

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

Chronic Biomonitoring Report

98985

Americamysis bahia
Menidia beryllina

December 18, 2025

Approved by: Joshua Reed
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: 98985
Date: December 18, 2025

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

SAMPLE COLLECTION: A grab effluent sample from the Natural Energy Laboratory of Hawaii Authority, Hawaii Ocean Science and Technology Park, was transported to by Eurofins Environment Testing Bio-Aquatics on December 18, 2025. The effluent sample was collected from the Shrimp Farm by facility personnel.

The effluent sample was analyzed for total residual chlorine using the Hanna Ion Specific Meter #711 and contained <0.10 mg/L. Effluent 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 16:50 hours on December 18, 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 14:00 hours on December 25, 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 effluent 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. Growth data were shown to be homogeneous using Bartlett's test at the alpha level of 0.01 (15.09) without data transformations. 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 effluent 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 16:01 hours on December 18, 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 14:10 hours on December 25, 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 effluent 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. Growth data were shown to be homogeneous using Bartlett's test at the alpha level of 0.01 (15.09) without data transformations. 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 effluent 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: 98985

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: 12/18/2025

Test Start Time: 16:50

Test End Time: 14:00

End Date: 12/25/2025

SURVIVAL

Effluent Con. %		Number of Alive								Avg% Surv.
		12/18	12/19	12/20	12/21	12/22	12/23	12/24	12/25	
Control	A	5	5	5	5	5	5	5	5	100.0%
	B	5	5	5	5	5	5	5	5	
	C	5	5	5	5	5	5	5	5	
	D	5	5	5	5	5	5	5	5	
	E	5	5	5	5	5	5	5	5	
100	A	5	5	5	5	5	5	5	5	100.0%
	B	5	5	5	5	5	5	5	5	
	C	5	5	5	5	5	5	5	5	
	D	5	5	5	5	5	5	5	5	
	E	5	5	5	5	5	5	5	5	
	A									
	B									
	C									
	D									
	E									
	A									
	B									
	C									
	D									
	E									

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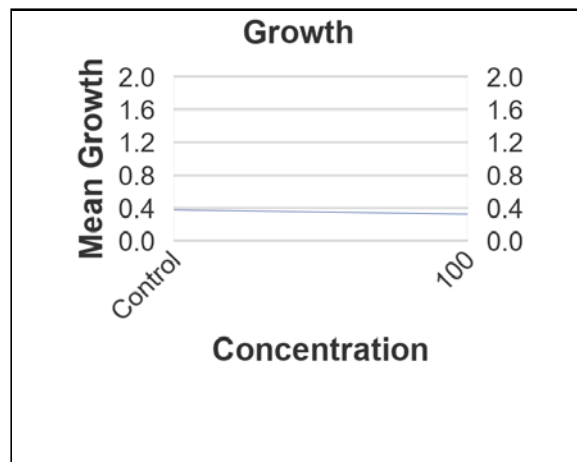
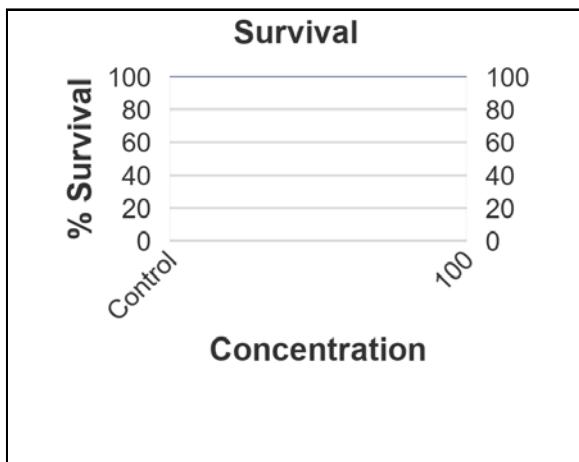
Effluent Con. %	Number Of Alive								Avg% Surv.
	12/18	12/19	12/20	12/21	12/22	12/23	12/24	12/25	
A									
B									
C									
D									
E									

A									
B									
C									
D									
E									

A									
B									
C									
D									
E									

A									
B									
C									
D									
E									

Concentration Response Relationships



Eurofins Environment Testing Bio-Aquatics

Chronic

Americamysis bahia SURVIVAL

Lab ID: 98985

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

Culture No. : MY-25-345

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	12-18-25 m-1650	5					5														
24Hr	12-19-25 SL 1909	5					5														
48Hr	12-20-25 1120 GJ	5					5														
72Hr	12-21-25 CAP 1020	5					5														
96Hr	12-22-25 ccc 1555	5					5														
5 days	12-23-25 8-1550	5					5														
6 days	12-24-25 0850 TM	5					5														
7 days	12-29-25 3-1400	5					5														

Dilution:

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

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Eurofins Environment Testing Bio-Aquatics

Chronic

Americamysis bahia SURVIVAL

Lab ID: **98985**Client: Natural Energy Laboratory of Hawaii Facility: Hawaii Ocean Science and TechnologyOutfall: 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	25.7	25.7	25.8	25.8	25.2	25.7	23.4	25.9	26.3	25.2	25.1	25.7	25.1	26.2
100														
TIME/DATE TECH	12-19-25 1650	12-19-25 SL 0909		12-20-25 1120 GJ		12-21-25 CAP 1020		12-22-25 CCL 1555		12-23-25 S 1550		12-24-25 0853 TM		12-25-25 51400
IR GUN ID #	013	013		013		013		013		013		013		013

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

Lab ID: 98985

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	2.18	0.436	0.436
B	5	5	1.39	0.278	0.278
C	5	5	1.85	0.370	0.370
D	5	5	2.20	0.440	0.440
E	5	5	1.84	0.368	0.368

Mean	C.V. %
0.378	17.42

SN Mean	SN C.V. %
0.378	17.4

	ON	Wt.	Avg.
A	5	1.84	0.368
B	5	1.32	0.264
C	5	1.40	0.280
D	5	2.11	0.422
E	5	1.45	0.290

Mean	C.V. %
0.325	20.78

	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.

Eurofins Environment Testing Bio-Aquatics TOXICITY TEST

Chronic

Americamysis bahia

Lab ID: 98985

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

Balance: BAL-010

Begin Date: 12/18/2025

End Date: 12/25/2025

Organism: Americamysis bahia

Analyst: JH

Date/Time placed in Oven: 12-24-25 1520

Weigh Date: 12-25-25

Date/Time removed from Oven: 12-25-25 1520

Control

100 %

	Qty.	Wt.
A	5	2.18
B	1	1.39
C	1	1.85
D	1	2.20
E	1	1.84
F		
G		
H		

	Qty.	Wt.
A	5	1.84
B	1	1.32
C	1	1.40
D	1	2.11
E	1	1.45
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		
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F		
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Eurofins Environment Testing Bio-Aquatics

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

Lab ID: 98985

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: 16:01

Test End Time: 14:10

Begin Date: 12/18/2025

End Date: 12/25/2025

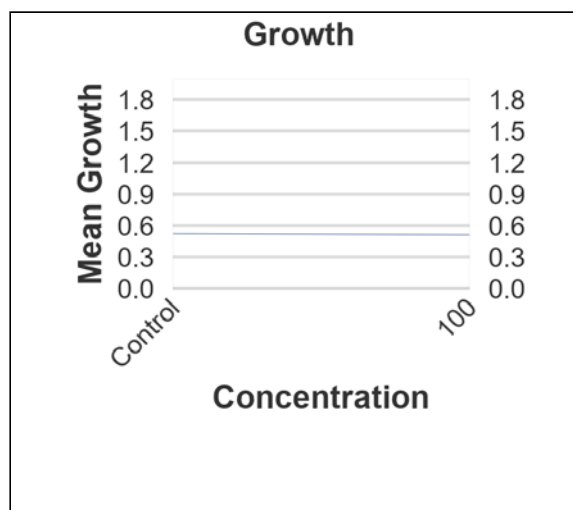
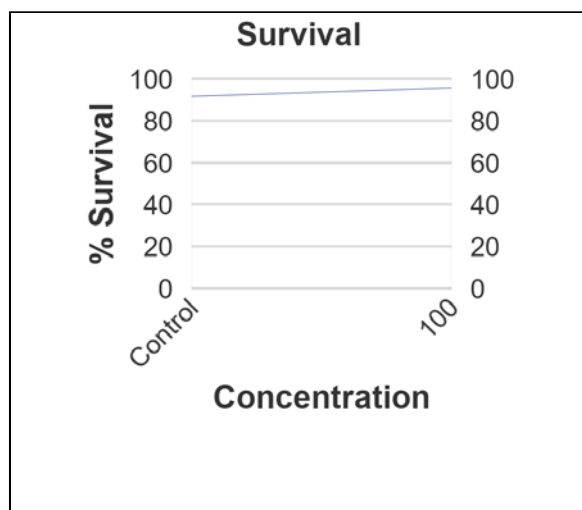
SURVIVAL

Effluent Concentration	Number Of Alive								Avg% Surv.
	12/18	12/19	12/20	12/21	12/22	12/23	12/24	12/25	
Control	A	8	8	8	8	8	8	7	91.7%
	B	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	7	
	D								
	E								
100	A	8	8	8	8	8	7	7	95.8%
	B	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	
	D								
	E								
	A								
	B								
	C								
	D								
	E								
	A								
	B								
	C								
	D								
	E								

Eurofins Environment Testing Bio-Aquatics

Effluent Concentration	Number Of Alive								Avg% Surv.
	12/18	12/19	12/20	12/21	12/22	12/23	12/24	12/25	
	A								
	B								
	C								
	D								
	E								
	A								
	B								
	C								
	D								
	E								
	A								
	B								
	C								
	D								
	E								

Concentration Response Relationships



Eurofins Environment Testing Bio-Aquatics

Chronic

Menidia beryllina SURVIVAL

Lab ID: **98985**

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

Culture No. : MM-25-342

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	12-18-25 1601	8			1	1/8	8				1/8										
24Hr	12-19-25 SU 1913	8			1	1/8	8				1/8										
48Hr	12-20-25 1123 GJ	8			1	1/8	8				1/8										
72Hr	12-21-25 CAP1025	8			1	1/8	8				1/8										
96Hr	12-22-25 CU 1600	8			1	1/8	8				1/8										
5 days	12-23-25 SU 1530	8					7	8	8												
6 days	12-24-25 0840 TM	8					7	8													
7 days	12-25-25 01410	7	8	8	7		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																				

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Eurofins Environment Testing Bio-Aquatics

Chronic

Menidia beryllina SURVIVAL

Lab ID: 98985

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

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	25.7	25.7	25.8	25.8	25.0	25.9	25.7	26.9	25.8	25.2	25.1	25.9	25.0	25.0
100														
TIME/DATE TECH	12-18-25 1609	12-19-25 FG 0913		12-20-25 1123 GJ		12-21-25 CAR 1025		12-22-25 CC 1400 /		12-23-25 S 1530		12-24-25 0943 0843 77		12-25-25 S 1413
IR GUN ID #	013	013		013		013		013		012		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: 98985

Permit Number: N/A

Sample Type: Grab

Outfall Name: Shrimp Farm

Receiving Water Name:

Synthetic**100**

	ON	SN	Wt.	Avg.	SN Avg.
A	8	7	3.92	0.490	0.560
B	8	8	4.88	0.610	0.610
C	8	7	3.80	0.475	0.543
D					
E					

Mean C.V. %

0.525	14.1
-------	------

SN Mean SN C.V. %

0.571	6.1
-------	-----

	ON	Wt.	Avg.
A	8	4.35	0.544
B	8	3.93	0.491
C	8	4.05	0.506
D			
E			

Mean C.V. %

0.514	5.3
-------	-----

	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.

Eurofins Environment Testing Bio-Aquatics TOXICITY TEST

Chronic

Menidia beryllina

Lab ID: 98985

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

Balance: BAL-010

Begin Date: 12/18/2025

End Date: 12/25/2025

Organism: Menidia beryllina

Analyst: JH
Weigh Date: 12-25-25Date/Time placed in Oven: 12-24-25 1520
Date/Time removed from Oven: 12-25-25 1520

Control

	Qty.	Wt.
A	2	3.92
B	8	#1 3.28 4.88
C	2	3.80
D		
E		

100 %

	Qty.	Wt.
A	2	4.35
B	8	2 nd 8.393
C	8	4.05
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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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: 98985.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	3	2	4	0

Calculated Chi-Square goodness of fit test statistic = 2.8702

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

Data PASS normality test. Continue analysis.

mysid growth

File: 98985.myg

Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.004	
2	100	0.005	1.049

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: 98985.myg

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B1 statistic = 0.00

Table Chi-square value = 6.63 (alpha = 0.01, df = 1)

Table Chi-square value = 3.84 (alpha = 0.05, df = 1)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

mysid growth
File: 98985.myg

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.007	0.007	1.614
Within (Error)	8	0.036	0.004	
Total	9	0.043		

Critical F value = 5.32 (0.05,1,8)

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

mysid growth
File: 98985.myg

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	0.378	0.378		
2	100	0.325	0.325	1.270	

2 Sample t table value = 1.86 (1 Tailed Value, $P=0.05$, $df=8,1$)

UNEQUAL VARIANCE t-TEST H_0 :Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.378	0.378		
2	100	0.325	0.325	1.270	

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

mysid growth
File: 98985.myg

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	5			
2	100	5	0.078	20.7	0.054

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	0.080	21.1	0.054

menidia growth

File: 98985.meg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.012

W = 0.911

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: 98985.meg Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.005	
2	100	0.001	7.336

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

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

Transform: NO TRANSFORMATION

Table Chi-square value = 6.63 (alpha = 0.01, df = 1)
Table Chi-square value = 3.84 (alpha = 0.05, df = 1)

```
menidia growth
File: 98985.meg      Transform: NO TRANSFORMATION
```

SOURCE	DF	SS	MS	F
Between	1	0.000	0.000	0.062
Within (Error)	4	0.012	0.003	
Total	5	0.013		

```
menidia growth
File: 98985.meg      Transform: NO TRANSFORMATION
```

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.525	0.525		
2	100	0.514	0.514	0.249	

Bio-Aquatic Lab ID: 98985

UNEQUAL VARIANCE t-TEST

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.525	0.525		
2	100	0.514	0.514	0.249	

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

menidia growth

File: 98985.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	0.097	18.5	0.011

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	0.133	25.3	0.011

Eurofins Environment Testing Bio-Aquatics

SALT WATER TEST SETUP FORM

Client: Natural Energy Laboratory of Hawaii Permit N/A

Facility: Hawaii Ocean Science and Lab Number 98985

Outfall Name: Shrimp Farm Number of samples 1

Dilution Water:	Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
				Begin Date	End Date	Start	End
Receiving Water:	1	12/18/25	15:00	12/15/25	12/15/25	08:11	08:11
Dechlorinate Sample:							

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

Start Sx # 1 Date: 12/18/2025

Renew Sx # 1 Date: 12/19/2025

Renew Sx # 1 Date: 12/20/2025

Renew Sx # 1 Date: 12/21/2025

Renew Sx # 1 Date: 12/22/2025

Renew Sx # 1 Date: 12/23/2025

Renew Sx # 1 Date: 12/24/2025

Controls: Synthetic

pH Match:

Hardness Match:

Test Start Date: 12/18/2025 Test End Date: 12/25/2025

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:

Eurofins Environment Testing Bio-Aquatics

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

Client: Natural Energy Laboratory of

Lab ID: 98985

Facility: Hawaii Ocean Science and

Dilution Water(s): Synthetic Lab

Outfall: Shrimp Farm

Test Date: December 18, 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	12/18/25	15:00	<0.10	N/A	<0.25	DT	2.9

¹Dechlorination Reagent: 0.025 N Sodium Thiosulfate

Effluent Sample #	pH	DO (mg/L)	Init. Salinity (ppt)	Adjusted Salinity	Analyst Initials
1	8.1	7.7	40.1	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: 98985

Facility: Hawaii Ocean Science and Dilution Water(s): Synthetic Lab
Outfall: Shrimp Farm Test Begin Date: December 18, 2025

NR indicates that the test was not renewed

Concentration

ANALYST	DATE	TIME	SX#	UNIT	%	Control	100						
JP	12/18	Start	1	pH		8.2	8.1						
				DO (mg/L)		7.6	7.0						
		25 ± 1		Salinity (ppt)		19.1	35.9						
CAP	12/19	24 Hr	1	pH		8.1	8.0						
				DO (mg/L)		7.4	6.6						
		25 ± 1		Salinity (ppt)		22.0	41.4						
		Renew	1	pH		8.2	8.2						
				DO (mg/L)		7.6	7.1						
				Salinity (ppt)		21.1	38.9						
GJ	12/20	48 Hr	1	pH		7.7	7.7						
				DO (mg/L)		7.0	6.3						
		25 ± 1		Salinity (ppt)		20.5	38.3						
		Renew	1	pH		7.9	7.9						
				DO (mg/L)		7.6	7.3						
				Salinity (ppt)		20.0	36.5						
SG	12/21	72 Hr	1	pH		7.9	7.9						
				DO (mg/L)		6.9	6.3						
		25 ± 1		Salinity (ppt)		22.1	40.8						
		Renew	1	pH		8.1	8.0						
				DO (mg/L)		7.2	7.2						
				Salinity (ppt)		20.1	37.1						
CCC	12/22	96 Hr	1	pH		7.9	7.8						
				DO (mg/L)		6.9	5.9						
		25 ± 1		Salinity (ppt)		21.1	39.9						
		Renew	1	pH		8.1	8.0						
				DO (mg/L)		7.2	6.8						
				Salinity (ppt)		18.9	36.0						
GJ	12/23	120 Hr	1	pH		7.8	7.8						
				DO (mg/L)		6.3	5.6						
		25 ± 1		Salinity (ppt)		22.8	43.3						
		Renew	1	pH		8.1	8.0						
				DO (mg/L)		7.1	6.7						
				Salinity (ppt)		19.7	36.4						
TM	12/24	144 Hr	1	pH		8.1	7.9						
				DO (mg/L)		7.1	5.9						
		25 ± 1		Salinity (ppt)		22.5	42.1						
		Renew	1	pH		8.2	8.0						
				DO (mg/L)		6.9	6.7						
				Salinity (ppt)		19.1	35.6						
MV	12/25	168 Hr	1	pH		7.9	7.9						
				DO (mg/L)		6.4	5.7						
		25 ± 1		Salinity (ppt)		22.3	42.1						

Eurofins Environment Testing Bio-Aquatics

pH, Dissolved Oxygen, Salinity

Chronic

Menidia beryllina

Client: Natural Energy Laboratory of

Lab Number: 98985

Facility: Hawaii Ocean Science and

Dilution Water(s): Synthetic Lab

Outfall: Shrimp Farm

Test Begin Date: December 18, 2025

NR indicates that the test was not renewed

ANALYST	DATE	TIME	SX#	UNIT	%	Concentration							
						Control	100						
JP	12/18	Start	1		pH	8.2	8.1						
					DO (mg/L)	7.6	7.0						
		25 ± 1			Salinity (ppt)	19.1	35.9						
CAP	12/19	24 Hr	1		pH	8.1	8.0						
					DO (mg/L)	7.7	6.8						
		25 ± 1			Salinity (ppt)	22.7	36.0						
		Renew	1		pH	8.2	8.2						
					DO (mg/L)	7.6	7.1						
					Salinity (ppt)	21.1	38.9						
GJ	12/20	48 Hr	1		pH	7.7	7.7						
					DO (mg/L)	7.4	6.8						
		25 ± 1			Salinity (ppt)	20.9	34.1						
		Renew	1		pH	7.9	7.9						
					DO (mg/L)	7.6	7.3						
					Salinity (ppt)	20.0	36.5						
SG	12/21	72 Hr	1		pH	8.0	7.9						
					DO (mg/L)	7.2	6.5						
		25 ± 1			Salinity (ppt)	21.5	35.8						
		Renew	1		pH	8.1	8.0						
					DO (mg/L)	7.2	7.2						
					Salinity (ppt)	20.1	37.1						
CCC	12/22	96 Hr	1		pH	7.9	7.8						
					DO (mg/L)	6.8	6.2						
		25 ± 1			Salinity (ppt)	20.4	35.6						
		Renew	1		pH	8.1	8.0						
					DO (mg/L)	7.2	6.8						
					Salinity (ppt)	18.9	36.0						
GJ	12/23	120 Hr	1		pH	7.8	7.7						
					DO (mg/L)	6.7	5.8						
		25 ± 1			Salinity (ppt)	20.8	36.6						
		Renew	1		pH	8.1	8.0						
					DO (mg/L)	7.1	6.7						
					Salinity (ppt)	19.7	36.4						
TM	12/24	144 Hr	1		pH	8.0	7.9						
					DO (mg/L)	6.5	5.9						
		25 ± 1			Salinity (ppt)	20.0	36.2						
		Renew	1		pH	8.2	8.0						
					DO (mg/L)	6.9	6.7						
					Salinity (ppt)	19.1	35.6						
MV	12/25	168 Hr	1		pH	8.0	7.9						
					DO (mg/L)	6.6	5.9						
		25 ± 1			Salinity (ppt)	20.1	36.4						

Appendix B

Americamysis bahia

EUROFINS ENVIRONMENT TESTING BIO-AQUATICS

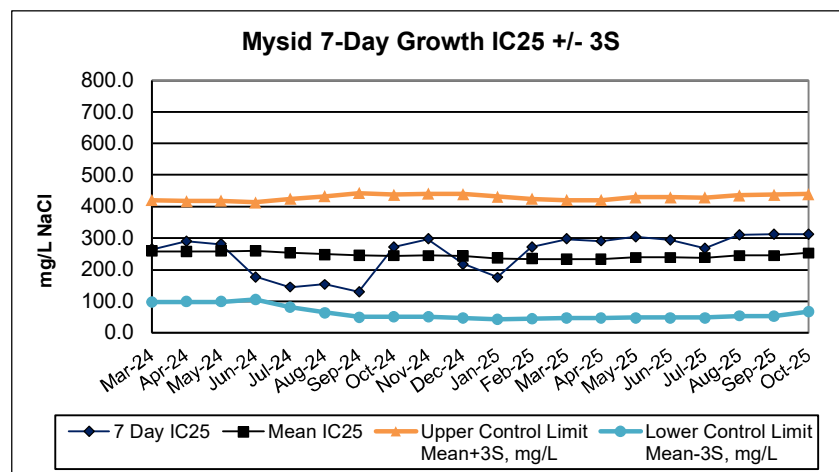
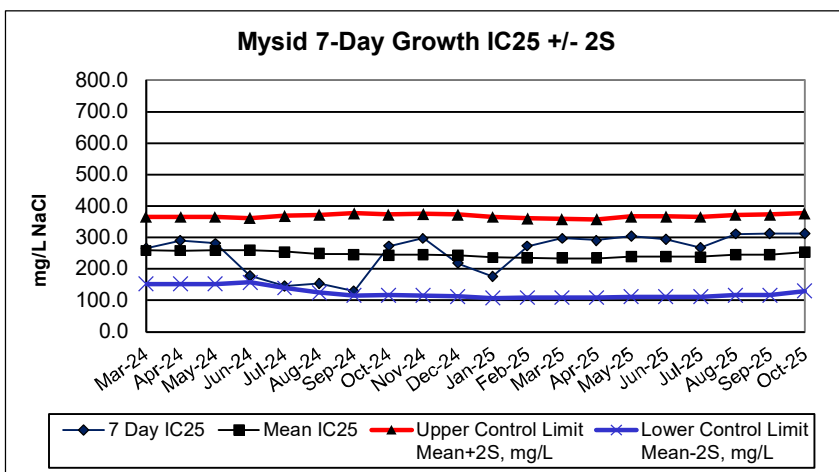
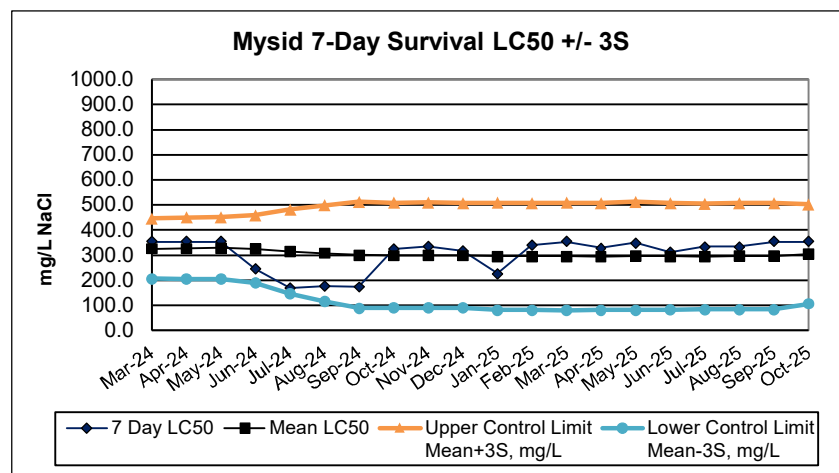
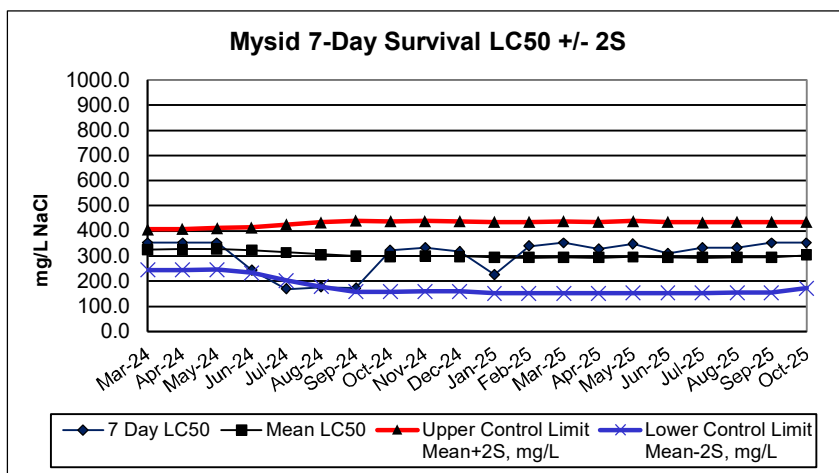
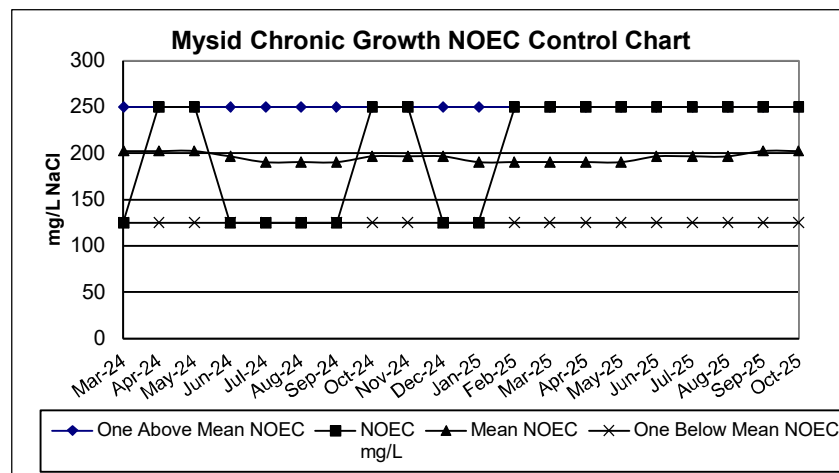
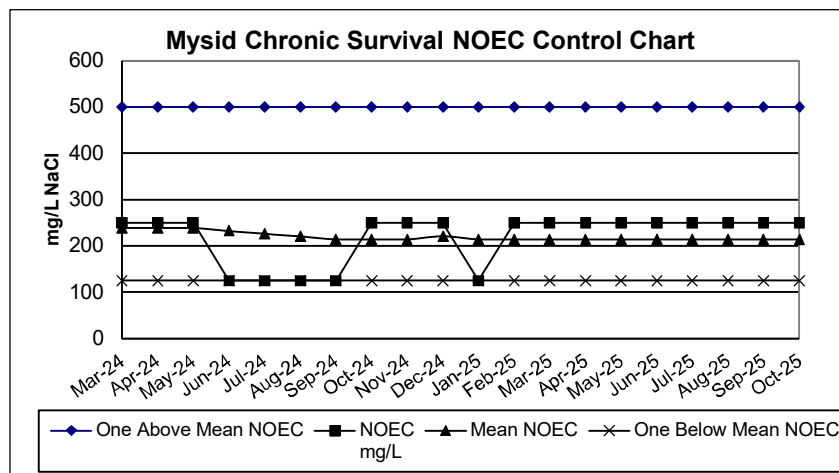
Carrollton, TX

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:	172						
PROJECT NUMBER:	98700						
START DATE:	10/29/2025						
START TIME:	11:27						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	25	50	125	250	500	1000
NUMBER DEAD PER CONCENTRATION:	2	2	0	2	0	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:	19.0						



Appendix B

Menidia beryllina

EUROFINS ENVIRONMENT TESTING BIO-AQUATICS

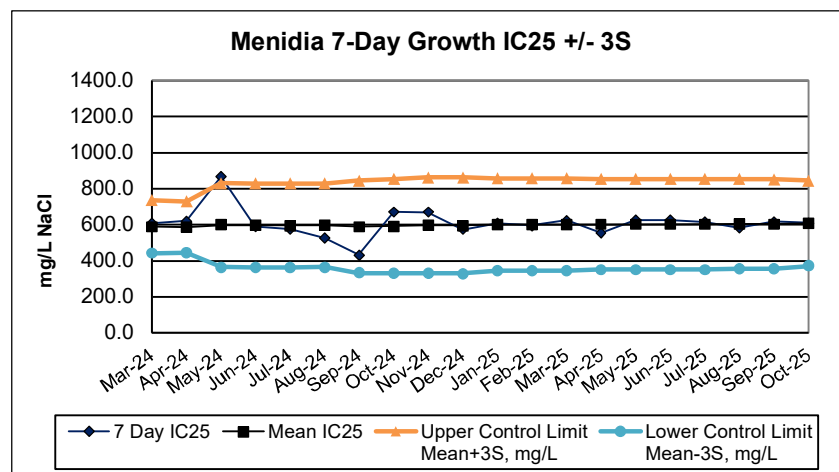
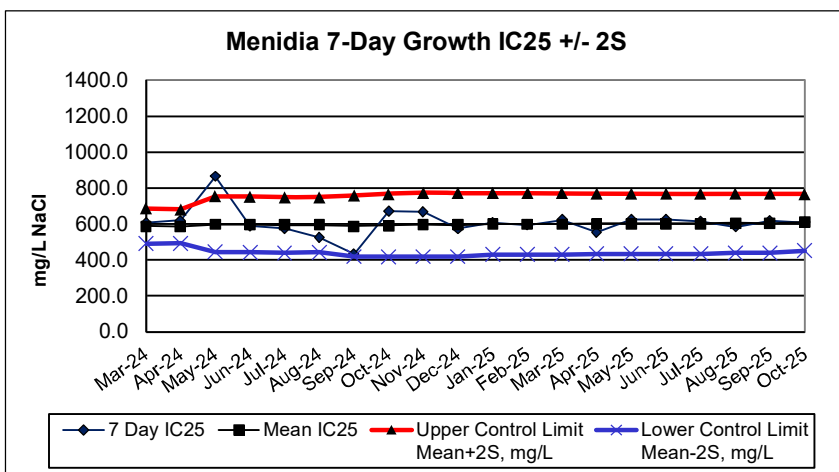
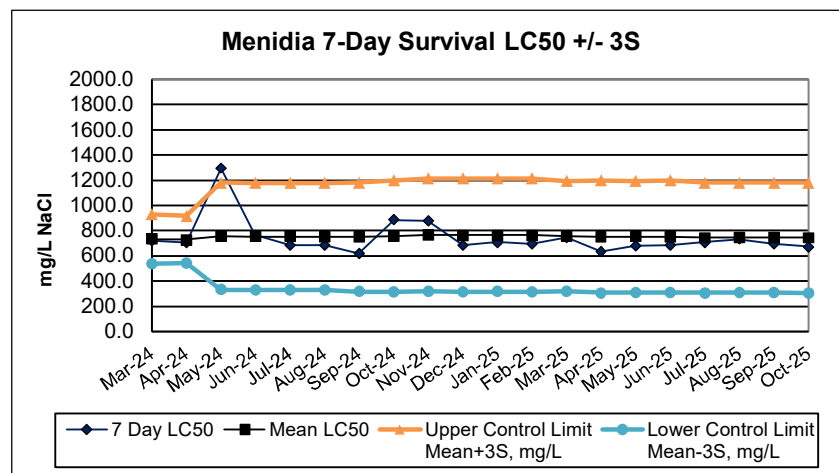
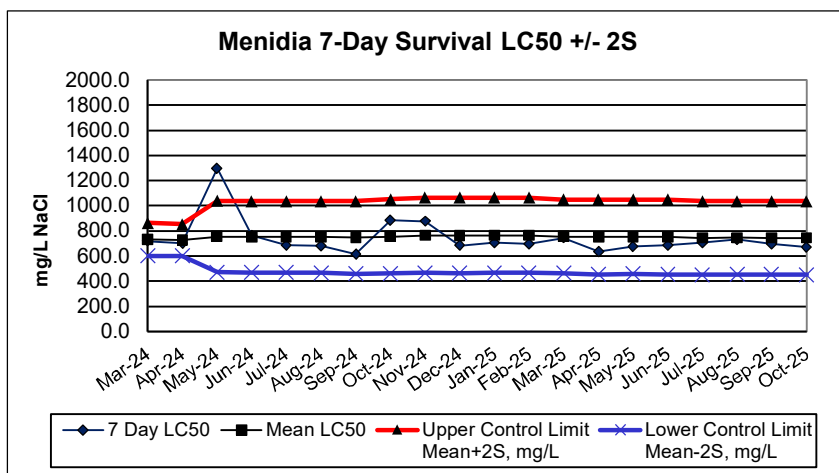
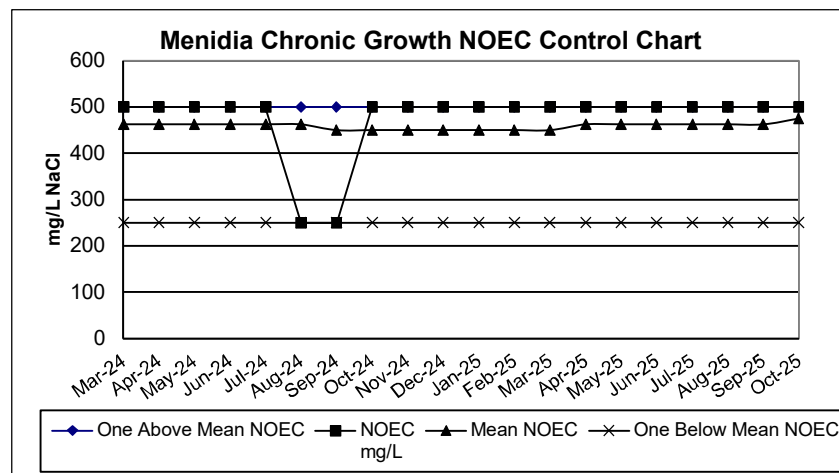
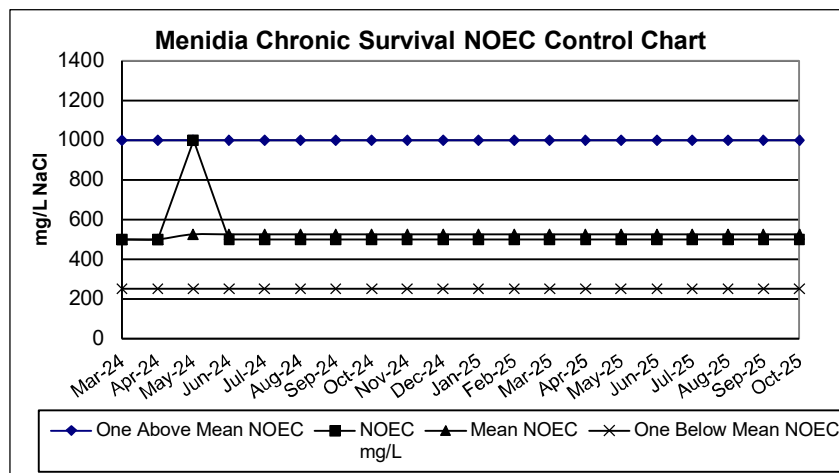
Carrollton, TX

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:	172						
PROJECT NUMBER:	98699						
START DATE:	10/29/2025						
START TIME:	11:10						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	125	250	500	1000	2000	4000
NUMBER DEAD PER CONCENTRATION:	0	0	2	1	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: 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:	16.1						



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



Environment Testing
Bio-Aquatics

Report Date 07/06/2026 Revision 0

2501 MAYES RD., STE. 100, CARROLLTON, TX 75006, PH: 972-242-7750

Client: Natural Energy Laboratory of Hawaii

Facility: Hawaii Ocean Science and Technology Park

Permit N/A

Outfall Shrimp Farm

Client Contact: Pam Madden

Client Phone: 808.327.9524

A. REVIEW SCHEDULED TEST(S):

Chronic	Americamysis bahia
Chronic	Menidia beryllina

To Ship the
1st Sample on:
12/1/2025

Concentration: 100

34 (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

Sample Time
(military)

From

To

Grab
or
Composite

Sampled By:
(Sign and Print Name)

Number Of
Containers
Shipped

1 Shrimp Farm

12/15/25

0811

G

Pam Madden

1

2

3

D.

Relinquished By:

Date

Time

Received By:

Date

Time

Pam Madden

12/15/25

1230

Fedex

12.18.25

1500

Bio-Aquatic Sample Login

BAT sample personnel:

☒ Yes ☐ No

Dechlorinate Sample:

☐ Yes ☐ No

Dilution Water:

☐ Receiving Stream

☒ Synthetic Lab

Date: 12.18.25

Chlorine: 2.01

pH: 8.1

DO: 7.7

Time: 1500

Ammonia: 2.25

Hardness:

Alkalinity:

By: DT

mg/l

mg/l

mg/l

Temperature: 22.9

Int. SalCond: 40.1

Other

Condition: good

(C) IR#026

Adj. Salinity

ppt

CHAIN OF CUSTODY



Bio Only
No Sample Left

Lab Id : 98985

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

Sample No: 98985

Revised 2

Effective Date 6/25/2017

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

P.O. No:

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

Freshwater Species				Saltwater Species			
	D. pullex (water flea)	D. magna (water flea)	P. promelas (minnow)	Selensastrum (green algae)	M. beryllina (minnow)	Mysidopsis (shrimp)	
<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> Chronic	<input type="checkbox"/> Chronic	
<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 96 Hour	
<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 48 Hour	
<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 24 Hour		<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 24 Hour	

Notes: Non-Routine/Specialty Testing for Information purpose

Bio-Aquatic Lab ID: 98985