Bio-Aquatic Testing, Inc.

TCEQ TNI Accredited

Natural Energy Laboratory of Hawaii Authority
Bivalve Farm

Chronic Biomonitoring Report

97326

Americamysis bahia
Menidia beryllina

May 02, 2025

Approved by: _____
Lab director

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

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

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TOXICITY TEST REPORT - Chronic

Client:Natural Energy Laboratory of Hawaii Authority
Facility: Hawaii Ocean Science Technology Park
Permit No. N/A

Sample: Bivalve Farm
Laboratory Number: 97326
Date: May 02, 2025

SAMPLE COLLECTION:

A grab sample from the Natural Energy Laboratory of Hawaii Authority, Bivalve Farm, was transported to Bio-Aquatic Testing on May 02, 2025. The effluent Shrimp Farm sample was collected from the pre-treatment inflow seawater stream into maturation tanks by facility and client personnel (nursery/larval tanks were not in use during sampling).

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:

EPA METHOD: 1007

Americamysis bahia

The seven-day Chronic *Americamysis bahia* survival and growth test was initiated at 14:00 hours on May 02, 2025. One effluent concentration of 100% effluent 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 13:30 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:

EPA METHOD: 1006

Menidia beryllina

The seven-day Chronic *Menidia beryllina* survival and growth test was initiated at 13:55 hours on May 02, 2025. One effluent concentration of 100% effluent 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 13:33 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: 97326**

Permit Number: N/A **Test Temperature (oC):** 25 ± 1

Sample Type: Grab **Outfall Name:** Bivalve Farm **Photo Period:** 16 Hours Light 8 Hours Dark

Receiving Water Name:

Begin Date: 5/2/2025

Test Start Time: 14:00 Test End Time: 13:30 **End Date:** 5/9/2025

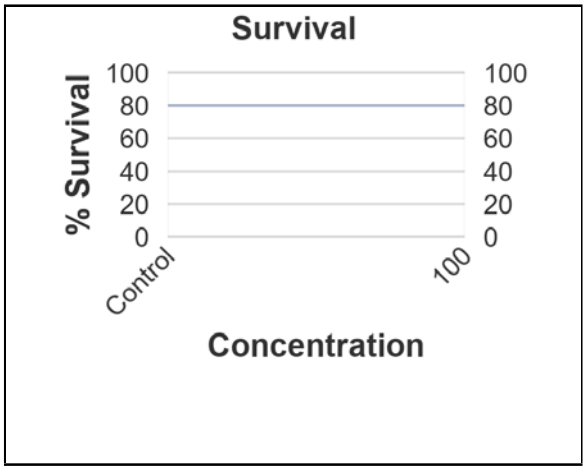
SURVIVAL

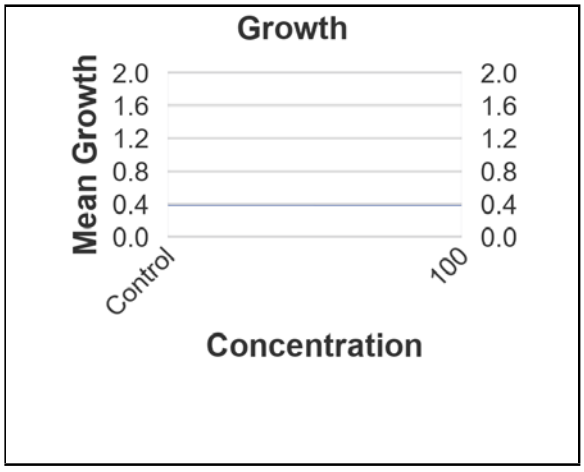
Effluent Con. %		Number of Alive 5/2	5/3	5/4	5/5	5/6	5/7	5/8	5/9	Avg% Surv.
Control	A	5	5	5	5	5	4	4	4	
	B	5	5	5	5	5	5	5	5	
	C	5	5	5	5	5	3	3	3	80.0%
	D	5	5	4	4	4	4	4	4	
	E	5	5	5	5	5	5	5	4	
100	A	5	5	4	4	4	4	4	4	
	B	5	5	5	5	5	5	5	5	
	C	5	5	5	5	4	4	4	4	80.0%
	D	5	5	3	3	3	3	3	3	
	E	5	5	4	4	4	4	4	4	
	A									
	B									
	C									
	D									
	E									
	A									
	B									
	C									
	D									
	E									

BIO-AQUATIC TESTING, INC.

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

Concentration Response Relationships





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Chronic Americamysis bahia SURVIVAL Lab ID: **97326**

Client: **Natural Energy Laboratory of Hawaii** Facility **Bivalve Farm** 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** **3**

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 1400	5	—	—	—	—	5	—	—	—	—										
24Hr	5-3-25 1257 m	5	—	—	—	—	5	—	—	—	—										
48Hr	5-4-25 SG 1024	5	—	—	4₁	5	4₁	5	5	3₂	4₁										
72Hr	5-5-25 1308 AE	5	—	—	4	5	4	5	—	3	4										
96Hr	5-6-25 AB 1034	5	—	—	4	5	4	5	4₀	3	4										
5 days	5-7-25 08 KM	4₁	5	3₂	4	5	4	5	4	3	4										
6 days	5-8-25 0915	4	5	3	4	5	4	5	4	3	4										
7 days	5-9-25 AB 1330	4	5	3	4	4₀	4	5	4	3	4										

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: **97326**

Client: **Natural Energy Laboratory of Hawaii** Facility **Bivalve Farm** 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	24.6	25.6	25.1	25.8	25.3	25.9	25.1	25.3	25.3	25.5	25.0	25.5	25.2	25.5
100														
TIME/DATE TECH	5-2-25 MB 1400	5-3-25 1300		5-4-25 86 1021		5-5-25 1300 AK		5-6-25 MB 1034		5-7-25 0819		5-8-25 1355		5-9-25 MB 13
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.

1st Organism **BIO-AQUATIC TESTING, INC.** **TOXICITY TEST**

Chronic *Americamysis bahia*

Client: Natural Energy Laboratory of Hawaii Ocean Science and Technology Park **Lab ID: 97326**

Permit Number: N/A

Sample Type: Grab **Outfall Name:** Bivalve Farm

Receiving Water Name:

Synthetic

	ON	SN	Wt.	Avg.	SN Avg.
A	5	4	1.770	0.354	0.443
B	5	5	2.680	0.536	0.536
C	5	3	1.820	0.364	0.607
D	5	4	1.680	0.336	0.420
E	5	4	1.670	0.334	0.418

Mean	C.V. %
0.385	22.21

SN Mean	SN C.V. %
0.485	17.3

100

	ON	Wt.	Avg.
A	5	2.240	0.448
B	5	2.140	0.428
C	5	1.860	0.372
D	5	1.450	0.290
E	5	1.910	0.382

Mean	C.V. %
0.384	15.96

	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: **97326**

Client: Natural Energy Laboratory of Hawaii - Bivalve Farm

Balance: Radwag BAL-007

Begin Date: 5/2/2025 End Date: 5/9/2025 Organism: Americamysis bahia

Analyst: SG

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

Weigh Date: 05/12/2025

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

Control

	Qty.	Wt.
A	4	1.770
B	5	2.080
C	3	1.820
D	4	1.680
E	4	1.670
F		
G		
H		

100 %

	Qty.	Wt.
A	4	2.240
B	5	2.140
C	4	1.860
D	3	1.450
E	4	1.910
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:** 97326

Permit Number: N/A

Outfall Name: Bivalve Farm **Sample Type:** Grab

Receiving Water Name:

Test Temperature (oC): 25 ± 1

Photo Period: 16 Hours Light 8 Hours Dark

Test Start Time: 13:55 Test End Time: 13:33

Begin Date: 5/2/2025

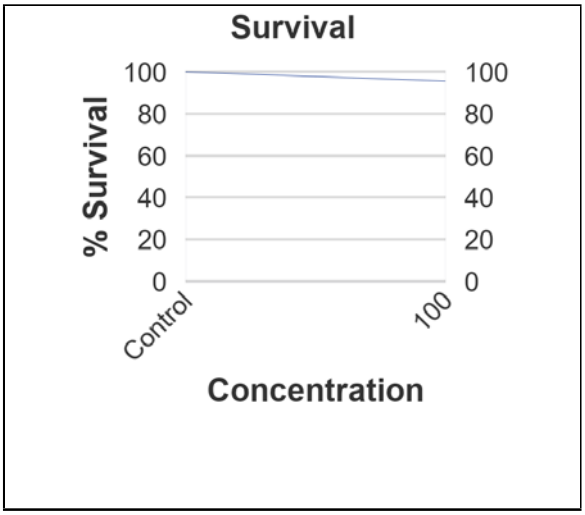
End Date: 5/9/2025

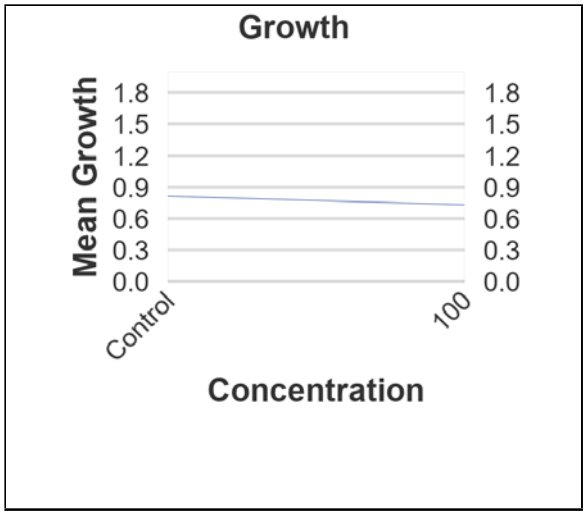
SURVIVAL

Effluent Concentration		Number Of Alive 5/2	5/3	5/4	5/5	5/6	5/7	5/8	5/9	Avg% Surv.
Control	A	8	8	8	8	8	8	8	8	100.0%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D									
	E									
100	A	8	8	8	8	8	8	8	8	95.8%
	B	8	8	8	8	8	8	8	7	
	C	8	8	8	8	8	8	8	8	
	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									
	A									
	B									
	C									
	D									
	E									
	A									
	B									
	C									
	D									
	E									

Concentration Response Relationships





BIO-AQUATIC TESTING, INC.

Chronic Menidia beryllina SURVIVAL Lab ID: **97326**

Client: **Natural Energy Laboratory of Hawaii** Facility **Bivalve Farm** 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	5-2-25 1355 DB	8	—	—			8	—	—												
24Hr	5-3-25 1300 m	8	—				8	—													
48Hr	5-4-25 SG 1023	8	—				8	—													
72Hr	5-5-25 1300 AM	8	—				8	—													
96Hr	5-6-25 DB 1044	8	—				8	—													
5 days	5-7-25 0945	8	—				8	—													
6 days	5-8-25 041359	8	—				8	—													
7 days	5-9-25 DB 1333	8	—				8	7	8												

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																				

Lined/boxed spaces preceded by a number represent the same numbered lined spaces without a preceding number indicating unused control solution.

BIO-AQUATIC TESTING, INC.

Chronic Menidia beryllina SURVIVAL Lab ID: **97326**

Client: **Natural Energy Laboratory of Hawaii** Facility **Bivalve Farm** 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	24.8	25.7	25.1	25.5	25.3	25.0	25.0	25.4	25.4	25.5	25.0	25.4	25.2	25.5
100														
				86										
				86										
TIME/DATE TECH	5-2-25 1355 DB	5-3-25 1303		5-4-25 86	1023	5-5-25 1308 AK		5-6-25 DB	1044	5-7-25 0815		5-8-25 1359		5-9-25 DB
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.

2nd Organism **BIO-AQUATIC TESTING, INC.** **TOXICITY TEST**

Chronic *Menidia beryllina*

Client: Natural Energy Laboratory of Hawaii Ocean Science and Technology **Lab ID:** **97326**

Permit Number: N/A

Sample Type: Grab **Outfall Name:** Bivalve Farm

Receiving Water Name:

Synthetic

	ON	SN	Wt.	Avg.	SN Avg.
A	8	8	6.940	0.868	0.868
B	8	8	6.730	0.841	0.841
C	8	8	5.900	0.738	0.738
D					
E					

Mean	C.V. %
0.815	8.4

SN Mean	SN C.V. %
0.815	8.4

100

	ON	Wt.	Avg.
A	8	6.610	0.826
B	8	5.950	0.744
C	8	5.060	0.633
D			
E			

Mean	C.V. %
0.734	13.2

	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: **97326**

Client: Natural Energy Laboratory of Hawaii - Bivalve Farm

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	8	6.940
B	(lined through)	6.730
C	(lined through)	5.900
D		
E		

100 %

	Qty.	Wt.
A	8	6.610
B	7	5.950
C	8	5.060
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		

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

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: 97326.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	1	2	4	2	1

Calculated Chi-Square goodness of fit test statistic = 0.4793
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

mysid growth
File: 97326.myg Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.007	
2	100	0.004	1.945

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

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

mysid growth
File: 97326.myg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.000	0.000	0.000
Within (Error)	8	0.044	0.006	
Total	9	0.044		

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

mysid growth
File: 97326.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	0.385	0.385		
2	100	0.384	0.384	0.017	

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	0.385	0.385		
2	100	0.384	0.384	0.017	

2 Sample t table value = 1.89 (1 Tailed Value, P=0.05, df=7,1)

mysid growth
File: 97326.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	0.087	22.7	0.001

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	0.089	23.2	0.001

---

menidia growth
File: 97326.meg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.028

W = 0.931

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: 97326.meg Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.005	
2	100	0.009	1.994

Critical F = 199.00 (P=0.01, 2, 2)

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

menidia growth
File: 97326.meg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.010	0.010	1.409
Within (Error)	4	0.028	0.007	

Total 5 0.038

Critical F value = 7.71 (0.05,1,4)
Since F < Critical F FAIL TO REJECT Ho: All equal

menidia growth
File: 97326.meg 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	0.816	0.816		
2	100	0.734	0.734	1.187	

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

UNEQUAL VARIANCE t-TEST Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.816	0.816		
2	100	0.734	0.734	1.187	

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

menidia growth
File: 97326.meg 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	3			
2	100	3	0.146	17.9	0.081

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	3			
2	100	3	0.161	19.8	0.081

Bio-Aquatic Testing, Inc.

SALT WATER TEST SETUP FORM

Client: Natural Energy Laboratory of Hawaii Permit N/A

Facility: Hawaii Ocean Science and Technology Lab Number 97326

Outfall Name: Bivalve Farm Number of samples 1

Dilution 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	10:22	04/29/25	04/29/25	08:14	08:14

Type of Test(s)	
Americamysis bahia	Chronic
Menidia beryllina	Chronic

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

Controls: Synthetic

pH Match:

Hardness Match:

Test Start Date: 5/2/2025 Test End Date: 5/9/2025

Americamysis Test Set Up: 5 Reps & 5 Organisms per Rep

Menidia beryllina Test Set Up: 3 Reps & 8 Organism per Rep

Concentrations: 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
Facility: Hawaii Ocean Science and
Outfall: Bivalve Farm

Lab ID: 97326
Dilution Water(s): Synthetic Lab
Test Date: May 2, 2025

EFFLUENT PARAMETERS

Effluent Sample #	Received		Residual Cl_2 (mg/L)	DeChlor (ml/L)[1]	Ammonia (mg/L)	Analyst Initials	Temp. Received
	Date	Time					
1	5/2/25	10:22	<0.10	N/A	<0.25	JR	2.9

[1]**Dechlorination Reagent:** 0.025 N Sodium Thiosulfate

Effluent Sample #	pH	DO (mg/L)	Init. Salinity (ppt)	Adjusted Salinity	Analyst Initials
1	8.1	7.3	32.1	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

Chronic **Americamysis bahia**

Client: Natural Energy Laboratory of Hawaii **Lab Number:** 97326

Facility: Hawaii Ocean Science and **Dilution Water(s):** Synthetic Lab

Outfall: Bivalve Farm **Test Begin Date:** May 2, 2025

						Concentration							
ANALYST	DATE	TIME	SX#	UNIT	%	Control	100						
GJ	5/2	Start	1	pH		7.9	7.7						
				DO (mg/L)		6.6	5.4						
		25 ± 1		Salinity (ppt)		20.3	36.7						
GJ	5/3	24 Hr	1	pH		7.9	7.7						
				DO (mg/L)		6.5	5.5						
		25 ± 1		Salinity (ppt)		21.5	37.0						
		Renew	1	pH		8.0	8.0						
				DO (mg/L)		6.9	6.4						
				Salinity (ppt)		20.5	36.8						
SG	5/4	48 Hr	1	pH		7.9	7.7						
				DO (mg/L)		6.9	6.2						
		25 ± 1		Salinity (ppt)		21.5	36.5						
		Renew	1	pH		7.8	8.0						
				DO (mg/L)		6.9	6.7						
				Salinity (ppt)		21.4	34.0						
AK	5/5	72 Hr	1	pH		7.7	7.7						
				DO (mg/L)		7.6	6.8						
		25 ± 1		Salinity (ppt)		23.7	31.8						
		Renew	1	pH		7.9	8.0						
				DO (mg/L)		7.8	7.2						
				Salinity (ppt)		20.6	31.5						
TT	5/6	96 Hr	1	pH		7.8	7.8						
				DO (mg/L)		6.6	6.2						
		25 ± 1		Salinity (ppt)		19.8	38.4						
		Renew	1	pH		7.8	7.9						
				DO (mg/L)		7.0	6.3						
				Salinity (ppt)		19.9	35.4						
GJ	5/7	120 Hr	1	pH		8.0	7.8						
				DO (mg/L)		7.1	6.0						
		25 ± 1		Salinity (ppt)		20.9	33.5						
		Renew	1	pH		8.0	8.0						
				DO (mg/L)		7.0	6.8						
				Salinity (ppt)		21.0	35.7						
GJ	5/8	144 Hr	1	pH		7.9	7.8						
				DO (mg/L)		7.0	6.2						
		25 ± 1		Salinity (ppt)		21.5	32.6						
		Renew	1	pH		8.0	8.0						
				DO (mg/L)		7.3	6.8						
				Salinity (ppt)		20.5	35.5						
CAP	5/9	168 Hr	1	pH		7.8	7.8						
				DO (mg/L)		7.2	6.3						
		25 ± 1		Salinity (ppt)		24.36	35.0						

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen, Salinity

Chronic **Menidia beryllina**

Client: Natural Energy Laboratory of
Facility: Hawaii Ocean Science and
Outfall: Bivalve Farm

Lab Number: 97326
Dilution Water(s): Synthetic Lab
Test Begin Date: May 2, 2025

ANALYST	DATE	TIME	SX#	UNIT	%	Concentration							
						Control	100						
GJ	5/2	Start	1	pH		7.9	7.7						
				DO (mg/L)		6.6	5.4						
		25 ± 1		Salinity (ppt)		20.3	36.7						
GJ	5/3	24 Hr	1	pH		7.9	7.8						
				DO (mg/L)		6.8	6.0						
		25 ± 1		Salinity (ppt)		20.5	32.4						
		Renew	1	pH		8.0	8.0						
				DO (mg/L)		6.9	6.4						
				Salinity (ppt)		20.5	36.8						
SG	5/4	48 Hr	1	pH		7.8	7.8						
				DO (mg/L)		6.1	5.9						
		25 ± 1		Salinity (ppt)		34.0	38.5						
		Renew	1	pH		7.8	8.0						
				DO (mg/L)		6.9	6.7						
				Salinity (ppt)		21.4	34.0						
AK	5/5	72 Hr	1	pH		7.9	7.8						
				DO (mg/L)		7.6	6.9						
		25 ± 1		Salinity (ppt)		22.7	38.2						
		Renew	1	pH		7.9	8.0						
				DO (mg/L)		7.8	7.2						
				Salinity (ppt)		20.6	31.5						
TT	5/6	96 Hr	1	pH		7.7	7.8						
				DO (mg/L)		6.4	6.1						
		25 ± 1		Salinity (ppt)		22.8	38.4						
		Renew	1	pH		7.8	7.9						
				DO (mg/L)		7.0	6.3						
				Salinity (ppt)		19.9	35.4						
GJ	5/7	120 Hr	1	pH		7.9	7.8						
				DO (mg/L)		7.2	6.3						
		25 ± 1		Salinity (ppt)		22.0	38.6						
		Renew	1	pH		8.0	8.0						
				DO (mg/L)		7.0	6.8						
				Salinity (ppt)		21.0	35.7						
GJ	5/8	144 Hr	1	pH		7.9	7.8						
				DO (mg/L)		7.2	6.5						
		25 ± 1		Salinity (ppt)		21.3	37.1						
		Renew	1	pH		8.0	8.0						
				DO (mg/L)		7.3	6.8						
				Salinity (ppt)		20.5	35.5						
CAP	5/9	168 Hr	1	pH		8.0	7.9						
				DO (mg/L)		7.2	7.3						
		25 ± 1		Salinity (ppt)		23.4	32.6						

Appendix B

Americamysis bahia

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

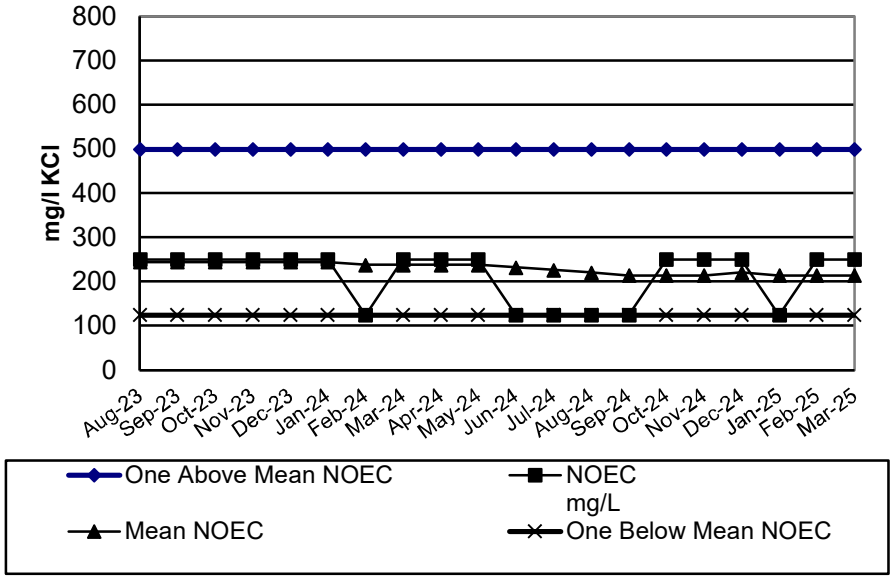
Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals 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

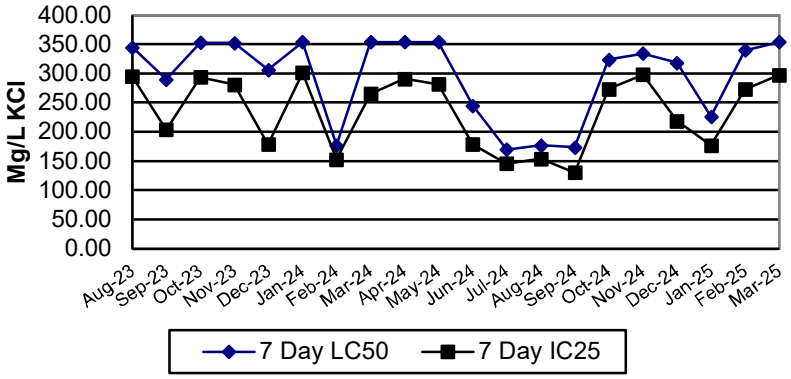
DILUTION WATER:	Standard Synthetic Saltwater						
CHEMICAL:	Potassium Chloride						
DURATION:	7 Days						
TEST NUMBER:	165						
PROJECT NUMBER:	97618						
START DATE:	3/25/2025						
START TIME:	15:26						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	25	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

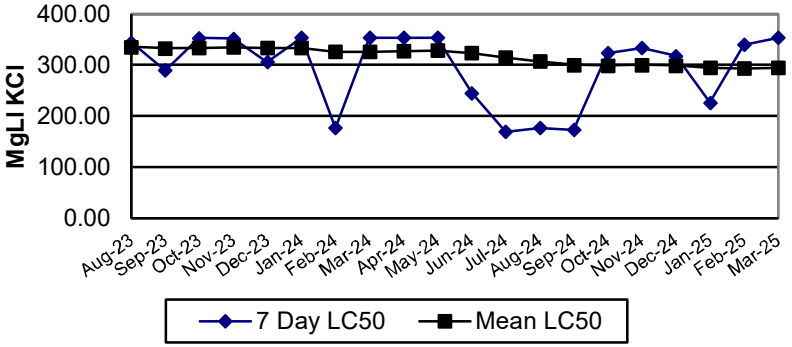
Mysid Chronic Survival Control Chart



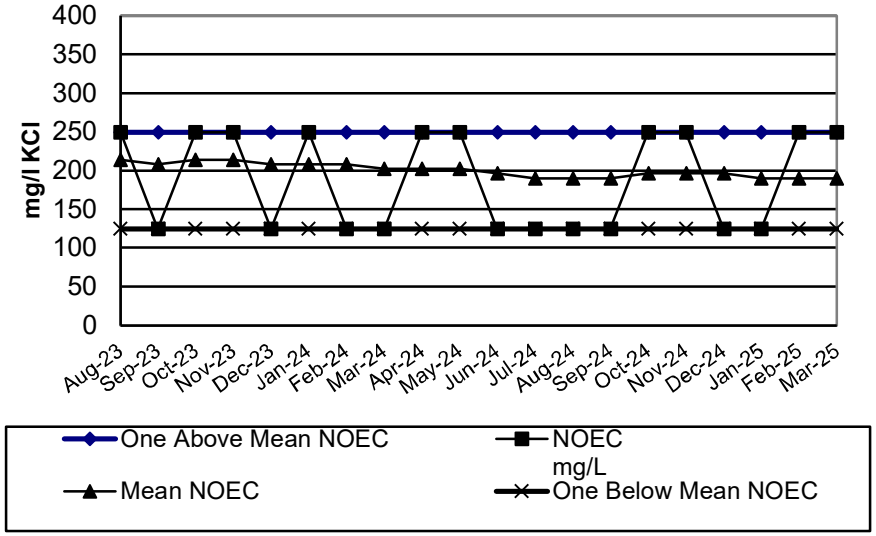
Mysid 7-Day LC50 & IC25



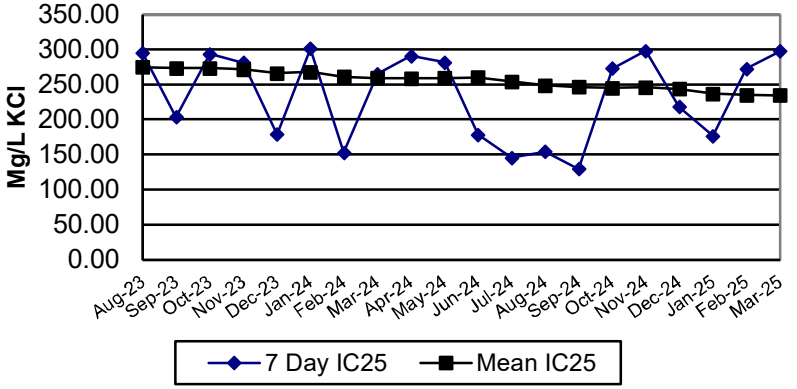
Mysid 7-Day LC50



Mysid Chronic Growth Control Chart



Mysid 7-Day IC25



Appendix B

Menidia beryllina

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

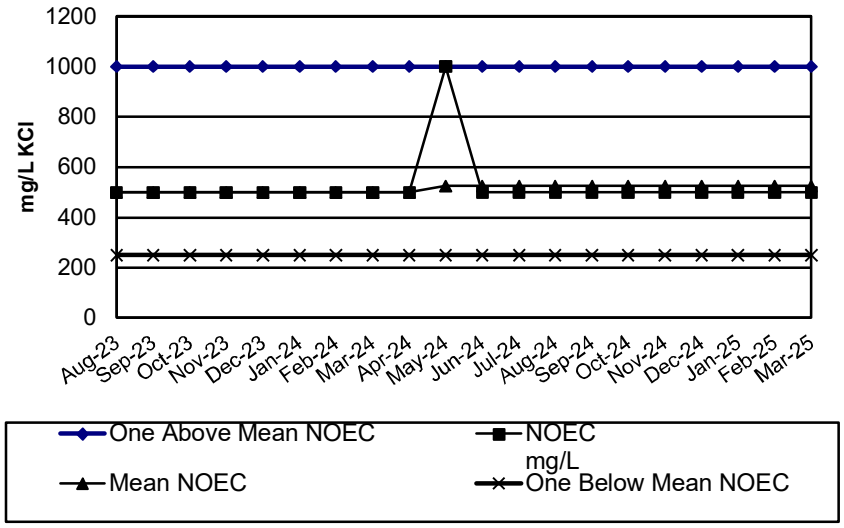
Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals 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

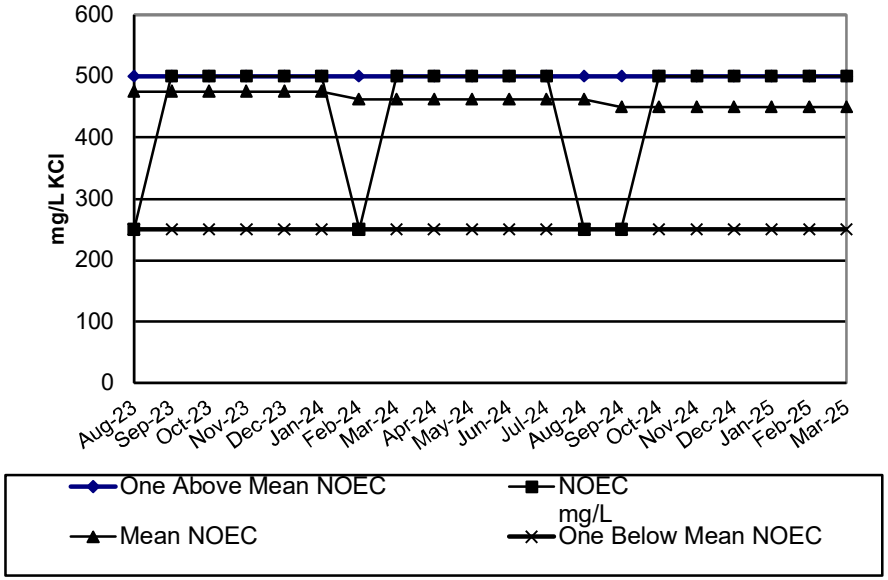
DILUTION WATER:	Standard Synthetic Saltwater						
CHEMICAL:	Potassium Chloride						
DURATION:	7 Days						
TEST NUMBER:	165						
PROJECT NUMBER:	97619						
START DATE:	3/25/2025						
START TIME:	15:42						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	125	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

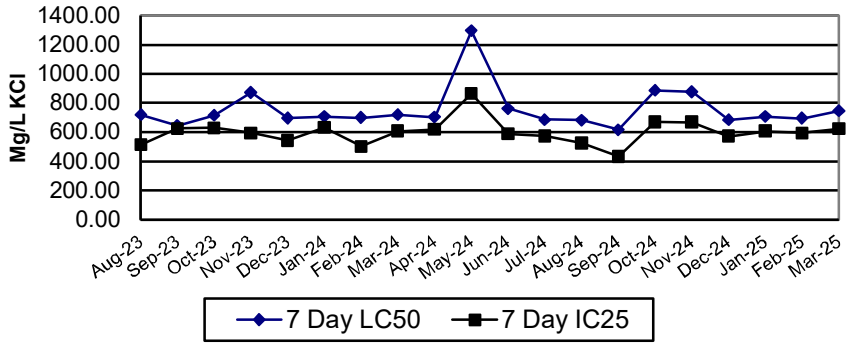
Menidia Chronic Survival Control Chart



Menidia Chronic Growth Control Chart



Menidia 7-Day LC50 & IC25



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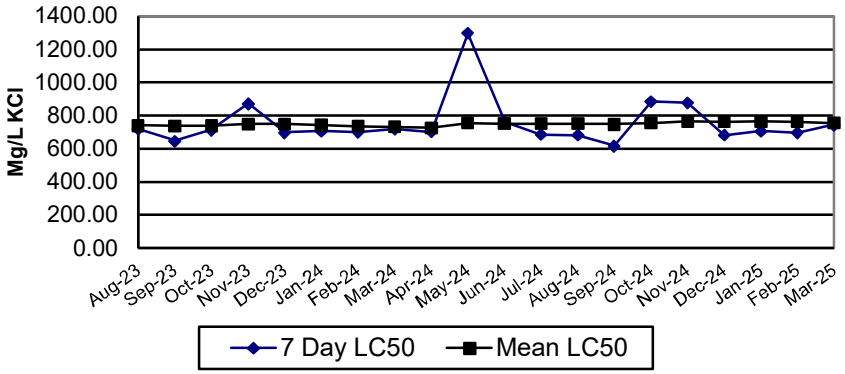

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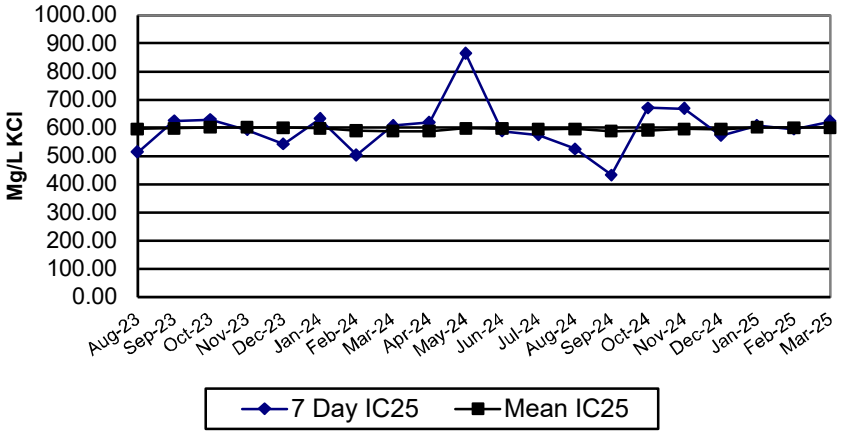

APPENDIX C

LITERATURE REFERENCES

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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.

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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 : 97326

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

Sample No: 97326 –

Revision 2 Effective Date 9/25/2017

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

P.O. No:

Client: Natural Energy Laboratory of Hawaii

Facility: Bivalve Farm

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

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

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

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex* (water flea)	*D. magna* (water flea)	*P. promelas* (minnow)	*Selenastrum* (green algae)	*M. beryllina* (minnow)	*Mysidopsis* (shrimp)
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: Non-Routine/Specialty Testing for Information purpose

C.

Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1 Bivalve Farm	other	4/29/25	—	0801	—	g	Pam Madden	1
2				0814 (Pam)	0814			
3								

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	Pam Madden	4/29/25	1215			
2						
3					5-2-25	1022

Bio-Aquatic Sample Login

BAT sample personnel: ● Yes ● No

Dechlorinate Sample: ○ Yes ○ No

Dilution Water: ○ Receiving Stream ◉ Synthetic Lab

Date: 5-2-25	Time: 1240	By: S	Temperature: 2.9 (C)	IR#026	
Chlorine: <0.1 mg/l	Ammonia: <0.25 mg/l	Int. Sal\Cond: 32.1 ppt/uS	Adj. Salinity	ppt	
pH: 8.1	Hardness: mg/l	Other			
DO: 7.3 mg/l	Alkalinity: mg/l	Condition: Good			

Report Date 06/30/2025 Revision 1

Bio-Aquatic Lab ID: 97326

Bio-Aquatic Testing, Inc.

TCEQ TNI ACCREDITED

Report Revision Form

Report Revision Number 0 **for Lab ID** 97326 **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

FORM 10.6 Revision 1 *Effective: 08/07/2018*