



**Natural Energy Laboratory of Hawaii Authority
Shrimp Farm**

Chronic Biomonitoring Report

97324

Americamysis bahia
Menidia beryllina

May 02, 2025

Approved by: Justin Reed
Lab director

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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: Shrimp Farm
Laboratory Number: 97324
Date: May 02, 2025

SAMPLE COLLECTION: A grab sample from the Natural Energy Laboratory of Hawaii Authority, Shrimp Farm, was transported to Bio-Aquatic Testing on May 02, 2025. The Bivalve Farm sample was collected from the pre-treatment inflow seawater stream into larval tanks by facility and client 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 14:12 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 13:45 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 14:10 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 13:48 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: 97324

Permit Number: N/A

Test Temperature (oC): 25 ± 1

Sample Type: Grab

Outfall Name: Shrimp Farm

Photo Period: 16 Hours Light
8 Hours Dark

Receiving Water Name:

Begin Date: 5/2/2025

Test Start Time:

Test End Time:

End Date: 5/9/2025

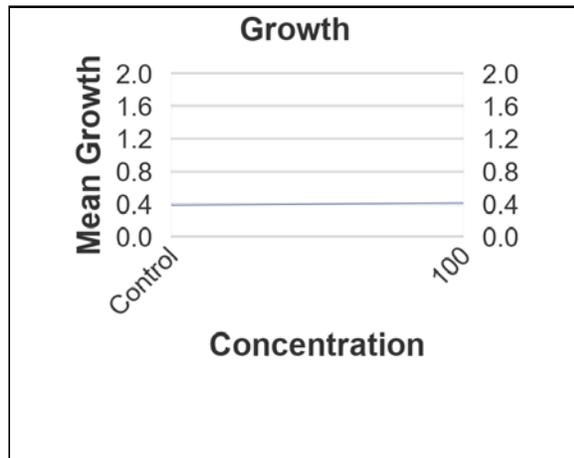
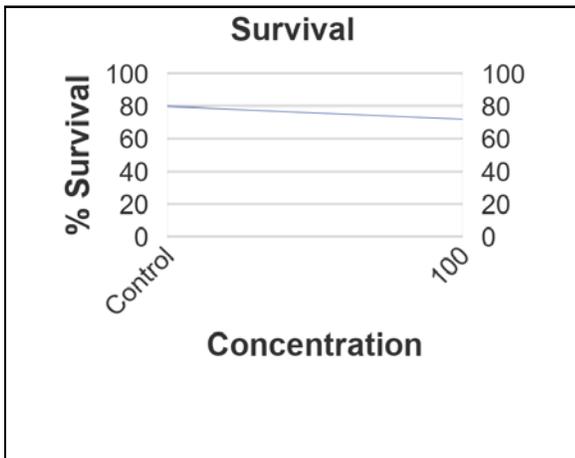
SURVIVAL

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	4	4	4	80.0%
	B	5	5	5	5	5	5	5	5	
	C	5	5	5	5	5	3	3	3	
	D	5	5	4	4	4	4	4	4	
	E	5	5	5	5	5	5	5	4	
100	A	5	5	5	5	5	5	5	5	72.0%
	B	5	5	5	5	4	3	3	2	
	C	5	5	5	5	5	5	5	5	
	D	5	5	3	3	3	3	3	3	
	E	5	5	4	4	3	3	3	3	
	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									
A									
B									
C									
D									
E									
A									
B									
C									
D									
E									
A									
B									
C									
D									
E									

Concentration Response Relationships



BIO-AQUATIC TESTING, INC.

Chronic Americamysis bahia SURVIVAL Lab ID: **97324**

Client: Natural Energy Laboratory of Hawaii Facility Shrimp 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 2

	DATE/TIME/ TECHNICIAN	Dilution: Control					Dilution: 100																			
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E					
0Hr	1412 AB 5-2-25	5	—	—	—	—	5	—	—	—	—															
24Hr	5-3-25 1112 M	5	—	—	—	—	5	—	—	—	—															
48Hr	5-4-25 SG 1008	5	—	4	5	5	5	—	3 ₂	4 ₁	5															
72Hr	5-5-25 1250 AK	5	—	4	5	5	5	—	3	4	5															
96Hr	5-6-25 AB1056	5	—	4	5	5	5	4	5	3	3 ₁															
5 days	5-7-25 020940	4	5	3 ₂	4	5	5	3 ₁	5	3	3															
6 days	5-8-25 07411	4	5	3	4	5	5	3	5	3	3															
7 days	5-9-25 AB1345	4	5	3	4	4 ₀	5	2 ₀	5	3	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	Americamysis bahia SURVIVAL	Lab ID: 97324
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Client: Natural Energy Laboratory of Hawaii Facility Shrimp 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	25.6	25.1	25.8	25.3	25.8	25.1	25.4	25.4	25.5	25.0	25.6	25.2	25.5
100														
	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/	/	/	/	/	/	/
TIME/DATE TECH	5-2-25 AB 1412	5-3-25 1114	5-3-25 1114	5-4-25 56	5-4-25 1008	5-5-25 1258	5-5-25 AK	5-6-25 AB 1030	5-6-25 1030	5-7-25 1549	5-7-25 1549	5-8-25 1549	5-8-25 1549	5-9-25 AB 134
IR GUN ID #	013	013	013	013	013	013	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 Americamysis bahia

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

Lab ID: 97324

Permit Number: N/A

Sample Type: Grab

Outfall Name: Shrimp 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	2.760	0.552
B	5	1.580	0.316
C	5	2.520	0.504
D	5	1.820	0.364
E	5	1.810	0.362
Mean		C.V. %	
0.420		24.36	

	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: **97324**

Client: Natural Energy Laboratory of Hawaii - Shrimp Farm

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	4	1.770
B	5	2.490
C	3	1.920
D	4	1.090
E	4	1.070
F		
G		
H		

	Qty.	Wt.
A	5	2.760
B	2	96% 1.590
C	5	2.520
D	3	1.820 ^{96%}
E	3	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: 97324

Permit Number: N/A

Test Temperature (oC): 25 ± 1

Outfall Name: Shrimp Farm

Sample Type: Grab

Photo Period: 16 Hours Light
8 Hours Dark

Receiving Water Name:

Test Start Time:

Test End Time:

Begin Date: 5/2/2025

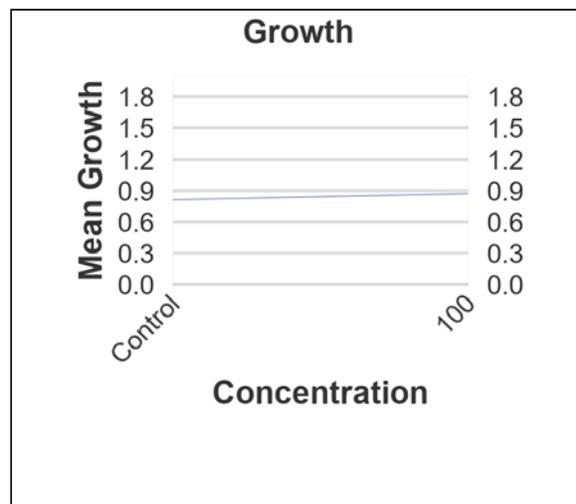
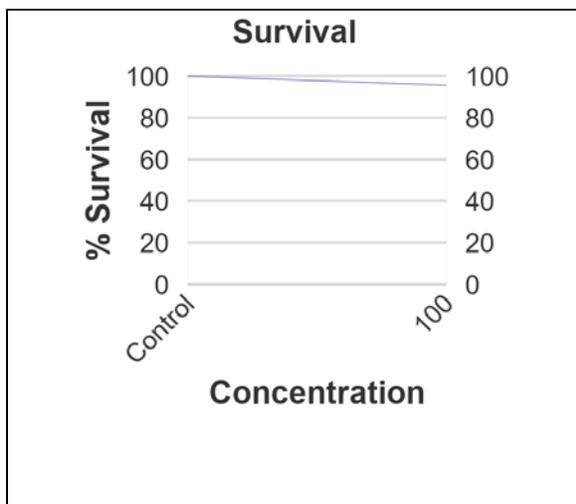
End Date: 5/9/2025

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	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	7	95.8%
	B	8	8	8	8	8	8	8	8	
	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: **97324**

Client: Natural Energy Laboratory of Hawaii Facility: Shrimp 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	<u>Control</u>					<u>100</u>																			
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E					
0Hr	5-2-25 OB 1410	8	—				8	—																		
24Hr	5-3-25 1114 M	8	—				8	—																		
48Hr	5-4-25 SB 1000	8	—				8	—																		
72Hr	5-5-25 1300 AM	8	—				8	—																		
96Hr	5-6-25 OB 1100	8	—				8	—																		
5 days	5-7-25 OB 370	8	—				8	—																		
6 days	5-8-25 OB 1314	8	—				8	—																		
7 days	5-9-25 OB 1345	8	—				7	8	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																				

BIO-AQUATIC TESTING, INC.

Chronic Menidia beryllina SURVIVAL Lab ID: **97324**

Client: Natural Energy Laboratory of Hawaii Facility Shrimp Farm Outfall: _____
 Sample Type: Grab

TEST INSTRUCTIONS: Mysisid 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	25.8 25.0	25.4 25.3	25.9 24.8	25.4 25.4	25.5 25.0	25.6 25.2	25.5
100								
	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/
TIME/DATE TECH	5-2-25 AB 1410	5-3-25 1117 M	5-4-25 8V 100W	5-5-25 1300 AL	5-6-25 AB 1100	5-7-25 2857	5-8-25 1344	5-9-25 AB 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.

Chronic *Menidia beryllina*

Client: Natural Energy Laboratory of Hawaii Ocean Science and Technology

Lab ID: 97324

Permit Number: N/A

Sample Type: Grab

Outfall Name: Shrimp Farm

Receiving Water Name:

Synthetic

100

	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					

	ON	Wt.	Avg.
A	8	6.820	0.853
B	8	7.580	0.948
C	8	6.610	0.826
D			
E			

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %
0.815	8.4

Mean	C.V. %
0.875	7.3

Mean	C.V. %

Mean	C.V. %

SN Mean	SN C.V. %
0.815	8.4

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

Mean	C.V. %

Mean	C.V. %

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: **97324**

Client: Natural Energy Laboratory of Hawaii - Shrimp 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

100 %

	Qty.	Wt.
A	9	6.940
B	1	6.730
C	1	5.900
D		
E		

	Qty.	Wt.
A	7	6.820
B	8	7.580
C	8	6.610
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: 97324.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	5	2	2	1

Calculated Chi-Square goodness of fit test statistic = 4.5231
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

mysid growth
 File: 97324.myg Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.007	
2	100	0.010	1.431

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

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

mysid growth
 File: 97324.myg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.003	0.003	0.341
Within (Error)	8	0.071	0.009	
Total	9	0.074		

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

mysid growth
 File: 97324.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	0.420	0.420	-0.584	

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	0.420	0.420	-0.584	

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

mysid growth
 File: 97324.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	0.111	28.8	-0.035

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	0.113	29.3	-0.035

menidia growth
File: 97324.meg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.018

W = 0.948

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: 97324.meg Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.005	
2	100	0.004	1.146

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

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

menidia growth
File: 97324.meg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.005	0.005	1.226
Within (Error)	4	0.018	0.004	

Total 5 0.023

Critical F value = 7.71 (0.05,1,4)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All equal

menidia growth

File: 97324.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	0.816	0.816		
2	100	0.876	0.876	-1.107	

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	0.816	0.816		
2	100	0.876	0.876	-1.107	

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

menidia growth

File: 97324.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	0.116	14.2	-0.060

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	0.116	14.2	-0.060

1	con	3			
2	100	3	0.128	15.6	-0.060

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 97324

Outfall Name: Shrimp 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	10:30	04/29/25	04/29/25	08:01	08:01

Type of Test(s)	
<u>Americamysis bahia</u>	<u>Chronic</u>
<u>Menidia beryllina</u>	<u>Chronic</u>

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

Lab ID: 97324

Facility: Hawaii Ocean Science and

Dilution Water(s): Synthetic Lab

Outfall: Shrimp Farm

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	10:30	<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	6.6	31.9	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: 97324

Facility: Hawaii Ocean Science and

Dilution Water(s): Synthetic Lab

Outfall: Shrimp Farm

Test Begin Date: May 2, 2025

ANALYST	DATE	TIME	SX#	UNIT	%	Concentration							
						Control	100						
GJ	5/2	Start	1	pH	7.8	7.9							
		25 ± 1		DO (mg/L)	7.0	6.5							
				Salinity (ppt)	20.7	36.7							
GJ	5/3	24 Hr	1	pH	7.9	7.9							
		25 ± 1		DO (mg/L)	7.0	6.6							
				Salinity (ppt)	20.7	36.7							
		Renew	1	pH	8.0	8.0							
				DO (mg/L)	7.1	6.5							
				Salinity (ppt)	20.6	37.0							
SG	5/4	48 Hr	1	pH	7.8	7.9							
		25 ± 1		DO (mg/L)	7.1	6.2							
				Salinity (ppt)	21.4	39.1							
		Renew	1	pH	8.2	8.1							
				DO (mg/L)	7.4	6.7							
				Salinity (ppt)	19.1	33.9							
AK	5/5	72 Hr	1	pH	7.8	7.8							
		25 ± 1		DO (mg/L)	6.9	6.6							
				Salinity (ppt)	21.0	33.1							
		Renew	1	pH	8.0	8.1							
				DO (mg/L)	7.6	7.1							
				Salinity (ppt)	21.0	32.7							
GJ	5/6	96 Hr	1	pH	7.8	7.8							
		25 ± 1		DO (mg/L)	6.6	5.9							
				Salinity (ppt)	19.8	35.2							
		Renew	1	pH	7.8	7.9							
				DO (mg/L)	7.0	6.4							
				Salinity (ppt)	19.9	35.5							
GJ	5/7	120 Hr	1	pH	7.8	7.9							
		25 ± 1		DO (mg/L)	6.8	6.1							
				Salinity (ppt)	22.8	32.3							
		Renew	1	pH	8.0	8.0							
				DO (mg/L)	7.2	6.8							
				Salinity (ppt)	21.4	34.7							
GJ	5/8	144 Hr	1	pH	7.9	7.8							
		25 ± 1		DO (mg/L)	7.0	6.1							
				Salinity (ppt)	21.5	34.5							
		Renew	1	pH	8.1	8.0							
				DO (mg/L)	7.1	6.8							
				Salinity (ppt)	20.6	34.8							
CAP	5/9	168 Hr	1	pH	7.8	7.6							
		25 ± 1		DO (mg/L)	8.0	6.4							
				Salinity (ppt)	23.9	37.7							

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen, Salinity

Chronic

Menidia beryllina

Client: Natural Energy Laboratory of

Lab Number: 97324

Facility: Hawaii Ocean Science and

Dilution Water(s): Synthetic Lab

Outfall: Shrimp Farm

Test Begin Date: May 2, 2025

ANALYST	DATE	TIME	SX#	UNIT	%	Concentration							
						Control	100						
GJ	5/2	Start	1	pH		7.8	7.9						
		25 ± 1		DO (mg/L)		7.0	6.5						
				Salinity (ppt)		20.7	36.7						
GJ	5/3	24 Hr	1	pH		7.9	7.8						
		25 ± 1		DO (mg/L)		7.0	6.4						
				Salinity (ppt)		20.6	32.6						
	Renew	1	pH		8.0	8.0							
			DO (mg/L)		7.1	6.5							
			Salinity (ppt)		20.6	37.0							
SG	5/4	48 Hr	1	pH		7.8	7.9						
		25 ± 1		DO (mg/L)		7.1	6.3						
				Salinity (ppt)		21.4	34.1						
	Renew	1	pH		8.2	8.1							
			DO (mg/L)		7.4	6.7							
			Salinity (ppt)		19.1	33.9							
AK	5/5	72 Hr	1	pH		7.9	7.8						
		25 ± 1		DO (mg/L)		6.6	6.8						
				Salinity (ppt)		21.8	38.6						
	Renew	1	pH		8.0	8.1							
			DO (mg/L)		7.6	7.1							
			Salinity (ppt)		21.0	32.7							
GJ	5/6	96 Hr	1	pH		7.7	7.8						
		25 ± 1		DO (mg/L)		6.4	6.2						
				Salinity (ppt)		22.8	39.1						
	Renew	1	pH		7.8	7.9							
			DO (mg/L)		7.0	6.4							
			Salinity (ppt)		19.9	35.5							
GJ	5/7	120 Hr	1	pH		7.9	7.8						
		25 ± 1		DO (mg/L)		7.2	6.3						
				Salinity (ppt)		21.9	37.9						
	Renew	1	pH		8.0	8.0							
			DO (mg/L)		7.2	6.8							
			Salinity (ppt)		21.4	34.7							
GJ	5/8	144 Hr	1	pH		7.9	7.9						
		25 ± 1		DO (mg/L)		7.3	6.4						
				Salinity (ppt)		21.4	36.7						
	Renew	1	pH		8.1	8.0							
			DO (mg/L)		7.1	6.8							
			Salinity (ppt)		20.6	34.8							
CAP	5/9	168 Hr	1	pH		7.6	7.1						
		25 ± 1		DO (mg/L)		7.2	8.1						
				Salinity (ppt)		20.2	35.6						

Appendix B

Americamysis bahia

BIO-AQUATIC TESTING, INC.

Carrollton, TX

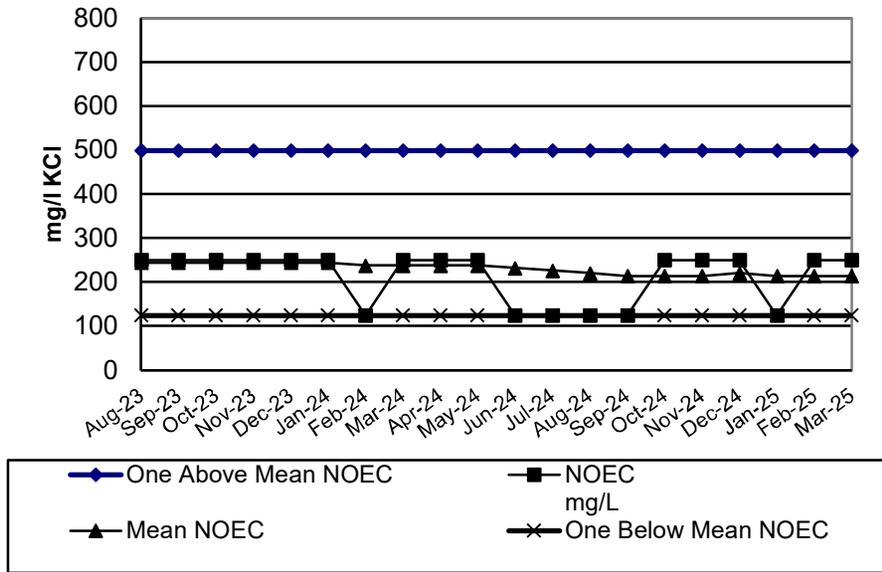
REFERENCE TOXICANTS

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.

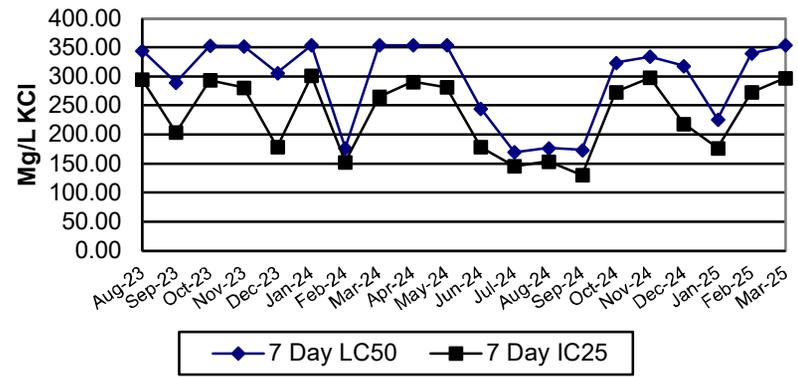
CHRONIC REFERENCE TOXICANT TEST RESULTS

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						

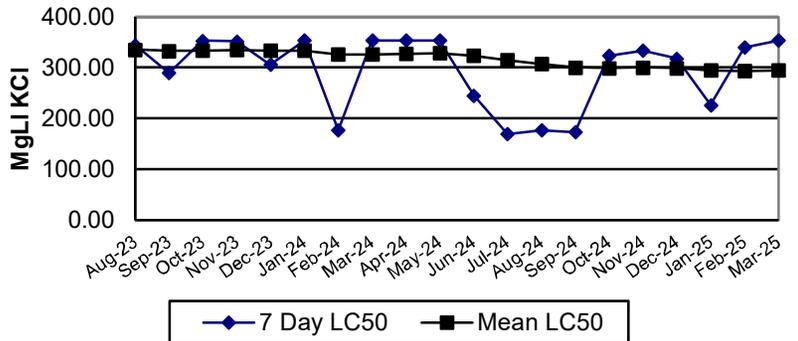
Mysid Chronic Survival Control Chart



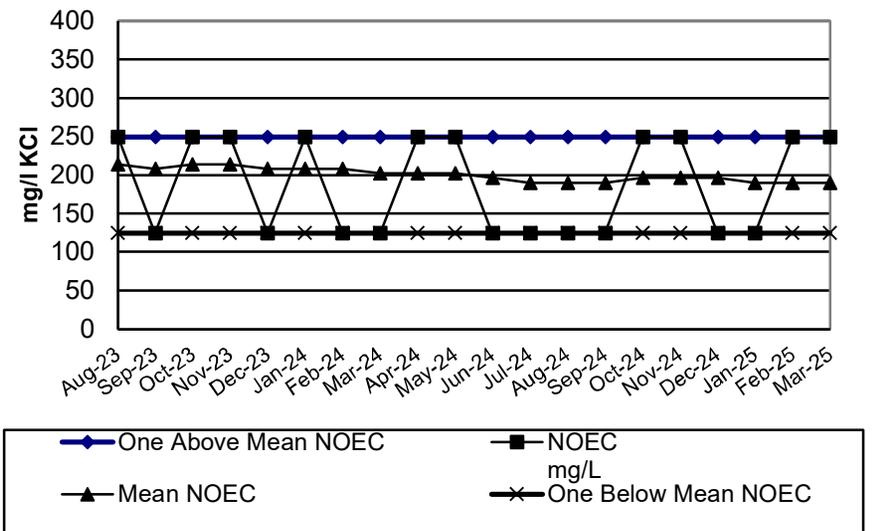
Mysid 7-Day LC50 & IC25



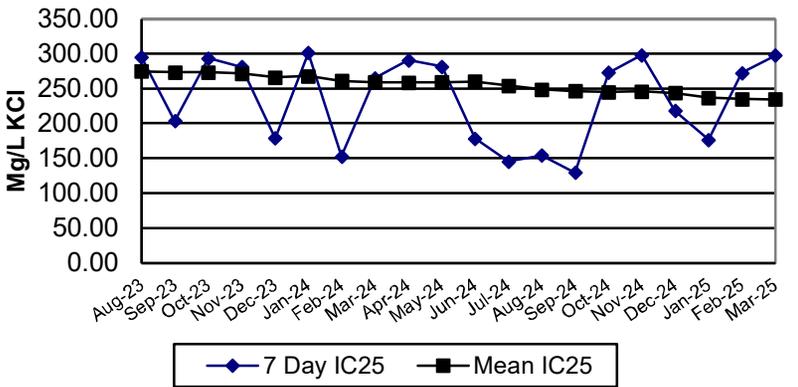
Mysid 7-Day LC50



Mysid Chronic Growth Control Chart



Mysid 7-Day IC25



Appendix B

Menidia beryllina

BIO-AQUATIC TESTING, INC.

Carrollton, TX

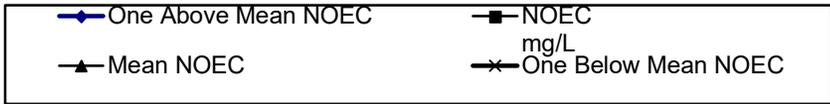
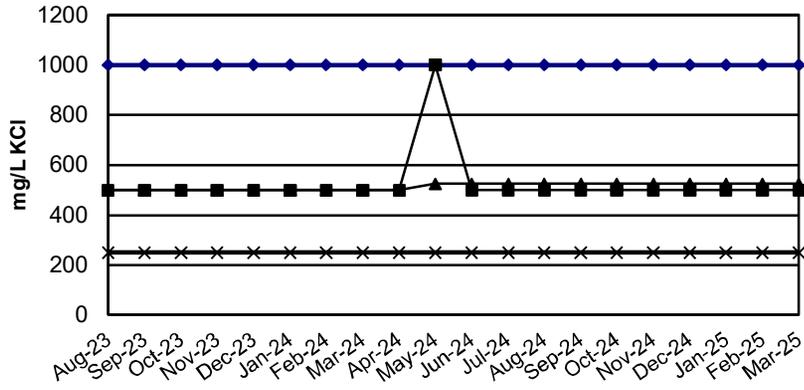
REFERENCE TOXICANTS

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.

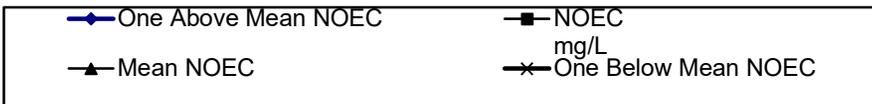
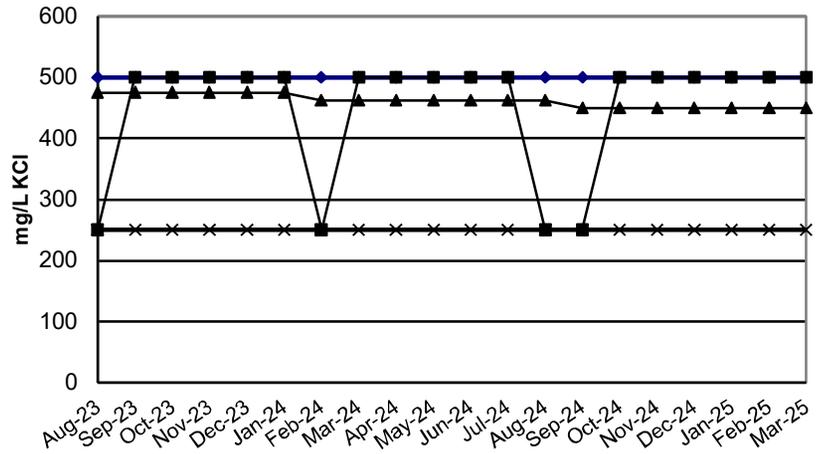
CHRONIC REFERENCE TOXICANT TEST RESULTS

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						

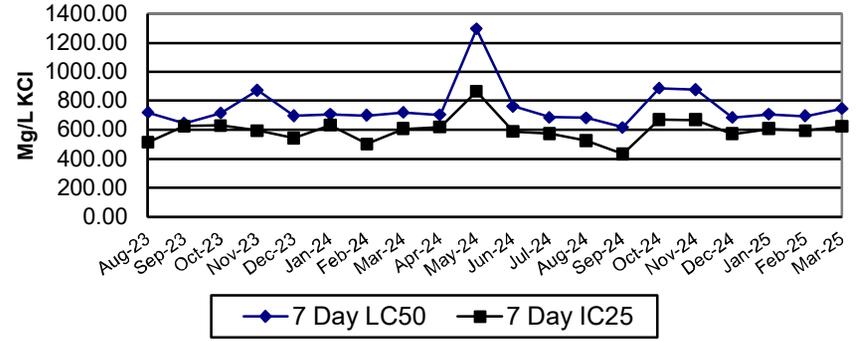
Menidia Chronic Survival Control Chart



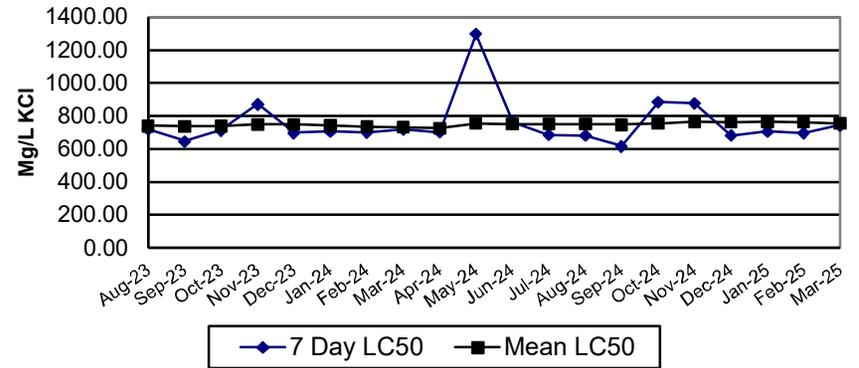
Menidia Chronic Growth Control Chart



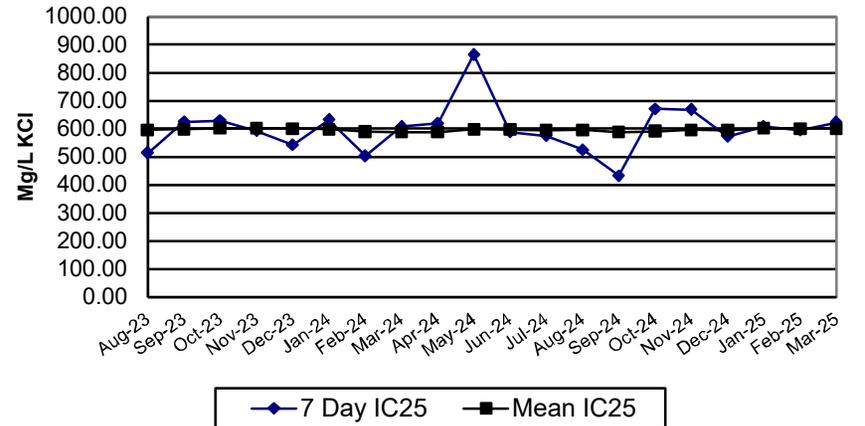
Menidia 7-Day LC50 & IC25



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

Lab Id : **97324**

Bio Only
 No Sample Left

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

Sample No: **97324**

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

Client: Natural Energy Laboratory of Hawaii

Facility: Shrimp Farm

Permit No: NA

Outfall:

Client Contact: Pam Madden

Client Phone: 808-322-9527

A. REVIEW SCHEDULED TEST(S):

Chronic	Americamysis bahia
Chronic	Menidia beryllina

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

Concentration: 100

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

C.

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

D.

Relinquished By:	Date	Time	Received By:	Date	Time
<u>Pam Madden</u>	4/29/25	12:15			

Bio-Aquatic Sample Login

BAT sample personnel:
 Yes No

Dechlorinate Sample:
 Yes No

Dilution Water:
 Receiving Stream
 Synthetic Lab

Chlorine: 10.1 mg/l Ammonia: 10.25 mg/l Int. SalCond: 31.9 ppt/uS Adj. Salinity ppt

pH: 8.1 Hardness: mg/l Other

DO: 6.6 mg/l Alkalinity: mg/l Condition: Good

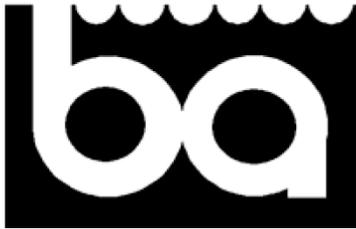
Date: 5-2-25 Time: 12:20 Temperature: 22.9 (C) IR#: 026

Signature: [Signature] Date: 5-2-25 Time: 10:30

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)	P. promelas (minnow)	Selastrium (green algae)	M. beryllina (minnow)	Mysidopsis (shrimp)
<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour

Notes: Non-Routine/Specialty Testing for Information purpose



Report Revision Form

Report Revision Number 0 for Lab ID 97324 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