applicable						
NOEC FOR SURVIVAL:	250	mg/L					
LOEC FOR SURVIVAL:	500	mg/L					
NOEC FOR GROWTH:	250	mg/L					
LOEC FOR GROWTH:	500	mg/L					
PMSD:	13.7						

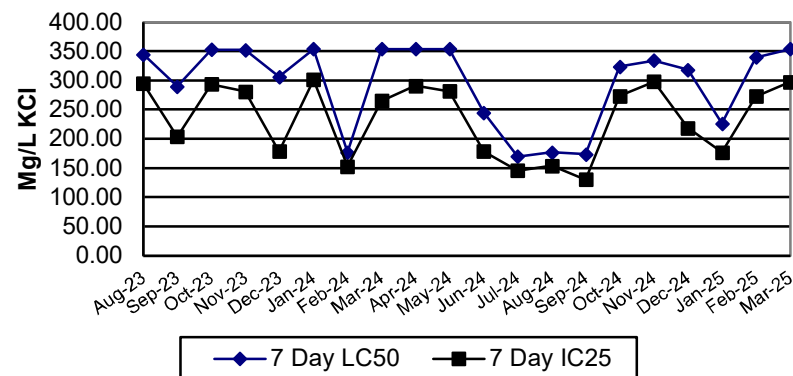
**Mysid Chronic Survival Control Chart**



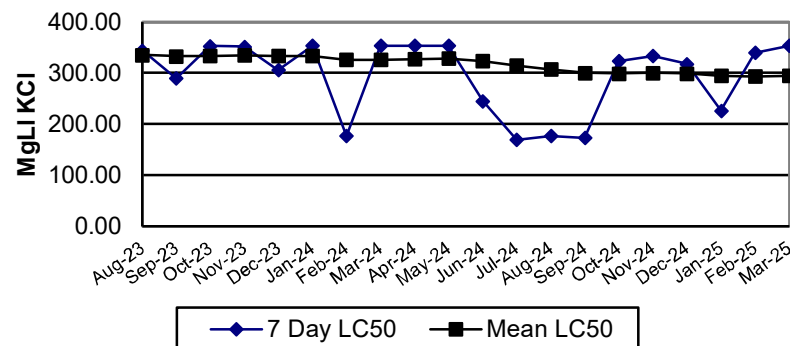
**Mysid Chronic Growth Control Chart**



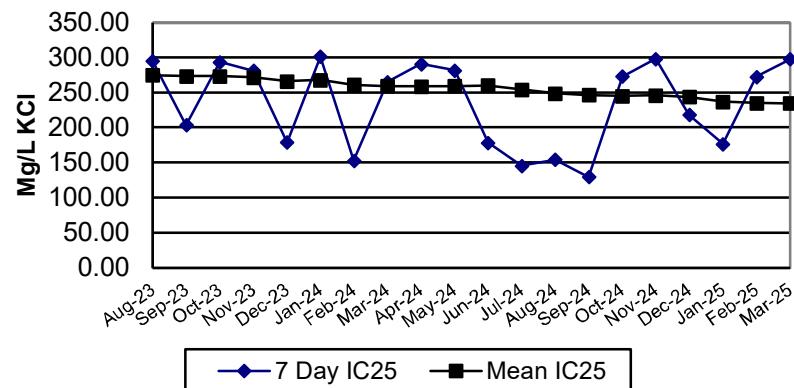
**Mysid 7-Day LC50 & IC25**



**Mysid 7-Day LC50**



**Mysid 7-Day IC25**



# Appendix B

*Menidia beryllina*

## BIO-AQUATIC TESTING, INC.

Carrollton, TX

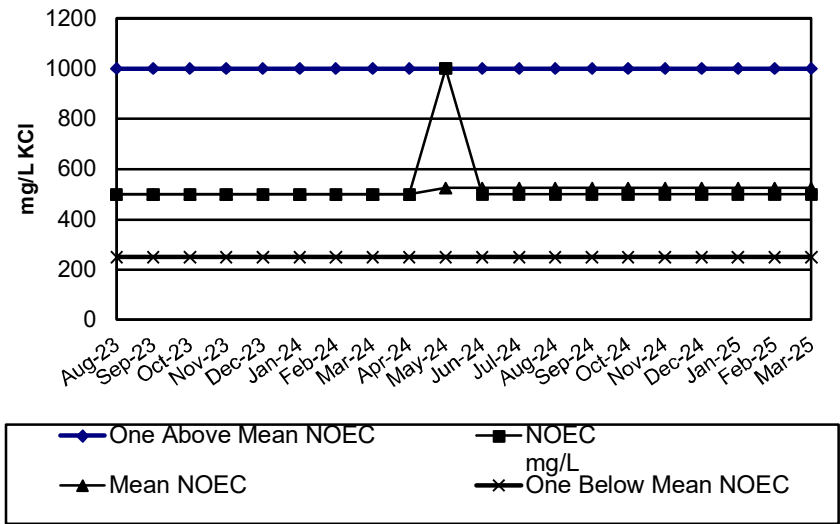
### REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, copper chloride, potassium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

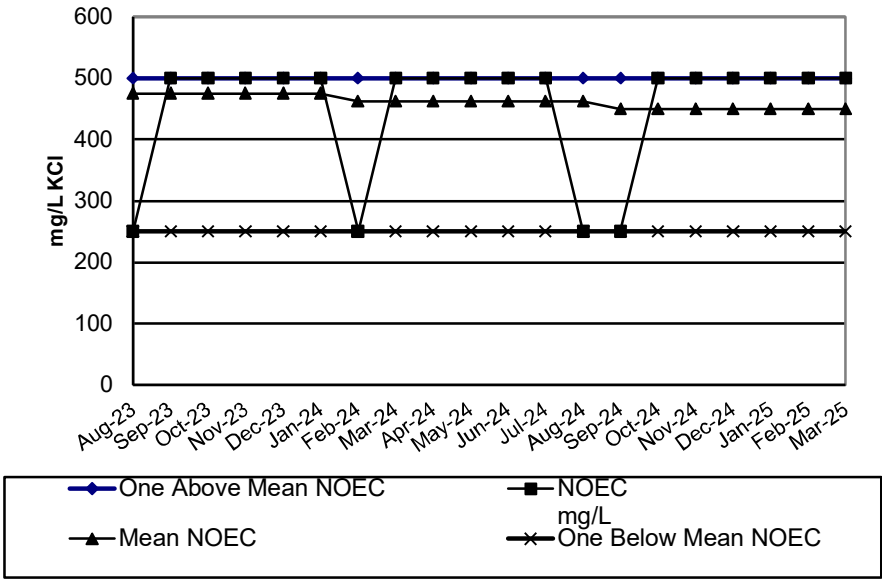
### CHRONIC REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Saltwater
CHEMICAL:	Potassium Chloride
DURATION:	7 Days
TEST NUMBER:	165
PROJECT NUMBER:	97619
START DATE:	3/25/2025
START TIME:	15:42
TOTAL NUMBER EXPOSED:	40 organisms per concentration
CONCENTRATIONS (mg/L):	CON 125 250 500 1000 2000 4000
NUMBER DEAD PER CONCENTRATION:	0 0 1 1 35 40 40
TEST METHODS:	As listed in EPA-821-R-02-014
STATISTICAL METHODS:	SURVIVAL: Steel's Many-One Rank Test GROWTH: ANOVA w/Dunnett's Test
NOEC FOR SURVIVAL:	500 mg/L
LOEC FOR SURVIVAL:	1000 mg/L
NOEC FOR GROWTH:	500 mg/L
LOEC FOR GROWTH:	1000 mg/L
PMSD:	14.5

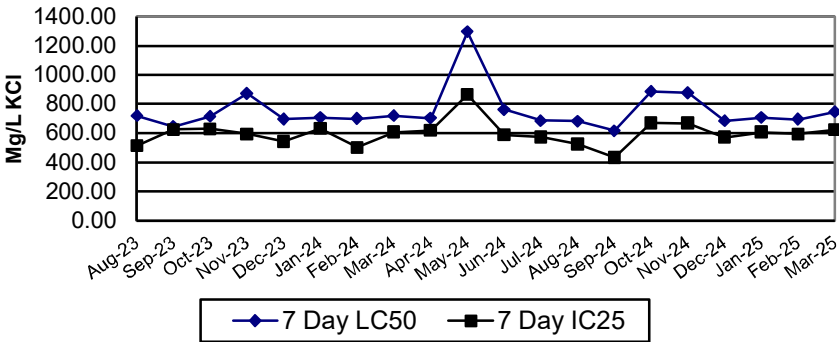
Menidia Chronic Survival Control Chart



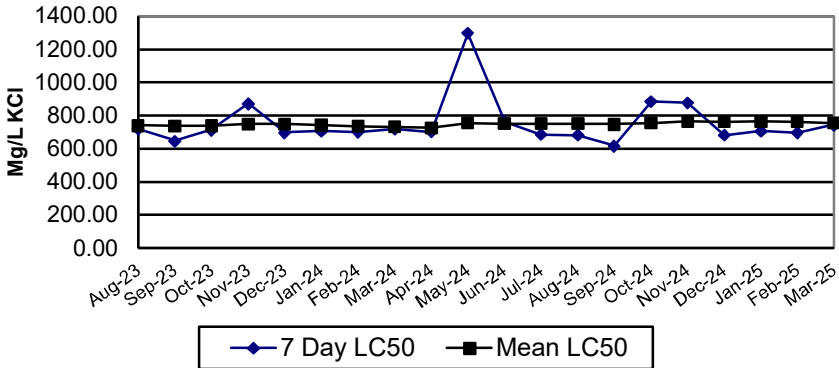
Menidia Chronic Growth Control Chart



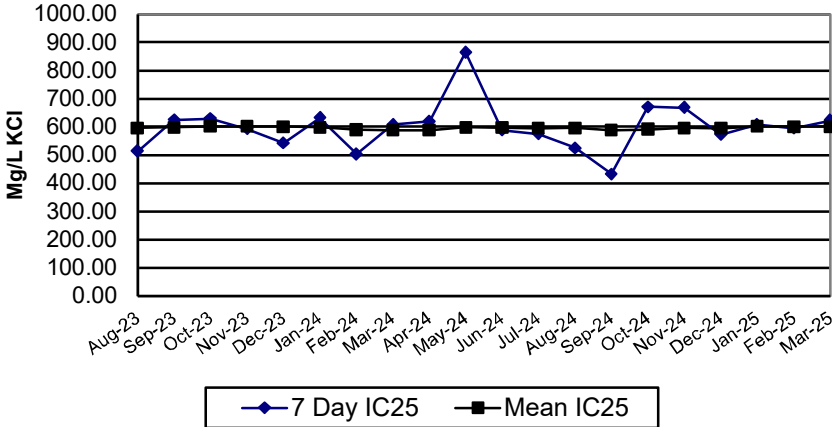
Menidia 7-Day LC50 & IC25



Menidia 7-Day LC50



Menidia 7-Day IC25



## APPENDIX C

### LITERATURE REFERENCES

- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fifth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-012.
- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents and Receiving Water To Marine And Estuarine Organisms (Third Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-014.
- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fourth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-013.
- U.S.E.P.A., 2012. Tropical Collector Urchin, *Tripneustes gratilla* (First Edition) U.S. Environmental Protection Agency, Office of Research and Development and Region 9, EPA-600-R-12-022.
- U.S.E.P.A., 1995. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To West Coast Marine and Estuarine Organisms (First Edition) U.S. Environmental Protection Agency, EPA-600-R-95-136.
- U.S.E.P.A., 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document, U.S. Environmental Protection Agency, Office of Wastewater, Washington D.C., EPA-833-R-10-004.
- U.S.E.P.A., 1991. Technical Support Document For Water Quality-Based Toxics Control, U.S. Environmental Protection Agency, EPA-505-2-90-001.
- Zarr, Jerrold, H., 1984. Biostatistical Analysis, (Second Edition). Prentice-Hall, Inc., Englewood Cliffs, N.J.



# **CHAIN-OF-CUSTODY SHEETS**

## *Appendix D*



BIO-AQUATIC TESTING, INC.  
2501 MAYES RD., STE. 100  
CARROLLTON, TX 75006  
PH: 972-242-7750 FAX: 972-242-7749

## CHAIN OF CUSTODY

Bio Only:  
No Sample Left

Lab Id :

97321

Please Review & Complete Sections A, B, C, & D.

Sample No: 97321 -

Reference Date 8/25/2021

Revision 2

Check Sample No. : First, Second, or Third.

P.O. No:

Client: Natural Energy Laboratory of Hawaii

Facility: SSW-28 Sump

Permit No: N/A

Outfall:

Client Contact: Pam Madden

Client Phone: 808.327.9524

### A. REVIEW SCHEDULED TEST(S):

Chronic	Americamysis bahia
Chronic	Menidia beryllina

Concentration: 100

To Ship the  
1st Sample on:  
2/25/2025

(For TX) Setup separate 24hr Acute Test? No

### C.

Sample ID or Location:  
(Outfall No. or Name)

1 SSW-28 Sump

4/29/25

From

To

Sample Time  
(military)

Grab  
or  
Composite

Sampled By:  
(Sign and Print Name)

Number Of  
Containers  
Shipped

1

1

1

### D.

Relinquished By:

Date

Time

Received By:

Date

Time

Received By:

Date

Time

1 Pam Madden

4/29/25

From

To

Sample Time  
(military)

Grab  
or  
Composite

Sampled By:  
(Sign and Print Name)

Number Of  
Containers  
Shipped

1

1

1

1 Pam Madden

4/29/25

From

To

Sample Time  
(military)

Grab  
or  
Composite

Sampled By:  
(Sign and Print Name)

Number Of  
Containers  
Shipped

1

1

1

### Bio-Aquatic Sample Login

BAT sample personnel:

Yes ☒ No ☐

Dechlorinate Sample:

Yes ☐ No ☒

Dilution Water:

Receiving Stream ☐

Synthetic Lab ☒

Date: 5-2-25

Time: 12:33

By: JR

Temperature: 2.7 (C)

IR#: 025

Chlorine: 60.1 mg/l

Ammonia: 20.25 mg/l

Int. SalCond: 32 ppt/μS

Adj. Salinity ppt

pH: 8.2

Hardness: mg/l

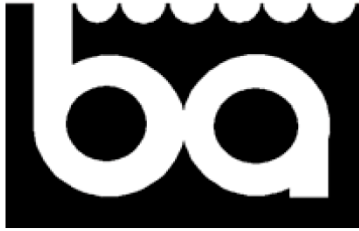
Other

Condition: Good

DO: 7.3 mg/l

Alkalinity: mg/l

Condition: Good



**Bio-Aquatic Testing, Inc.**



TCEQ TNI ACCREDITED

## Report Revision Form

Report Revision Number 0 for Lab ID 97321 was revised on 06/30/2025.

The revision was issued for the following reason(s):

- ☐ Typo in the report document or tables
- ☐ Missing sheets or tables
- ☐ Hard data was not scanned in as required by the client
- ☐ Missing specially requested forms or data for the client

☒ Other (Please Specify):

Updated sampling information