

**Natural Energy Laboratory of Hawaii Authority
Hawaii Ocean Science and Technology Park
OUTFALL Shrimp Farm**

Chronic Biomonitoring Report

98041

Americamysis bahia
Menidia beryllina

August 22, 2025

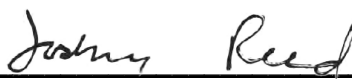
Approved by: 
Lab director

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

Eurofins Environment Testing Bio-Aquatics

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TOXICITY TEST REPORT - Chronic

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

Sample: Shrimp Farm
Laboratory Number: 98041
Date: August 22, 2025

Americamysis bahia **passed** survival and growth testing requirements. *Menidia beryllina* **passed** survival and growth testing requirements.

SAMPLE COLLECTION: A grab sample from the Natural Energy Laboratory of Hawaii Authority, Hawaii Ocean Science and Technology Park, was transported to Eurofins Environment Testing Bio-Aquatics on August 22, 2025. The effluent sample was collected from the Shrimp Farm 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 18:00 hours on August 22, 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 15:59 hours on August 29, 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 any of the sample concentrations 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 any of the sample concentrations 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 17:34 hours on August 22, 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 17:32 hours on August 29, 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 *Menidia beryllina* survival data demonstrated no statistically significant differences between the control and any of the sample concentrations 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 *Menidia beryllina* growth data demonstrated no statistically significant differences between the control and any of the sample concentrations tested.

LOEC: Not Calculable (Q)

NOEC: 100%

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TOXICITY TEST

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

Lab ID: 98041

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: 8/22/2025

Test Start Time: 18:00

Test End Time: 15:59

End Date: 8/29/2025

SURVIVAL

Effluent Con. %	Number of Alive								Avg% Surv.	
	8/22	8/23	8/24	8/25	8/26	8/27	8/28	8/29		
Control	A	5	5	5	5	5	5	5	5	88.0%
	B	5	5	5	5	5	5	4	3	
	C	5	5	5	5	5	5	5	5	
	D	5	5	5	5	5	5	5	5	
	E	5	5	4	4	4	4	4	4	
100	A	5	5	5	5	5	5	5	5	92.0%
	B	5	5	5	5	5	5	5	5	
	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	4	4	
	A									
	B									
	C									
	D									
	E									
	A									
	B									
	C									
	D									
	E									

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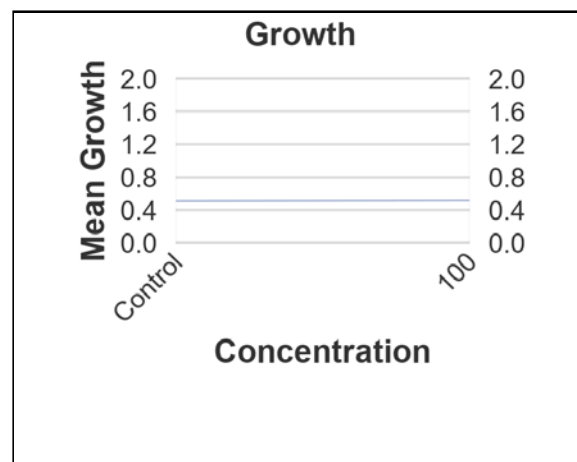
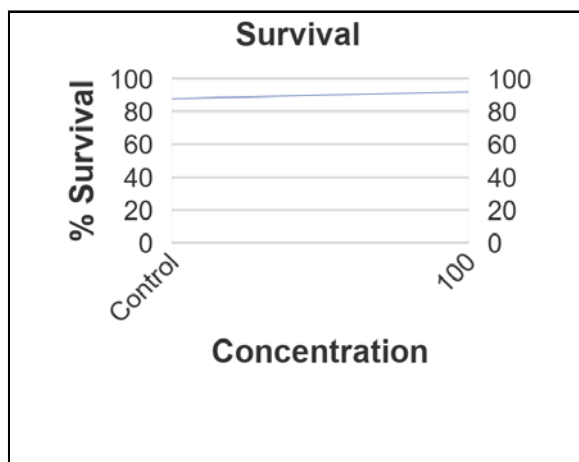
Effluent Con. %	Number Of Alive								Avg% Surv.
	8/22	8/23	8/24	8/25	8/26	8/27	8/28	8/29	
A									
B									
C									
D									
E									

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: **98041**

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

Outfall: Shrimp Farm
Sample Type: Grab

TEST INSTRUCTIONS: Mysisid test is Abbreviated Reps (only need 5 NOT 8) Menidia is 3 reps of 8

Culture No. : MY-25-227

Photo Period: 16hr light, 8hr dark

RANDOMIZATION: SC-5 2

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	8-22-25 SB 1800	5	---	---	---	---	5	---	---	---	---										
24Hr	8-23-25 DT 1048	5	---	---	---	---	5	---	---	---	---										
48Hr	8-24-25 SB 1037	5	---	---	---	40	5	---	---	---	---										
72Hr	8-25-25 CC 1530	5	---	---	---	4	5	---	---	---	---										
96Hr	8-26-25 AB 0801	5	---	---	---	4	5	---	---	---	---										
5 days	8-27-25 5 1100	5	---	---	---	4	5	5	4	5	5										
6 days	8-28-25 AB 1420	5	40	5	5	4	5	5	4	5	40										
7 days	8-29-25 SB 1551	5	3	5	5	4	5	5	4	5	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																			

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BIO-AQUATIC TESTING, INC.

Chronic

Americamysis bahia SURVIVAL

Lab ID: **98041**

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

Outfall: Shrimp Farm
Sample Type Grab

TEST INSTRUCTIONS: Mysid test is Abbreviated Reps (only need 5 NOT 8) Menidia is 3 reps of 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	25.4	25.6 25.5	25.5 25.6	25.5 25.7	25.5 25.5	25.3 25.4	25.5 25.5	25.8
100	25.8	1 25.6	1 1	1 1	1 1	1 1	1 1	1
TIME/DATE TECH	8-22-25 JB 1800	8-23-25 DT 1049	8-24-25 JB 1037	8-25-25 CC 1930	8-26-25 AB 0801	8-27-25 JB 1100	8-28-25 AB 1420	8-29-25 JB 1539
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 *Americamysis bahia*Client: Natural Energy Laboratory of Hawaii Ocean Science and Technology Park

Lab ID: 98041

Permit Number: N/A

Sample Type: Grab

Outfall Name: Shrimp Farm

Receiving Water Name:

Synthetic**100**

	ON	SN	Wt.	Avg.	SN Avg.
A	5	5	2.79	0.558	0.558
B	5	3	1.82	0.364	0.607
C	5	5	2.99	0.598	0.598
D	5	5	2.78	0.556	0.556
E	5	4	2.39	0.478	0.598

Mean	C.V. %
0.511	18.18

SN Mean	SN C.V. %
0.583	4.2

	ON	Wt.	Avg.
A	5	2.13	0.426
B	5	2.59	0.518
C	5	2.59	0.518
D	5	2.92	0.584
E	5	2.68	0.536

Mean	C.V. %
0.516	11.10

	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.

Chronic

Americamysis bahia TOXICITY TEST

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

Lab ID: **98041**

Balance: Radwag BAL-007

Begin Date: 8/22/2025

End Date: 8/29/2025

Organism: Americamysis bahia

Analyst: FD
Weigh Date: 9/12/25

Date/Time placed in Oven: 8/29/25 1740
Date/Time removed from Oven: 8/30/25 1740

Control

100 %

	Qty.	Wt.
A	5	2.79
B	3	1.82
C	5	2.99
D	5	2.78
E	4	2.39

	Qty.	Wt.
A	5	2.13
B	5	2.59
C	4	2.59
D	5	2.92
E	4	2.68

	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		

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Eurofins Environment Testing Bio-Aquatics

TOXICITY TEST

Chronic *Menidia beryllina*

Client: Natural Energy Laboratory of Hawaii Ocean Science and Technology

Lab ID: 98041

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: 8/22/2025

Test Start Time: 17:34

Test End Time: 17:32

End Date: 8/29/2025

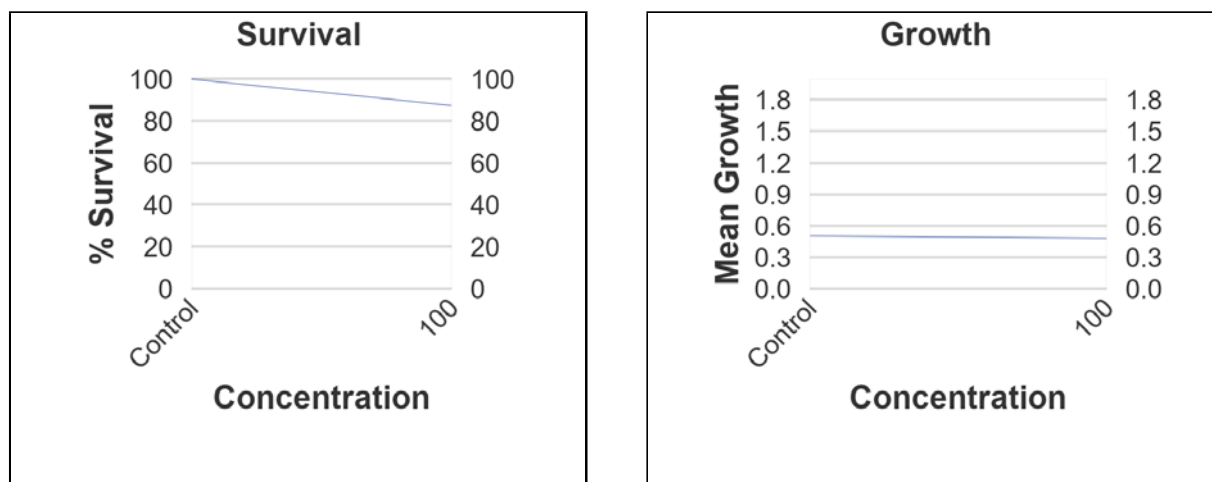
SURVIVAL

Effluent Con.	Number Of Alive								Avg% Surv.
	8/22	8/23	8/24	8/25	8/26	8/27	8/28	8/29	
Control	A	8	8	8	8	8	8	8	100.0%
	B	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	
	D								
100	A	8	8	8	8	8	7	7	87.5%
	B	8	8	8	8	8	7	7	
	C	8	8	8	8	8	7	7	
	D								
	A								
	B								
	C								
	D								
	A								
	B								
	C								
	D								

Eurofins Environment Testing Bio-Aquatics

Effluent Con.	Number Of Alive								Avg% Surv.
	8/22	8/23	8/24	8/25	8/26	8/27	8/28	8/29	
	A								
	B								
	C								
	D								
	A								
	B								
	C								
	D								
	A								
	B								
	C								
	D								
	A								
	B								
	C								
	D								

Concentration Response Relationships



Eurofins Environment Testing Bio-Aquatics

Chronic

Menidia beryllina SURVIVAL

Lab ID: 98041

Client: Natural Energy Laboratory of Hawaii Facility: Hawaii Ocean Science andOutfall: Shrimp Farm
Sample Type: Grab

TEST INSTRUCTIONS: Mysid test is Abbreviated Reps (only need 5 NOT 8) Menidia is 3 reps of 8

Culture No. : PN-25-223

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	8-22-25	8	—	—	—	—	8	—	—	—	—										
	SG 1734																				
24Hr	8-23-25	8	—	—	—	—	8	—	—	—	—										
	DT 1040																				
48Hr	8-24-25	8	—	—	—	—	8	—	—	—	—										
	SG 1044																				
72Hr	8-25-25	8	—	—	—	—	8	—	—	—	—										
	CC 1540																				
96Hr	8-26-25	8	—	—	—	—	8	—	—	—	—										
	AB 0745																				
5 days	8-27-25	8	—	—	—	—	8	7	7	—	—										
	SG 1210																				
6 days	8-28-25	8	—	—	—	—	7	7	7	—	—										
	AB 1428																				
7 days	8-29-25	8	—	—	—	—	7	—	—	—	—										
	SG 1732																				

Dilution:

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

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BIO-AQUATIC TESTING, INC.

Chronic

Menidia beryllina SURVIVAL

Lab ID: 98041

Client: Natural Energy Laboratory of Hawaii Facility Hawaii Ocean Science and

Outfall: Shrimp Farm
Sample Type: Grab

TEST INSTRUCTIONS: Mysis test is Abbreviated Reps (only need 5 NOT 8) Menidia is 3 reps of 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	25.10	25.6 21.6	25.5 25.4	25.5 25.7	25.3 25.5	25.3 25.3	25.3 25.3	25.3
100	1	1 1	1 1	1 1	1 1	1 1	1 1	1
TIME/DATE TECH	8-22-25 SB 1734	8-23-25 DT 1640	8-24-25 SB 1044	8-25-25 CC 1540	8-26-25 AB 0745	8-27-25 J 1210	8-28-25 AB 1428	8-29-25 SB 1732
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: 98041

Permit Number: N/A

Sample Type: Grab

Outfall Name: Shrimp Farm

Receiving Water Name:

Synthetic						100											
	ON	SN	Wt.	Avg.	SN		ON	Wt.	Avg.		ON	Wt.	Avg.		ON	Wt.	Avg.
A	8	8	3.62	0.453	0.453	A	8	3.89	0.486	A				A			
B	8	8	4.24	0.530	0.530	B	8	3.99	0.499	B				B			
C	8	8	4.26	0.533	0.533	C	8	3.72	0.465	C				C			
D						D				D				D			
Mean		C.V. %				Mean		C.V. %		Mean		C.V. %		Mean		C.V. %	
0.505		9.01				0.483		3.53									
SN Mean		SN C.V. %															
0.505		9.01															

	ON	Wt.	Avg.		ON	Wt.	Avg.		ON	Wt.	Avg.		ON	Wt.	Avg.
A				A				A				A			
B				B				B				B			
C				C				C				C			
D				D				D				D			
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 comple

Chronic

Menidia beryllina

Lab ID: 98041

Client: Natural Energy Laboratory of Hawaii - Hawaii Ocean Science and

Balance: Radwag BAL-007

Begin Date: 8/22/2025

End Date: 8/29/2025

Organism: Menidia beryllina

Analyst: DOO
Weigh Date: 9/2/25Date/Time placed in Oven: 8/29/25 1740
Date/Time removed from Oven: 8/30/25 1740

Control

	Qty.	Wt.
A	8	3.62
B	8	4.24
C	8	4.26
D		

100 %

	Qty.	Wt.
A	7	3.89
B	7	3.99
C	7	3.72
D		

	Qty.	Wt.
A		
B		
C		
D		

	Qty.	Wt.
A		
B		
C		
D		

	Qty.	Wt.
A		
B		
C		
D		

	Qty.	Wt.
A		
B		
C		
D		

	Qty.	Wt.
A		
B		
C		
D		

	Qty.	Wt.
A		
B		
C		
D		

	Qty.	Wt.
A		
B		
C		
D		

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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: 98041.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	2	0	5	3	0

Calculated Chi-Square goodness of fit test statistic = 6.2337

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

Data PASS normality test. Continue analysis.

mysid growth

File: 98041.myg

Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.009	
2	100	0.003	2.627

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: 98041.myg

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.000	0.000	0.013
Within (Error)	8	0.048	0.006	
Total	9	0.048		

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

mysid growth
 File: 98041.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	0.511	0.511		
2	100	0.516	0.516	-0.115	
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	0.511	0.511		
2	100	0.516	0.516	-0.115	
2 Sample t table value = 1.94 (1 Tailed Value, P=0.05, df=6,1)					

mysid growth
 File: 98041.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	0.091	17.8	-0.006

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	0.095	18.6	-0.006

menidia growth
File: 98041.meg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.005

W = 0.889

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: 98041.meg Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.002	
2	100	0.000	6.986

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

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

menidia growth
File: 98041.meg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.001	0.001	0.618
Within (Error)	4	0.005	0.001	

Total 5 0.005

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

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

menidia growth

File: 98041.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	0.505	0.505		
2	100	0.483	0.483	0.786	

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	0.505	0.505		
2	100	0.483	0.483	0.786	

2 Sample t table value = 2.92 (1 Tailed Value, $P=0.05$, $df=2,1$)

menidia growth

File: 98041.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	0.060	11.8	0.022

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	0.082	16.2	0.022

Eurofins Environment Testing Bio-Aquatics

SALT WATER TEST SETUP FORM

Client: Natural Energy Laboratory of HawaiiPermit N/AFacility: Hawaii Ocean Science and TechnologyLab Number 98041Outfall Name: Shrimp FarmNumber 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	08/22/25	13:00	08/20/25	08/20/25	07:42	07:42

Type of Test(s)

Americamysis bahiaChronicMenidia beryllinaChronicStart Sx # 1 Date: 8/22/2025Renew Sx # 1 Date: 8/23/2025Renew Sx # 1 Date: 8/24/2025Renew Sx # 1 Date: 8/25/2025Renew Sx # 1 Date: 8/26/2025Renew Sx # 1 Date: 8/27/2025Renew Sx # 1 Date: 8/28/2025

Test Start Date: _____ Test End Date: _____

8/22/2025 8/29/2025Controls: 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: _____

Eurofins Environment Testing Bio-Aquatics

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

Client: Natural Energy Laboratory of

Lab ID: 98041

Facility: Hawaii Ocean Science and

Dilution Water(s): Synthetic Lab

Outfall: Shrimp Farm

Test Date: August 22, 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	8/22/25	13:00	<0.10	N/A	<0.25	DT	3.1

¹Dechlorination Reagent: 0.025 N Sodium Thiosulfate

Effluent Sample #	pH	DO (mg/L)	Init. Salinity (ppt)	Adjusted Salinity	Analyst Initials
1	8.3	7.1	32.2	N/A	DT

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

Eurofins Environment Testing Bio-Aquatics

pH, Dissolved Oxygen, Salinity

Chronic

Americamysis bahia

Client: Natural Energy Laboratory of Hawaii

Lab Number: 98041

Facility: Hawaii Ocean Science and

Dilution Water(s): Synthetic Lab

Outfall: Shrimp Farm

Test Begin Date: August 22, 2025

NR indicates that the test was not renewed

						Concentration							
ANALYST	DATE	TIME	SX#	UNIT	%	Control	100						
SG	8/22	Start	1	pH		8.1	8.2						
				DO (mg/L)		7.1	6.9						
		25 ± 1		Salinity (ppt)		21.5	34.6						
GJ	8/23	24 Hr	1	pH		7.9	7.9						
				DO (mg/L)		7.1	6.6						
		25 ± 1		Salinity (ppt)		21.7	35.3						
		Renew	1	pH		7.8	8.0						
				DO (mg/L)		7.2	6.7						
				Salinity (ppt)		21.3	35.9						
CAP	8/24	48 Hr	1	pH		7.6	7.8						
				DO (mg/L)		6.9	6.3						
		25 ± 1		Salinity (ppt)		21.2	36.2						
		Renew	1	pH		7.9	8.0						
				DO (mg/L)		7.2	6.7						
				Salinity (ppt)		20.2	34.0						
CCC	8/25	72 Hr	1	pH		7.7	7.7						
				DO (mg/L)		7.0	6.3						
		25 ± 1		Salinity (ppt)		22.1	37.0						
		Renew	1	pH		7.9	7.9						
				DO (mg/L)		6.8	6.7						
				Salinity (ppt)		23.3	34.2						
JR	8/26	96 Hr	1	pH		8.0	7.9						
				DO (mg/L)		7.5	6.8						
		25 ± 1		Salinity (ppt)		24.6	36.0						
		Renew	1	pH		8.0	8.0						
				DO (mg/L)		6.9	7.0						
				Salinity (ppt)		24.6	32.5						
CCC	8/27	120 Hr	1	pH		7.8	7.8						
				DO (mg/L)		6.5	5.9						
		25 ± 1		Salinity (ppt)		25.5	37.5						
		Renew	1	pH		8.1	8.1						
				DO (mg/L)		6.8	6.9						
				Salinity (ppt)		23.2	35.3						
MM	8/28	144 Hr	1	pH		8.1	8.1						
				DO (mg/L)		7.5	6.8						
		25 ± 1		Salinity (ppt)		21.1	33.3						
		Renew	1	pH		8.1	8.1						
				DO (mg/L)		7.3	6.8						
				Salinity (ppt)		21.4	33.2						
SG	8/29	168 Hr	1	pH		7.9	7.9						
				DO (mg/L)		6.9	6.2						
		25 ± 1		Salinity (ppt)		24.6	37.3						

Eurofins Environment Testing Bio-Aquatics

pH, Dissolved Oxygen, Salinity

Chronic

Menidia beryllina

Client: Natural Energy Laboratory of

Lab Number: 98041

Facility: Hawaii Ocean Science and

Dilution Water(s): Synthetic Lab

Outfall: Shrimp Farm

Test Begin Date: August 22, 2025

NR indicates that the test was not renewed

ANALYST	DATE	TIME	SX#	UNIT	%	Concentration							
						Control	100						
SG	8/22	Start	1		pH	8.1	8.2						
					DO (mg/L)	7.1	6.9						
		25 ± 1			Salinity (ppt)	21.5	34.6						
GJ	8/23	24 Hr	1		pH	7.4	8.0						
					DO (mg/L)	7.5	6.7						
		25 ± 1			Salinity (ppt)	21.5	35.5						
		Renew	1		pH	7.8	8.0						
					DO (mg/L)	7.2	6.7						
					Salinity (ppt)	21.3	35.9						
CAP	8/24	48 Hr	1		pH	7.8	7.8						
					DO (mg/L)	6.9	6.4						
		25 ± 1			Salinity (ppt)	21.4	36.0						
		Renew	1		pH	7.9	8.0						
					DO (mg/L)	7.2	6.7						
					Salinity (ppt)	20.2	34.0						
CCC	8/25	72 Hr	1		pH	7.7	7.7						
					DO (mg/L)	6.9	6.2						
		25 ± 1			Salinity (ppt)	22.3	36.9						
		Renew	1		pH	7.9	7.9						
					DO (mg/L)	6.8	6.7						
					Salinity (ppt)	23.3	34.2						
JR	8/26	96 Hr	1		pH	8.0	7.9						
					DO (mg/L)	7.6	6.9						
		25 ± 1			Salinity (ppt)	24.6	35.2						
		Renew	1		pH	8.0	8.0						
					DO (mg/L)	6.9	7.0						
					Salinity (ppt)	24.6	32.5						
CCC	8/27	120 Hr	1		pH	7.8	7.9						
					DO (mg/L)	7.0	6.2						
		25 ± 1			Salinity (ppt)	24.2	36.4						
		Renew	1		pH	8.1	8.1						
					DO (mg/L)	6.8	6.9						
					Salinity (ppt)	23.2	35.3						
MM	8/28	144 Hr	1		pH	8.1	8.1						
					DO (mg/L)	7.3	6.8						
		25 ± 1			Salinity (ppt)	21.4	33.3						
		Renew	1		pH	8.1	8.1						
					DO (mg/L)	7.3	6.8						
					Salinity (ppt)	21.4	33.2						
SG	8/29	168 Hr	1		pH	7.9	7.8						
					DO (mg/L)	6.4	5.9						
		25 ± 1			Salinity (ppt)	24.8	37.2						

Appendix B

Americamysis bahia

EUROFINS ENVIRONMENT TESTING BIO-AQUATICS

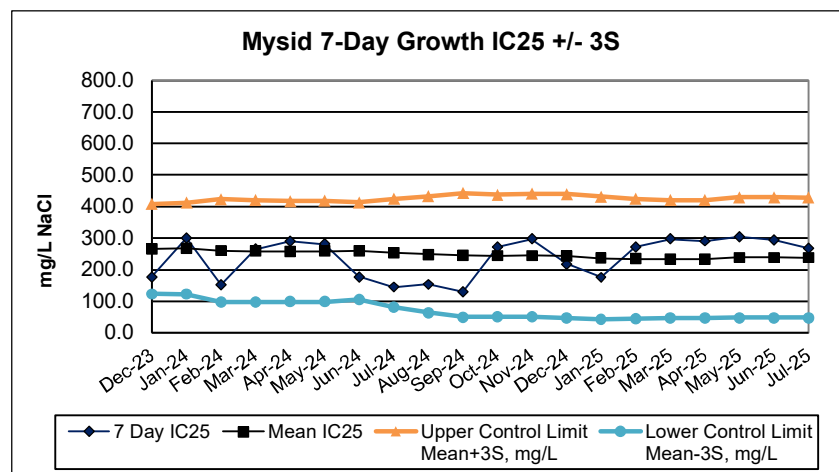
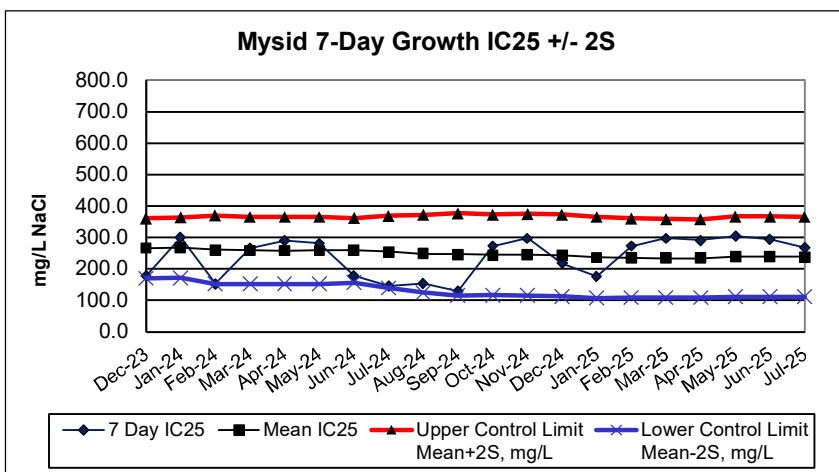
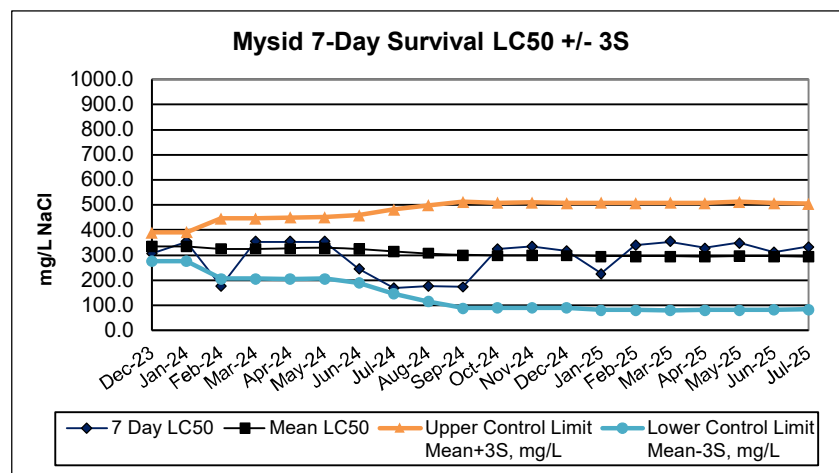
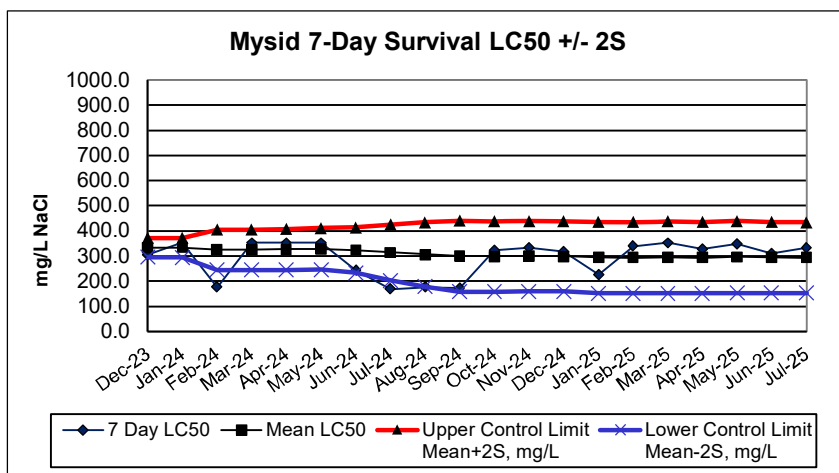
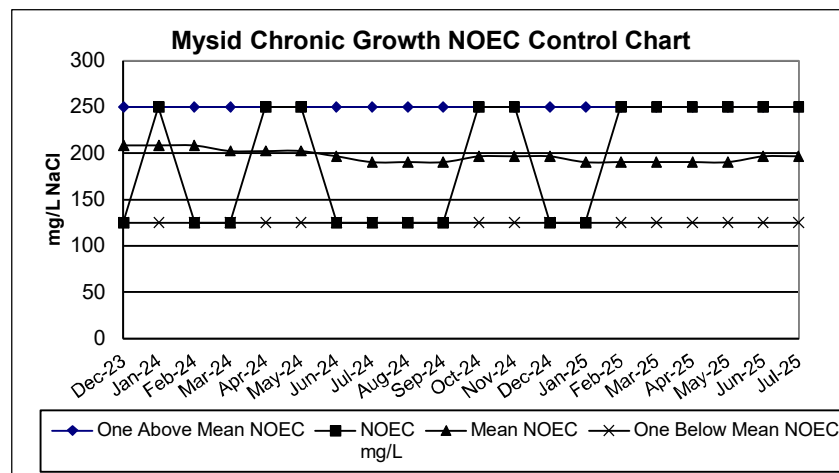
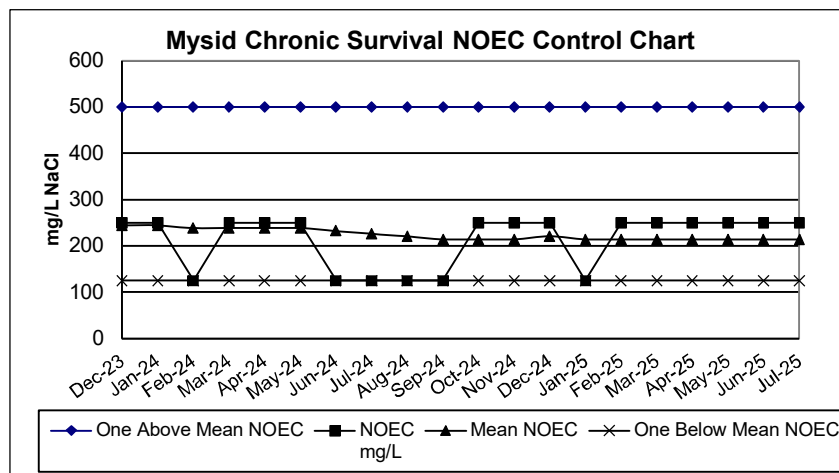
Carrollton, TX

REFERENCE TOXICANTS

Eurofins Environment Testing Bio-Aquatics 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:	169						
PROJECT NUMBER:	98122						
START DATE:	7/30/2025						
START TIME:	14:08						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	25	50	125	250	500	1000
NUMBER DEAD PER CONCENTRATION:	0	1	0	1	3	40	40
TEST METHODS:	Method 1007.0 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:	20.6						



Appendix B

Menidia beryllina

EUROFINS ENVIRONMENT TESTING BIO-AQUATICS

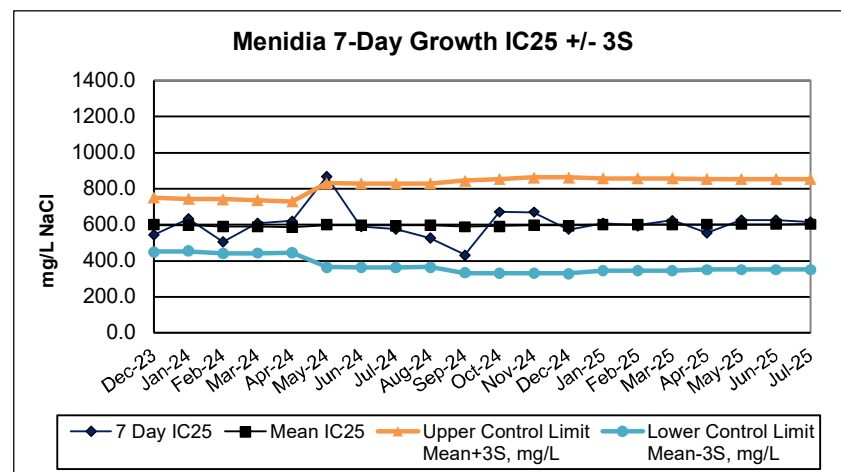
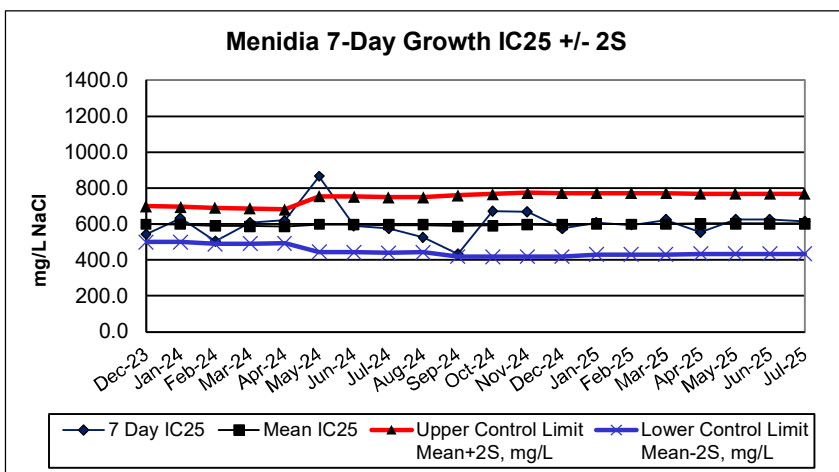
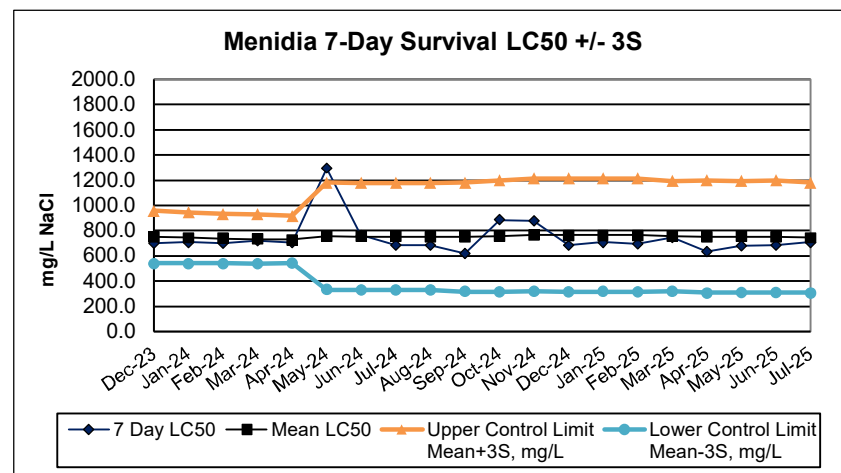
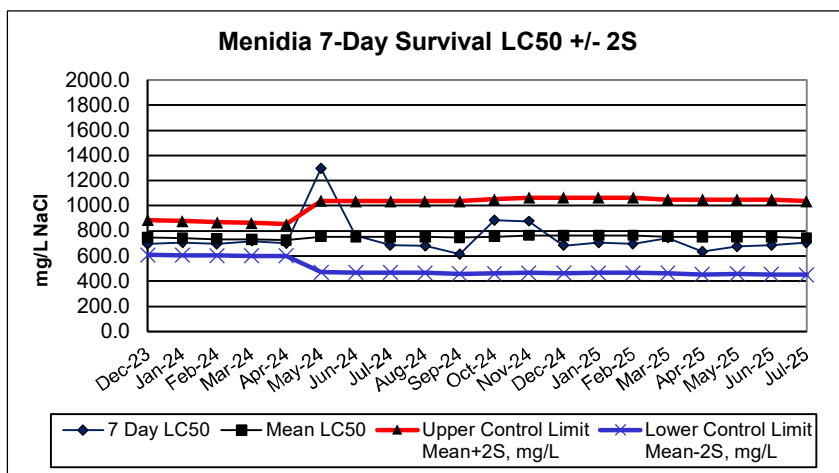
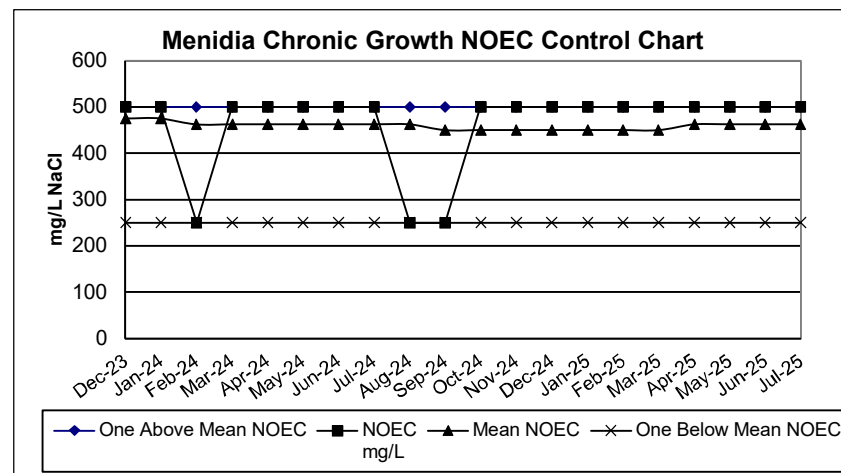
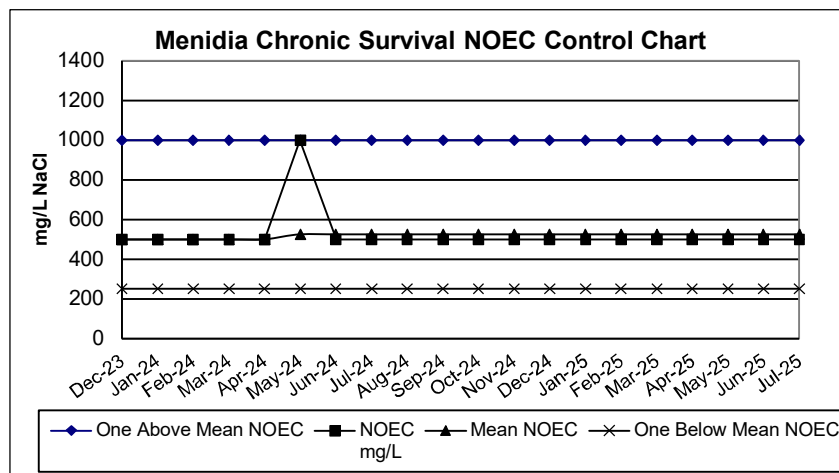
Carrollton, TX

REFERENCE TOXICANTS

Eurofins Environment Testing Bio-Aquatics 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:	169
PROJECT NUMBER:	98123
START DATE:	7/30/2025
START TIME:	13:40
TOTAL NUMBER EXPOSED:	40 organisms per concentration
CONCENTRATIONS (mg/L):	CON 125 250 500 1000 2000 4000
NUMBER DEAD PER CONCENTRATION:	0 0 0 0 40 40 40
TEST METHODS:	Method 1006.0 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:	21.4



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 : **98041**

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

Sample No: **98041** -

Revised 2

Effective Date 8/20/25

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

P.O. No:

Client: Natural Energy Laboratory of Hawaii

Facility: Hawaii Ocean Science and Technology Park

Permit No: N/A

Outfall: Shrimp Farm

Client Contact:

Client Phone:

A. REVIEW SCHEDULED TEST(S):

☒ Chronic	Americamysis bahia
☒ Chronic	Menidia beryllina

Concentration: 100

To Ship the
1st Sample on:
7/21/2025

(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

From To

1 Shrimp Farm - 8/20/25 -

2

3

D. Relinquished By:

Time

Received By:

Date

Time

1 *Donner*

8/24/25

1230

Fede

DT

1300

1300

Bio-Aquatic Sample Login

BAT sample personnel:
☒ Yes ☐ No

Dechlorinate Sample:
☐ Yes ☐ No

Dilution Water:
☐ Receiving Stream
☒ Synthetic Lab

Date: 8/22/25

Chlorine: Low

pH: 8.3

DO: 7.1

Time: 1300

Ammonia: Low

Hardness: mg/l

Alkalinity: mg/l

By: DT

mg/l

mg/l

mg/l

Temperature: 31

Int. Sal/Cond: 32.2 ppt/uS

Other

Condition: good

(C) IR#: 026

Adj. Salinity

ppt

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

Freshwater Species

Saltwater Species

☐ 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

☐ 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

Notes: Non-Routine/Specialty Testing for Information purpose

Grab or Composite

Sample Time (military)

From To

Sampled By:
(Sign and Print Name)

Number Of Containers Shipped

g

Donner

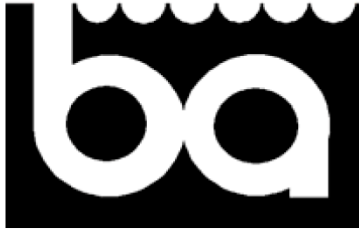
Donner

Donner

Donner

Donner

Donner



Bio-Aquatic Testing, Inc.



TCEQ TNI ACCREDITED

Report Revision Form

Report Revision Number 0 for Lab ID 98041 was revised on 09/11/2025.

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