

**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: Johnny Reed
Lab director

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

Eurofins Environment Testing Bio-Aquatics

2501 Mayes Road, Suite 100

Carrollton, Texas 75006

Tel: (972) 242-7750

Fax: (972) 242-7749

TOXICITY TEST REPORT - Chronic

Client: Natural Energy Laboratory of Hawaii Authority
Facility: Hawaii Ocean Science 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%

Eurofins Environment Testing Bio-Aquatics**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:

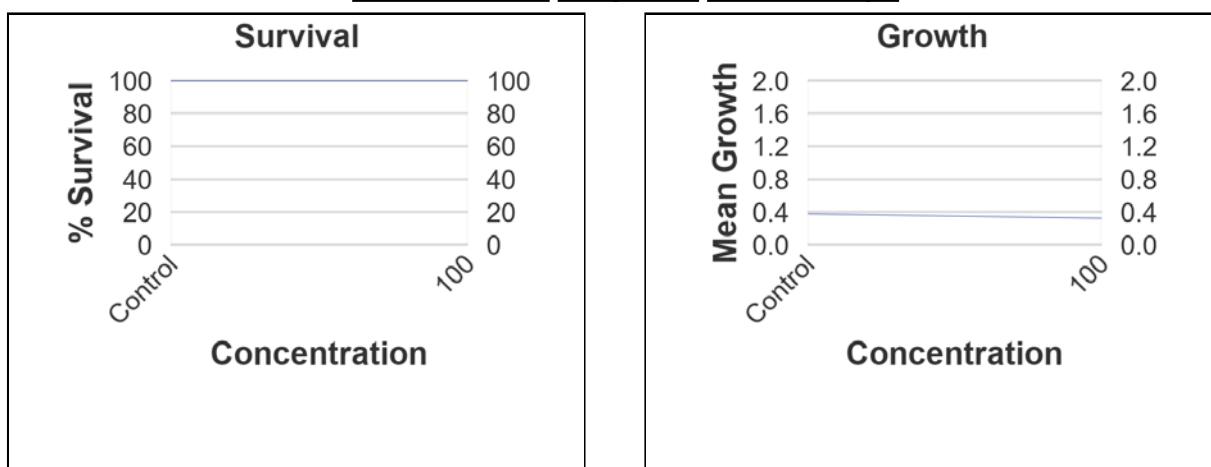
Test Start Time: Test End Time: **Begin Date:** 12/18/2025**End Date:** 12/25/2025**SURVIVAL**

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									

Eurofins Environment Testing Bio-Aquatics

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



Eurotins 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 on 1650	5					5														
24Hr 12-19-25 86 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 86 1550	5					5														
6 days 12-24-25 0850 TM	5					5														
7 days 12-25-25 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

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

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

Test Temperatures

	0Hr	24Hr	48Hr	72Hr	96Hr	5 days	6 days	7 days
Control	new 25.7	old / new 25.7 / 25.8	old / new 25.8 / 25.8	old / new 25.7 / 25.8	old / new 25.9 / 26.2	old / new 25.2 / 25.1	old / new 25.7 / 25.1	old 26.3
100								
TIME/DATE TECH	12-19-25 1600	12-19-25 SL 0909	12-20-25 1120 GJ	12-21-25 CAP 1021	12-22-25 cc 1595	12-23-25 ✓ 1595	12-23-25 5 1550	12-24-25 0853 TM
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: 98985

Permit Number: N/A

Sample Type: Grab

Outfall Name: Shrimp Farm

Receiving Water Name:

Synthetic

	ON	SN	Wt.	Avg.	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

100

	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. %

SN Mean	SN C.V. %
0.378	17.4

	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

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

100 %

Qty.	Wt.
A	5
B	1.32
C	1.40
D	2.11
E	1.95
F	
G	
H	

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

Qty. Wt.

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

Qty. Wt.

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

Qty. Wt.

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

Qty. Wt.

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

Qty. Wt.

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

Qty. Wt.

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

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TOXICITY TEST**Eurofins Environment Testing Bio-Aquatics****Chronic *Menidia beryllina***Client: Natural Energy Laboratory of Hawaii Hawaii Ocean Science and TechnologyLab ID: **98985**

Permit Number: N/A

Test Temperature (oC): **25 ± 1**

Outfall Name: Shrimp Farm

Sample Type: Grab

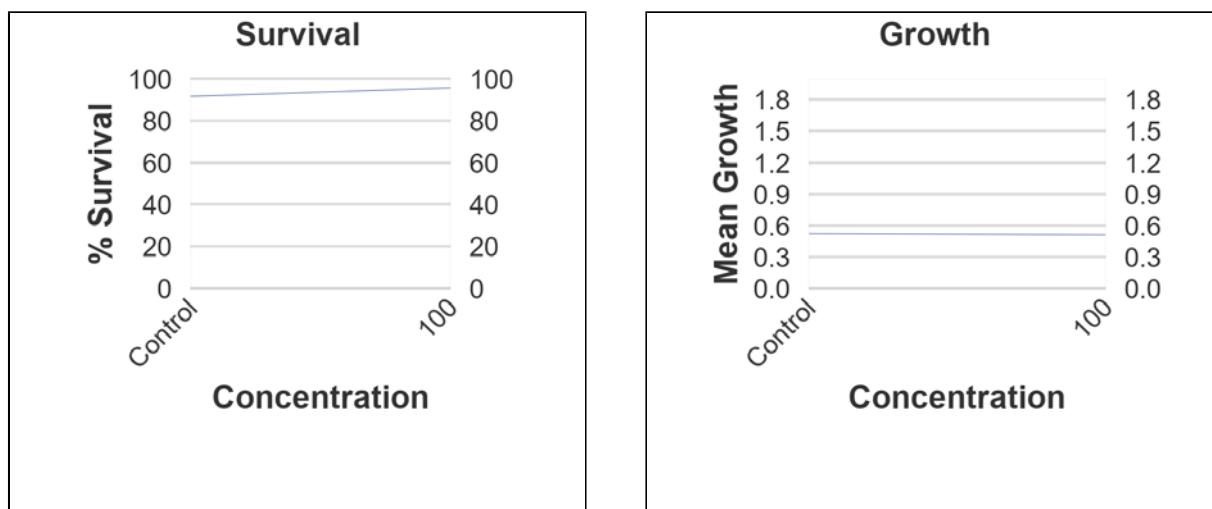
Photo Period: 16 Hours Light
8 Hours Dark

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								

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: Mysid 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
12-19-25 ~1601	8			1	100	8			5	100										
12-19-25 SU 1913	8			1	9	8			1	9										
12-20-25 1123 GJ	8			3	3	8			100	100										
12-21-25 CAP 1025	8			0	0	8			6	6										
12-22-25 CC 1600	8			0	0	8			0	0										
12-23-25 SU 1530	8						7	8	8											
12-24-25 0840 M	8						7	8												
12-25-25 0 1410	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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Eurotins 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	27	28.7 25.3	25.8 25.0	25.9 25.7	26.2 26.8	25.2 24.7	25.9 25.0	25.9
100	1	1	1	1	1	1	1	1
TIME/DATE TECH	12-18-25 ~1601	12-19-25 16 1013	12-20-25 1123 GJ	12-21-25 08 1025	12-22-25 06 1400	12-23-25 5 1530	12-24-25 0843 0545	12-25-25 5 1410
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.	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					

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

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean**C.V. %**

0.525

14.1

Mean**C.V. %**

0.514

5.3

Mean**C.V. %****Mean****C.V. %****SN Mean****SN C.V. %**

0.571

6.1

	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			

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

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

	Qty.	Wt.
A	2	3.92
B	8	3.28 4.80
C	2	3.80
D		
E		

100 %

	Qty.	Wt.
A	2	4.35
B	8	2.83 3.93
C	8	4.05
D		
E		

Qty. **Wt.**

	Qty.	Wt.
A		
B		
C		
D		
E		

Qty. **Wt.**

	Qty.	Wt.
A		
B		
C		
D		
E		

Qty. **Wt.**

	Qty.	Wt.
A		
B		
C		
D		
E		

Qty. **Wt.**

	Qty.	Wt.
A		
B		
C		
D		
E		

Qty. **Wt.**

	Qty.	Wt.
A		
B		
C		
D		
E		

Qty. **Wt.**

	Qty.	Wt.
A		
B		
C		
D		
E		

Qty. **Wt.**

	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 Steel's 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 Shapiro Wilks Test and Bartlett's Test then Steel's 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 Steel's 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 Steel's 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 Shapiro Wilks Test or Bartlett's Test then Steel's 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 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 Steel's 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 Steel's 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 Steel's 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 <= Critical F, FAIL TO REJECT Ho: 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 < Critical F FAIL TO REJECT Ho: All equal

mysid growth
File: 98985.myg Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED		MEAN CALCULATED IN		T STAT	SIG
		MEAN	ORIGINAL UNITS				
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 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED		MEAN CALCULATED IN		T STAT	SIG
		MEAN	ORIGINAL UNITS				
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 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	0.078	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.

menidia growth
File: 98985.meg Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 1.38

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.

menidia growth
File: 98985.meg Transform: NO TRANSFORMATION

ANOVA TABLE

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

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

menidia growth
File: 98985.meg Transform: NO TRANSFORMATION

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

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

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

UNEQUAL VARIANCE t-TEST

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED	MEAN CALCULATED IN	T STAT	SIG
		MEAN	ORIGINAL UNITS		
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	Minimum Sig Diff	% of	DIFFERENCE
		REPS	(IN ORIG. UNITS)	CONTROL	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	Minimum Sig Diff	% of	DIFFERENCE
		REPS	(IN ORIG. UNITS)	CONTROL	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 HawaiiPermit N/AFacility: Hawaii Ocean Science andLab Number 98985Outfall Name: Shrimp FarmNumber of samples 1Dilution Water: Synthetic Lab

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

Receiving Water: Dechlorinate Sample:

Type of Test(s)

<u>Americamysis bahia</u>	<u>Chronic</u>
<u>Menidia beryllina</u>	<u>Chronic</u>

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: SyntheticpH Match: Hardness Match:

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

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:

<input type="radio"/> Express Delivery	<input type="radio"/> UPS Next Day	<input type="radio"/> via Air Cargo	<input type="radio"/> DHL
<input checked="" type="radio"/> Federal Express	<input type="radio"/> the Client	<input type="radio"/> 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

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

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

ANALYST	DATE	TIME	SX#	UNIT	%	Concentration									
						Control	100								
JP	12/18	Start	1	pH	8.2	8.1									
		25 ± 1		DO (mg/L)	7.6	7.0									
				Salinity (ppt)	19.1	35.9									
CAP	12/19	24 Hr	1	pH	8.1	8.0									
		25 ± 1		DO (mg/L)	7.4	6.6									
				Salinity (ppt)	22.0	41.4									
GJ	12/20	Renew	1	pH	8.2	8.2									
		48 Hr		DO (mg/L)	7.6	7.1									
		25 ± 1		Salinity (ppt)	21.1	38.9									
SG	12/21	Renew	1	pH	7.7	7.7									
		72 Hr		DO (mg/L)	7.0	6.3									
		25 ± 1		Salinity (ppt)	20.5	38.3									
CCC	12/22	Renew	1	pH	7.9	7.9									
		96 Hr		DO (mg/L)	6.9	6.3									
		25 ± 1		Salinity (ppt)	22.1	40.8									
GJ	12/23	Renew	1	pH	8.1	8.0									
		120 Hr		DO (mg/L)	7.2	7.2									
		25 ± 1		Salinity (ppt)	20.1	37.1									
TM	12/24	Renew	1	pH	7.8	7.8									
		144 Hr		DO (mg/L)	6.3	5.6									
		25 ± 1		Salinity (ppt)	22.8	43.3									
MV	12/25	Renew	1	pH	8.1	8.0									
		168 Hr		DO (mg/L)	7.1	6.7									
		25 ± 1		Salinity (ppt)	22.5	42.1									
TM	12/24	Renew	1	pH	8.2	8.0									
		144 Hr		DO (mg/L)	6.9	6.7									
		25 ± 1		Salinity (ppt)	19.1	35.6									
MV	12/25	Renew	1	pH	7.9	7.9									
		168 Hr		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	DO (mg/L)	Control	100								
		25 ± 1				8.2	8.1								
		24 Hr				7.6	7.0								
CAP	12/19	25 ± 1	1	pH	DO (mg/L)	19.1	35.9								
		Renew				8.1	8.0								
		48 Hr				7.7	6.8								
GJ	12/20	25 ± 1	1	pH	DO (mg/L)	7.6	7.1								
		Renew				21.1	38.9								
		72 Hr	1	pH	DO (mg/L)	7.7	7.7								
SG	12/21	25 ± 1				7.4	6.8								
		Renew				20.9	34.1								
		96 Hr	1	pH	DO (mg/L)	7.9	7.9								
CCC	12/22	25 ± 1				7.6	7.3								
		Renew				20.0	36.5								
		120 Hr	1	pH	DO (mg/L)	7.9	7.8								
GJ	12/23	25 ± 1				6.8	6.2								
		Renew				20.4	35.6								
		144 Hr	1	pH	DO (mg/L)	8.1	8.0								
TM	12/24	25 ± 1				7.2	6.8								
		Renew				18.9	36.0								
		168 Hr	1	pH	DO (mg/L)	7.8	7.7								
MV	12/25	25 ± 1				6.7	5.8								
		Renew				20.8	36.6								
		120 Hr	1	pH	DO (mg/L)	8.1	8.0								
GJ	12/23	25 ± 1				7.1	6.7								
		Renew				19.7	36.4								
		144 Hr	1	pH	DO (mg/L)	8.0	7.9								
TM	12/24	25 ± 1				6.5	5.9								
		Renew				20.0	36.2								
		168 Hr	1	pH	DO (mg/L)	8.2	8.0								
MV	12/25	25 ± 1				6.9	6.7								
		Renew				19.1	35.6								
		120 Hr	1	pH	DO (mg/L)	8.0	7.9								
MV	12/25	25 ± 1				6.6	5.9								
		Renew				20.1	36.4								
		144 Hr	1	pH	DO (mg/L)	8.0	7.9								

Appendix B

Americamysis bahia

EUROFINS ENVIRONMENT TESTING BIO-AQUATICS

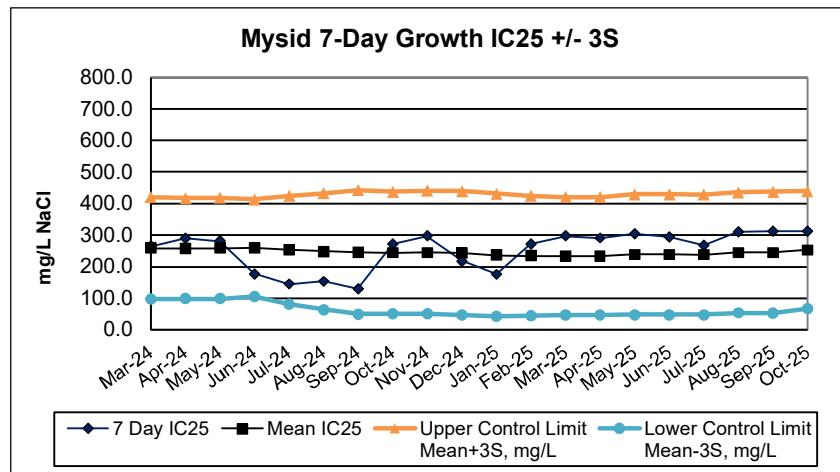
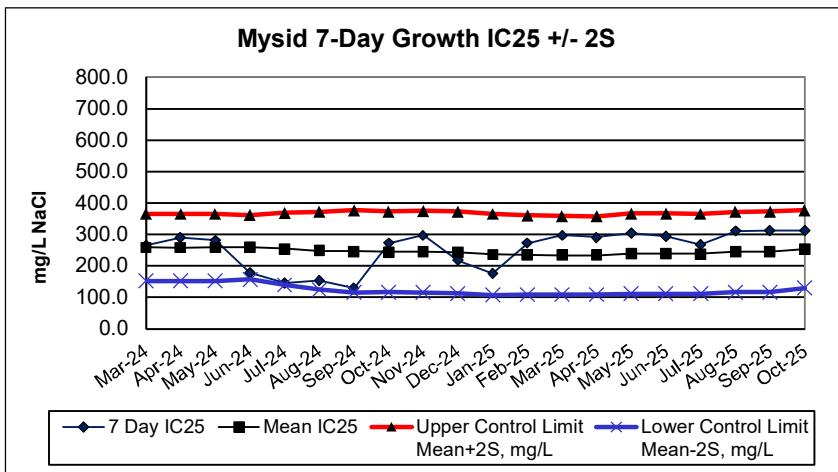
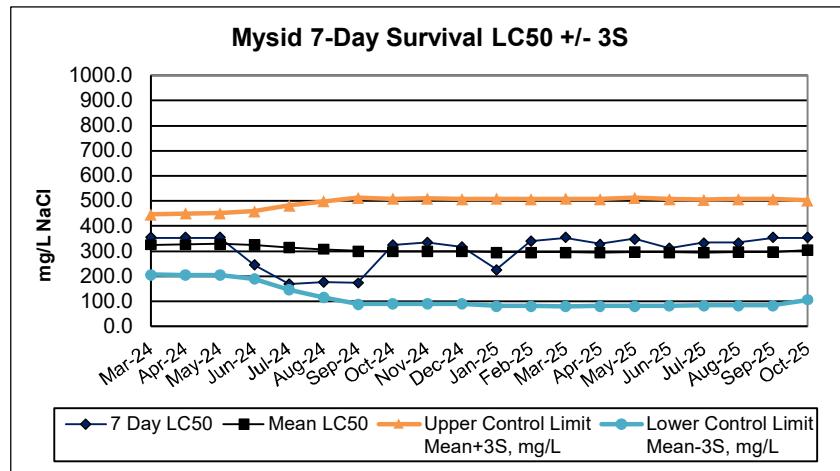
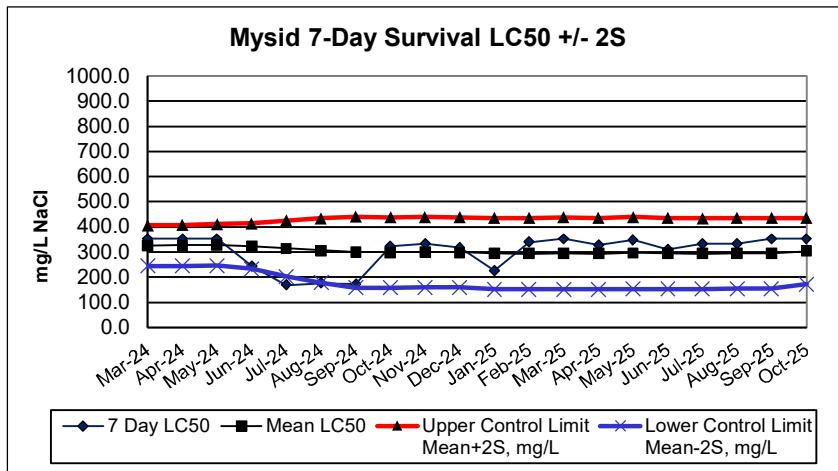
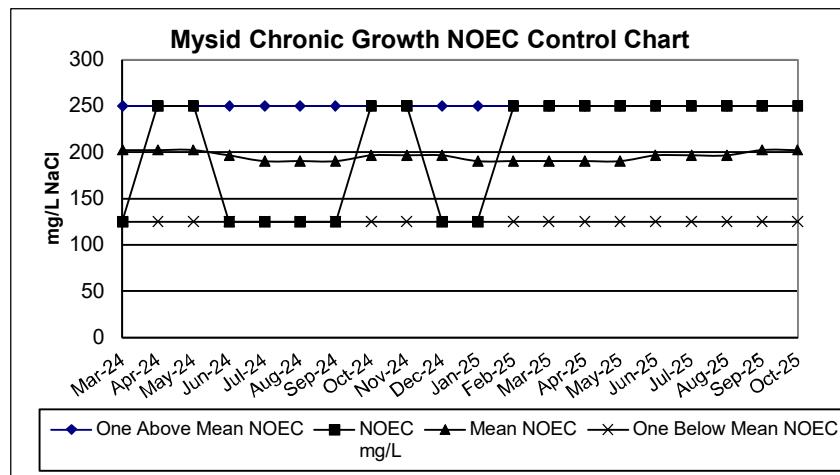
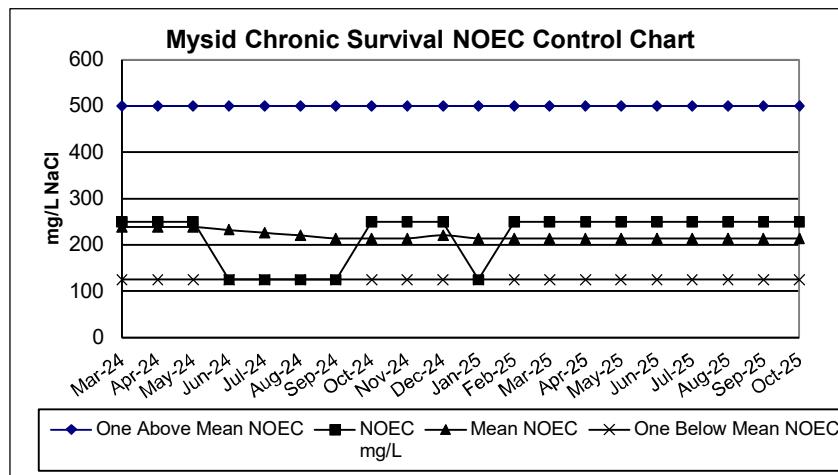
Carrollton, TX

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

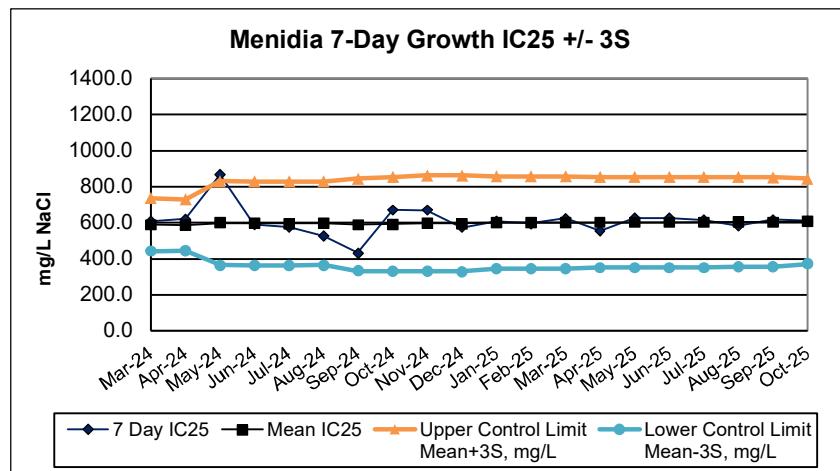
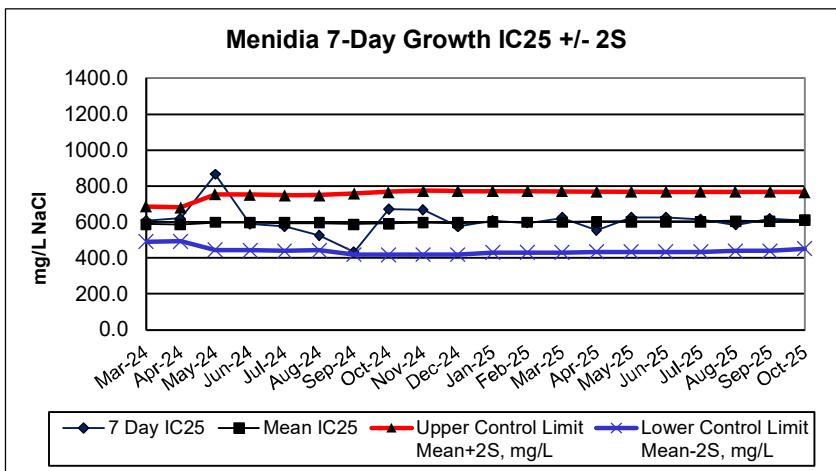
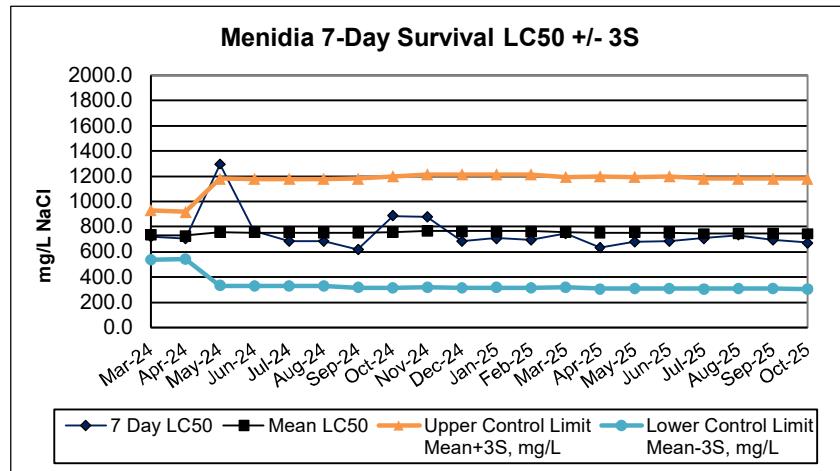
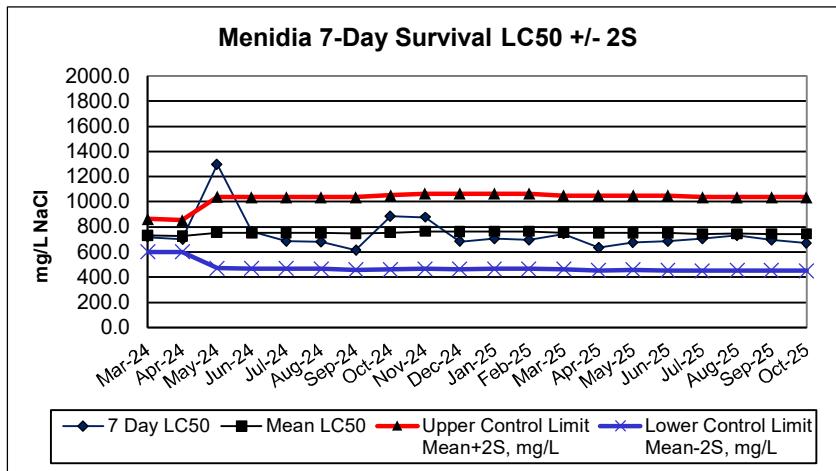
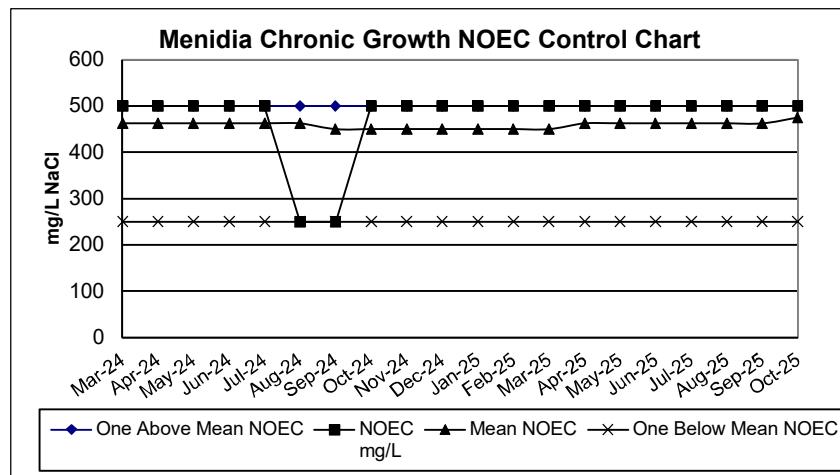
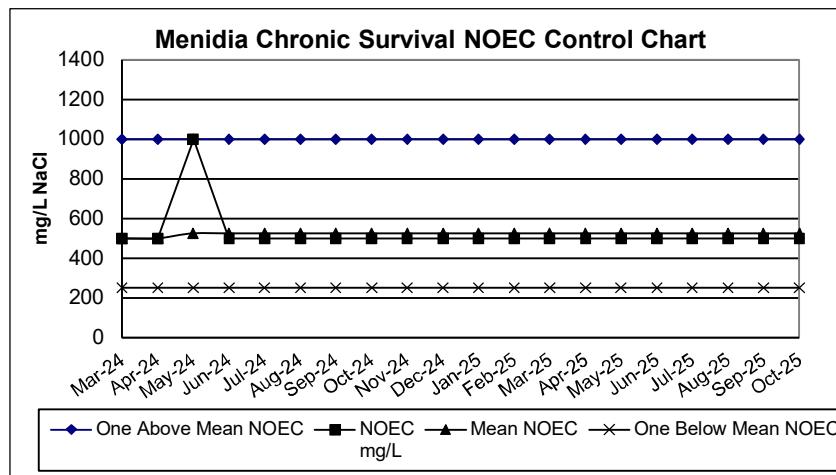
Carrollton, TX

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

2501 MAYES RD., STE. 100, CARRROLLTON, 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 Mcdermott

Client Phone: 808.327.9524

A. REVIEW SCHEDULED TEST(S):

Chronic Americamysis bahia

Chronic Menidia beryllina

Concentration: 100

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

34 of 34

Report Date 07/06/2026 Revision 0

CHAIN OF CUSTODY

Environment Testing
Bio-Aquatics

Please Review & Complete Sections A, B, C, & D.	Check Sample No. : <input type="text"/> First, <input type="checkbox"/> Second, or <input type="checkbox"/> Third.	Lab Id : <input type="text"/> 98985
Sample No: <input type="text"/> 98985	P.O. No: <input type="text"/>	Bio Only: <input type="checkbox"/> No Sample Left

Effective Date 5/25/2017

Revision 2

Page 1 of 2

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

Facility: Hawaii Ocean Science and Technology Park		Freshwater Species				Saltwater Species			
Permit	Outfall	C. dubia (water flea)	D. pullex (water flea)	D. magna (water flea)	P. promelas (minnow)	P. chilensis (minnow)	S. selenastylum (green algae)	M. beryllina (minnow)	M. beryllina (shrimp)
Permit	N/A	<input type="checkbox"/> Chronic	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> Chronic				
Outfall	Shrimp Farm	<input type="checkbox"/> 96 Hour	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 96 Hour					
Client Contact:	Pam Mcdermott	<input type="checkbox"/> 48 Hour	<input type="checkbox"/> 24 Hour	<input type="checkbox"/> 48 Hour					
Client Phone:	808.327.9524	<input type="checkbox"/> 24 Hour							<input type="checkbox"/> 24 Hour

Notes: Non-Routine/Specialty Testing for Information purpose

C. Sample ID or Location: (Outfall No. or Name)				Sample Date		Sample Time (military)		Grab or Composite		Sampled By: (Sign and Print Name)		Number Of Containers Shipped	
		From	To	From	To	From	To	From	To	From	To	From	To
1	Shrimp Farm	Other	12/15/25	—	0800	—	—	—	—	—	—	Pam Mcdermott	1
2													
3													
D. Relinquished By:				Date	Time	Time	Time	Received By:	Date	Time	Date	Time	
				12/15/25	1230	1230	1230		12/15/25	1230	12/15/25	1230	
Bio-Aquatic Lab ID: 98985													
Bio-Aquatic Sample Login				BAT sample personnel:		Date: <input type="text"/> 12/15/25	Time: <input type="text"/> 1500	By: <input type="text"/> DT	Temperature: <input type="text"/> 24	(C) IR# <input type="text"/> 006			
<input type="radio"/> Yes <input type="radio"/> No		Dechlorinate Sample:		<input type="radio"/> Yes <input type="radio"/> No		Chlorine: <input type="text"/> 0.1 mg/l	Ammonia: <input type="text"/> 0.1 mg/l	Int. Sal/Cond: <input type="text"/> 0.1 ppt/s	Adj. Salinity: <input type="text"/> ppt				
<input type="radio"/> Yes <input type="radio"/> No		Dilution Water:		<input type="radio"/> Receiving Stream <input type="radio"/> Synthetic Lab		pH: <input type="text"/> 8.1	Hardness: <input type="text"/> mg/l	Other					
<input type="radio"/> Yes <input type="radio"/> No		DO:		<input type="radio"/> Synthetic Lab		DO: <input type="text"/> 7.7 mg/l	Alkalinity: <input type="text"/> mg/l	Condition: <input type="text"/> good					