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TCEQ TNI Accredited

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

Chronic Biomonitoring Report

98986

Americamysis bahia
Menidia beryllina

December 18, 2025

Approved by: _____
Lab director

Eurofins Environment Testing Bio-Aquatics • 2501 Mayes Rd. Ste. 100 • Carrollton, Texas • 75006

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

Eurofins Environment Testing Bio-Aquatics
2501 Mayes Road, Suite 100
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Tel: (972) 242-7750
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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: Fish Farm
Laboratory Number: 98986
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 Fish 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:

EPA METHOD: 1007

Americamysis bahia

The seven-day Chronic *Americamysis bahia* survival and growth test was initiated at 16:56 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 15:40 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:

EPA METHOD: 1006

Menidia beryllina

The seven-day Chronic *Menidia beryllina* survival and growth test was initiated at 15:56 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 13:45 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: 98986**

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

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

Receiving Water Name:

Begin Date: 12/18/2025

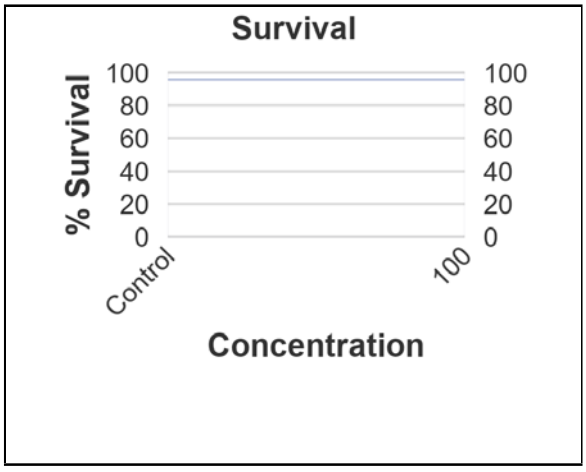
Test Start Time: 16:56 Test End Time: 15:40 **End Date:** 12/25/2025

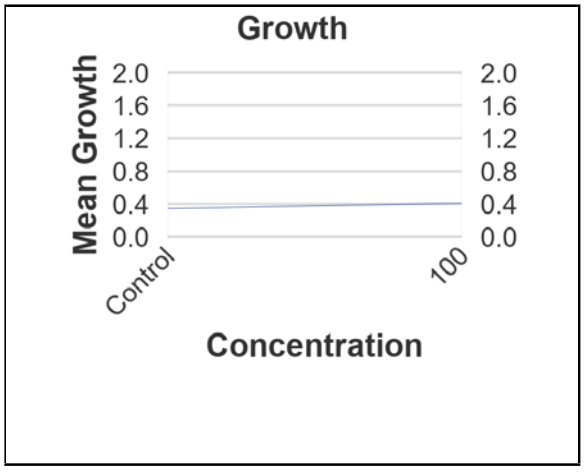
SURVIVAL

Effluent Con. %		Number of Alive 12/18	12/19	12/20	12/21	12/22	12/23	12/24	12/25	Avg% Surv.
Control	A	5	5	5	5	5	5	5	5	96.0%
	B	5	5	5	5	5	5	5	4	
	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	96.0%
	B	5	5	5	5	5	4	4	4	
	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									

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





Chronic Americamysis bahia SURVIVAL Lab ID: **98986**

Client: **Natural Energy Laboratory of Hawaii** Facility **Hawaii Ocean Science and Technology** Outfall: Fish 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	12-19-25 1050	5	—	—	—	—	5	—	—	—	—										
24Hr	12-19-25 SG 1900	5	—	—	—	—	5	—	—	—	—										
48Hr	12-20-25 1106 GJ	5	—	—	—	—	5	—	—	—	—										
72Hr	12.21.25 CAP 1030	5	—	—	—	—	5	—	—	—	—										
96Hr	12.22.25 CC C 1545	5	—	—	—	—	5	—	—	—	—										
5 days	12-23-25 SG 1305	5	—	—	—	—	5	4o	5	—	—										
6 days	12-24-25 0905 TM	5	—	—	—	—	5	4	5	—	—										
7 days	12-25-25 1540 KM	5	4o	5	—	—	5	4	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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Chronic Americamysis bahia SURVIVAL Lab ID: **98986**

Client: **Natural Energy Laboratory of Hawaii** Facility **Hawaii Ocean Science and Technology** Outfall: Fish 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	25.2	25.7	25.8	25.9	25.9	26.1	25.3	25.7	26.2	25.1	25.1	25.8	25.8	20.9
100														
TIME/DATE TECH	12-19-25 1650	12-19-25 BV	0900	12-20-25 1106	GJ	12-21-25 CAP	1030	12-22-25 CCL	1345	12-23-25 BV	1305	12-24-25 0708	TM	12-25-25 1340
IR GUN ID #	013	013		013		013		013		013		013		013

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1st Organism | **Eurofins Environment Testing Bio-Aquatics** | **TOXICITY TEST**

Chronic *Americamysis bahia*

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

Lab ID: **98986**

Permit Number: N/A

Sample Type: Grab

Outfall Name: Fish Farm

Receiving Water Name:

Synthetic

	ON	SN	Wt.	Avg.	SN Avg.
A	5	5	1.80	0.360	0.360
B	5	4	1.25	0.250	0.313
C	5	5	2.02	0.404	0.404
D	5	5	1.72	0.344	0.344
E	5	5	1.88	0.376	0.376

Mean	C.V. %
0.347	16.86

SN Mean	SN C.V. %
0.359	9.5

100

	ON	Wt.	Avg.
A	5	3.02	0.604
B	5	1.85	0.370
C	5	1.81	0.362
D	5	1.83	0.366
E	5	1.80	0.360

Mean	C.V. %
0.412	25.99

	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: **98986**

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	1.80
B	4	1.25
C	5	2.02
D	(lined through)	1.72
E	(lined through)	1.88
F		
G		
H		

100 %

	Qty.	Wt.
A	5	3.02
B	4	1.85
C	5	1.81
D	(lined through)	1.83
E	(lined through)	1.80
F		
G		
H		

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

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

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

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

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

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

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

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Chronic *Menidia beryllina*

Client: Natural Energy Laboratory of Hawaii Hawaii Ocean Science and Technology **Lab ID:** 98986

Permit Number: N/A

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

Receiving Water Name:

Test Temperature (oC): 25 ± 1

Photo Period: 16 Hours Light 8 Hours Dark

Test Start Time: 15:56 Test End Time: 13:45

Begin Date: 12/18/2025

End Date: 12/25/2025

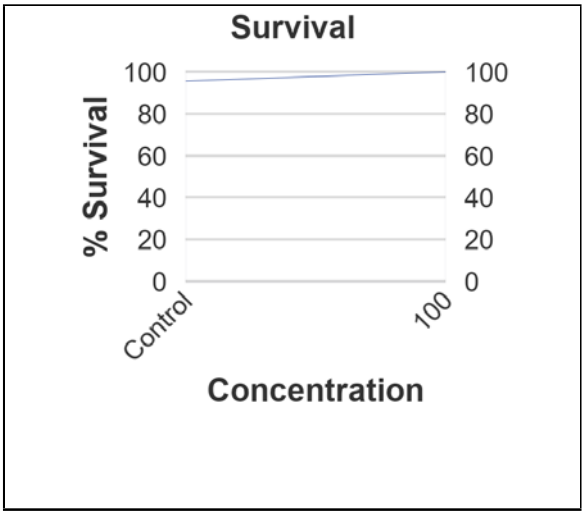
SURVIVAL

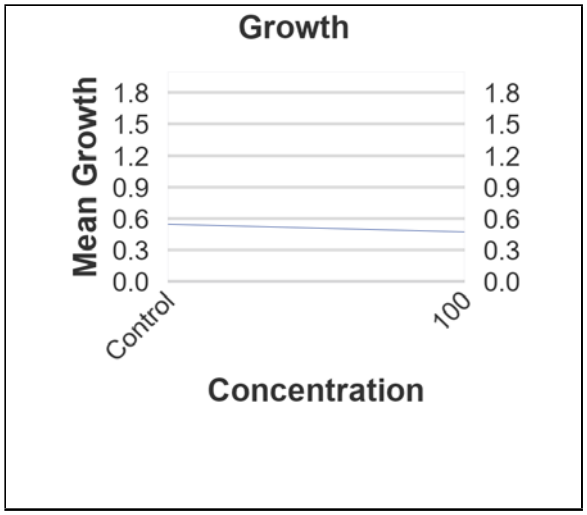
Effluent Concentration		Number Of Alive 12/18	12/19	12/20	12/21	12/22	12/23	12/24	12/25	Avg% Surv.
Control	A	8	8	8	8	8	8	8	8	95.8%
	B	8	8	8	8	8	7	7	7	
	C	8	8	8	8	8	8	8	8	
	D									
	E									
100	A	8	8	8	8	8	8	8	8	100.0%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D									
	E									
	A									
	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





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Chronic Menidia beryllina SURVIVAL Lab ID: **98986**

Client: **Natural Energy Laboratory of Hawaii** Facility **Hawaii Ocean Science and** Outfall: Fish 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-25-312 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	12-19-25 1356	8			1		8			1											
24Hr	12-19-25 SO PDS	8			1		8			1											
48Hr	12-20-25 1115 OJ	8			1		8			1											
72Hr	12.21.25 CAP 1035	8			CAP	1	8			CAP	1										
96Hr	12-22-25 CCC 1550	8			CCC	1	8			CCC											
5 days	12-23-25 1300	8	7	8			8														
6 days	12-24-25 CMCC M	8	7	8			8														
7 days	12-25-25 1345	8	7	8			8														

Dilution:

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

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Eurofins Environment Testing Bio-Aquatics

Chronic Menidia beryllina SURVIVAL Lab ID: **98986**

Client: **Natural Energy Laboratory of Hawaii** Facility **Hawaii Ocean Science and** Outfall: Fish 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	25.2	25.7	25.5	25.8	25.2	25.9	25.3	25.9	26.1	25.1	25.1	25.7	25.9	25.4
100														
TIME/DATE TECH	12-19-25 1556	12-19 SG	-25 0905	12-20 1115	-25 GJ	12-21 CAP	-25 1035	12-22-25 CCL 1550	/	12-23-25 1300		12-24 0904	-25 TM	12-28 1345
IR GUN ID #	013	013		013		013		013		013		013		013

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

2nd Organism **Eurofins Environment Testing Bio-Aquatics** **TOXICITY TEST**

Chronic *Menidia beryllina*

Client: Natural Energy Laboratory of Hawaii Ocean Science and Technology **Lab ID:** **98986**

Permit Number: N/A

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

Receiving Water Name:

Synthetic

	ON	SN	Wt.	Avg.	SN Avg.
A	8	8	4.16	0.520	0.520
B	8	8	4.96	0.620	0.620
C	8	8	4.05	0.506	0.506
D					
E					

Mean	C.V. %
0.549	11.3

SN Mean	SN C.V. %
0.549	11.3

100

	ON	Wt.	Avg.
A	8	3.71	0.464
B	8	3.99	0.499
C	8	3.64	0.455
D			
E			

Mean	C.V. %
0.473	4.9

	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: **98986**

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: 11-25-25

Date/Time placed in Oven: 11-24-25 1520

Date/Time removed from Oven: 11-25-25 1520

Control

	Qty.	Wt.
A	8	4.16
B	8	4.96
C	8	4.05
D		
E		

100 %

	Qty.	Wt.
A	8	3.71
B	8	3.99
C	8	3.64
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

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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: 98986.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	0	7	1	1

Calculated Chi-Square goodness of fit test statistic = 6.2255
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

mysid growth
File: 98986.myg Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.003	
2	100	0.011	3.359

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

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

mysid growth
File: 98986.myg Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 1.23

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: 98986.myg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.011	0.011	1.443
Within (Error)	8	0.060	0.007	
Total	9	0.070		

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

mysid growth
File: 98986.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	0.347	0.347		
2	100	0.412	0.412	-1.201	

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	0.347	0.347		
2	100	0.412	0.412	-1.201	

2 Sample t table value = 1.94 (1 Tailed Value, P=0.05, df=6,1)

mysid growth
File: 98986.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	0.102	29.3	-0.066

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	0.106	30.6	-0.066

mendida growth
File: 98986.meg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.009

W = 0.910

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.

mendida growth
File: 98986.meg Transform: NO TRANSFORMATION

F-Test for equality of two variances

GROUP	IDENTIFICATION	VARIANCE	F
1	con	0.004	
2	100	0.001	7.154

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

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

mendida growth
File: 98986.meg Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 1.35

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.

mendida growth
File: 98986.meg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	1	0.009	0.009	3.933
Within (Error)	4	0.009	0.002	
Total	5	0.017		

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

mendida growth
File: 98986.meg Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.549	0.549		
2	100	0.473	0.473	1.983	

---

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

UNEQUAL VARIANCE t-TEST Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.549	0.549		
2	100	0.473	0.473	1.983	

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

mendida growth
File: 98986.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	0.082	14.9	0.076

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	0.112	20.4	0.076

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 98986

Outfall Name: Fish Farm Number of samples 1

Dilution Water: Synthetic Lab

Receiving Water:

Dechlorinate Sample:

Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
			Begin Date	End Date	Start	End
1	12/18/25	13:56	12/15/25	12/15/25	08:33	08:33

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
Facility: Hawaii Ocean Science and
Outfall: Fish Farm

Lab ID: 98986
Dilution Water(s): Synthetic Lab
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	13:56	<0.10	N/A	<0.25	DT	2.8

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

Effluent Sample #	pH	DO (mg/L)	Init. Salinity (ppt)	Adjusted Salinity	Analyst Initials
1	8.2	7.6	39.6	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:** 98986

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

Outfall: Fish 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)		7.7	7.1						
		25 ± 1		Salinity (ppt)		19.3	36.0						
CAP	12/19	24 Hr	1	pH		8.1	8.0						
				DO (mg/L)		7.6	6.7						
		25 ± 1		Salinity (ppt)		21.9	41.3						
		Renew	1	pH		8.2	8.3						
				DO (mg/L)		7.4	6.8						
				Salinity (ppt)		21.2	38.8						
GJ	12/20	48 Hr	1	pH		7.8	7.7						
				DO (mg/L)		6.7	6.3						
		25 ± 1		Salinity (ppt)		20.5	38.6						
		Renew	1	pH		7.8	8.0						
				DO (mg/L)		7.5	7.1						
				Salinity (ppt)		20.0	36.2						
SG	12/21	72 Hr	1	pH		8.0	7.9						
				DO (mg/L)		7.4	6.3						
		25 ± 1		Salinity (ppt)		22.4	41.9						
		Renew	1	pH		8.1	8.1						
				DO (mg/L)		7.4	7.3						
				Salinity (ppt)		19.9	36.4						
CCC	12/22	96 Hr	1	pH		7.8	7.8						
				DO (mg/L)		6.8	5.9						
		25 ± 1		Salinity (ppt)		22.0	41.6						
		Renew	1	pH		8.0	8.1						
				DO (mg/L)		7.2	6.8						
				Salinity (ppt)		19.1	36.2						
GJ	12/23	120 Hr	1	pH		7.8	7.7						
				DO (mg/L)		6.3	5.6						
		25 ± 1		Salinity (ppt)		23.3	43.4						
		Renew	1	pH		8.1	8.1						
				DO (mg/L)		7.1	6.8						
				Salinity (ppt)		19.7	36.5						
TM	12/24	144 Hr	1	pH		8.0	8.0						
				DO (mg/L)		6.7	5.7						
		25 ± 1		Salinity (ppt)		23.7	43.6						
		Renew	1	pH		8.2	8.2						
				DO (mg/L)		7.0	7.1						
				Salinity (ppt)		19.1	35.3						
MV	12/25	168 Hr	1	pH		7.9	7.9						
				DO (mg/L)		6.5	7.0						
		25 ± 1		Salinity (ppt)		20.1	44.1						

Eurofins Environment Testing Bio-Aquatics

pH, Dissolved Oxygen, Salinity

Chronic **Menidia beryllina**

Client: Natural Energy Laboratory of **Lab Number:** 98986

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

Outfall: Fish 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)		7.7	7.1						
				Salinity (ppt)		19.3	36.0						
CAP	12/19	24 Hr	1	pH		8.2	8.0						
		25 ± 1		DO (mg/L)		7.6	6.8						
				Salinity (ppt)		22.6	34.6						
		Renew	1	pH		8.2	8.3						
				DO (mg/L)		7.4	6.8						
				Salinity (ppt)		21.2	38.8						
GJ	12/20	48 Hr	1	pH		7.8	7.7						
		25 ± 1		DO (mg/L)		7.4	6.8						
				Salinity (ppt)		21.2	35.4						
		Renew	1	pH		7.8	8.0						
				DO (mg/L)		7.5	7.1						
				Salinity (ppt)		20.0	36.2						
SG	12/21	72 Hr	1	pH		8.0	7.9						
		25 ± 1		DO (mg/L)		7.3	6.6						
				Salinity (ppt)		21.2	34.7						
		Renew	1	pH		8.1	8.1						
				DO (mg/L)		7.4	7.3						
				Salinity (ppt)		19.9	36.4						
CCC	12/22	96 Hr	1	pH		7.9	7.8						
		25 ± 1		DO (mg/L)		6.8	6.2						
				Salinity (ppt)		20.2	35.5						
		Renew	1	pH		8.0	8.1						
				DO (mg/L)		7.2	6.8						
				Salinity (ppt)		19.1	36.2						
GJ	12/23	120 Hr	1	pH		7.9	8.0						
		25 ± 1		DO (mg/L)		6.7	6.9						
				Salinity (ppt)		23.4	35.9						
		Renew	1	pH		8.1	8.1						
				DO (mg/L)		7.1	6.8						
				Salinity (ppt)		19.7	36.5						
TM	12/24	144 Hr	1	pH		8.0	7.9						
		25 ± 1		DO (mg/L)		6.4	5.8						
				Salinity (ppt)		19.8	35.7						
		Renew	1	pH		8.2	8.2						
				DO (mg/L)		7.0	7.1						
				Salinity (ppt)		19.1	35.3						
MV	12/25	168 Hr	1	pH		7.9	7.9						
		25 ± 1		DO (mg/L)		6.5	5.8						
				Salinity (ppt)		20.2	36.6						

Appendix B

Americamysis bahia

EUROFINS ENVIRONMENT TESTING BIO-AQUATICS

Carrollton, TX

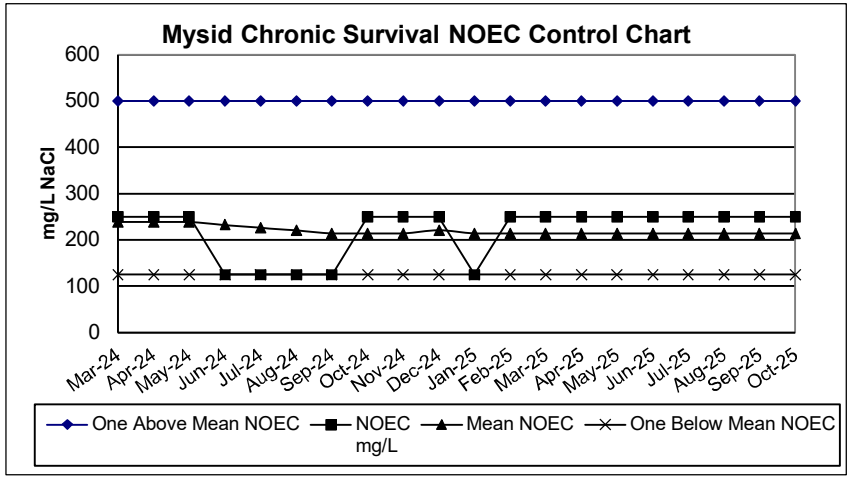
REFERENCE TOXICANTS

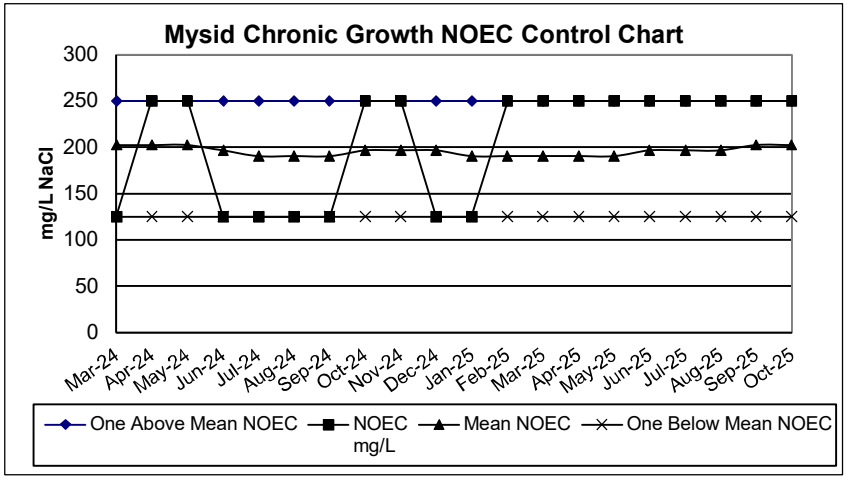
Eurofins Environment Testing Bio-Aquatics conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent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

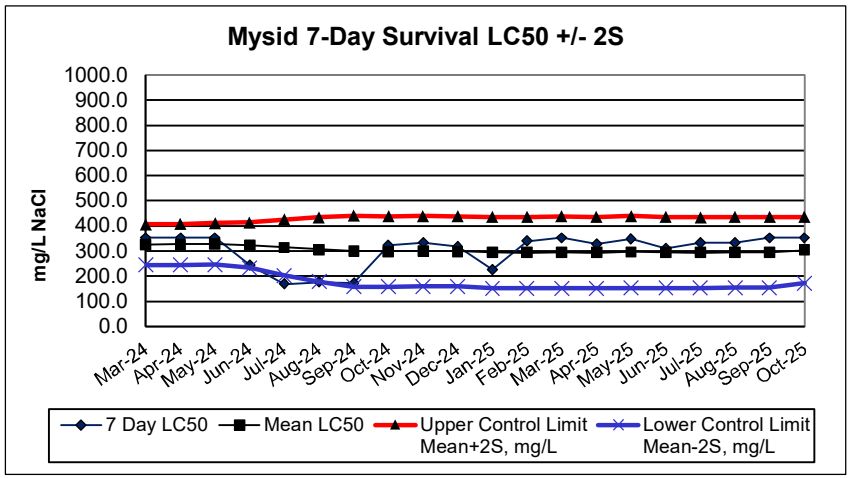
CHRONIC REFERENCE TOXICANT TEST RESULTS

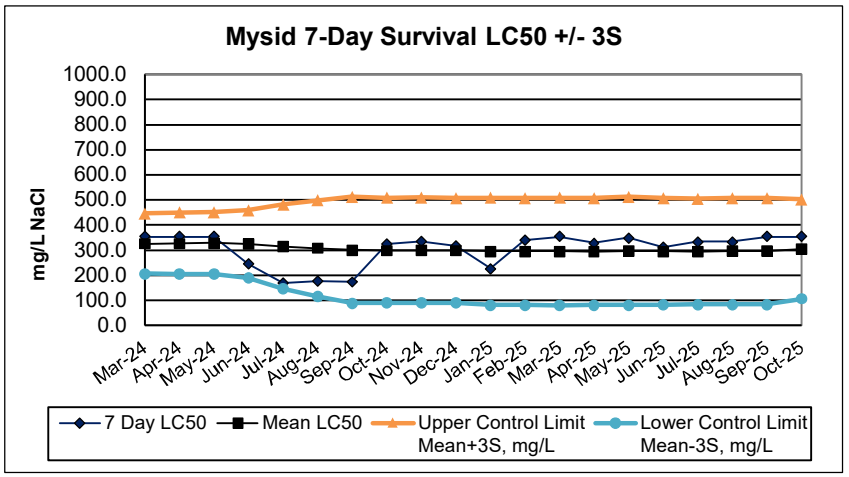
DILUTION WATER:	Standard Synthetic Saltwater						
CHEMICAL:	Potassium Chloride						
DURATION:	7 Days						
TEST NUMBER:	172						
PROJECT NUMBER:	98700						
START DATE:	10/29/2025						
START TIME:	11:27						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	25	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						

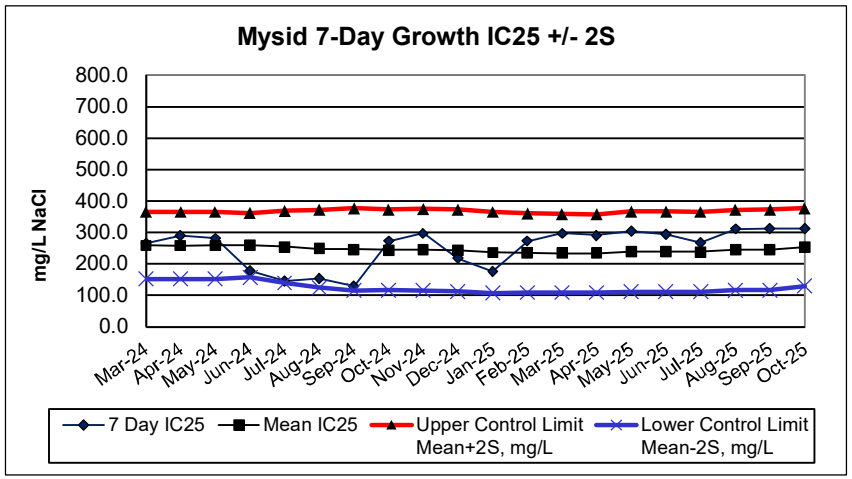
PMSD: 19.0

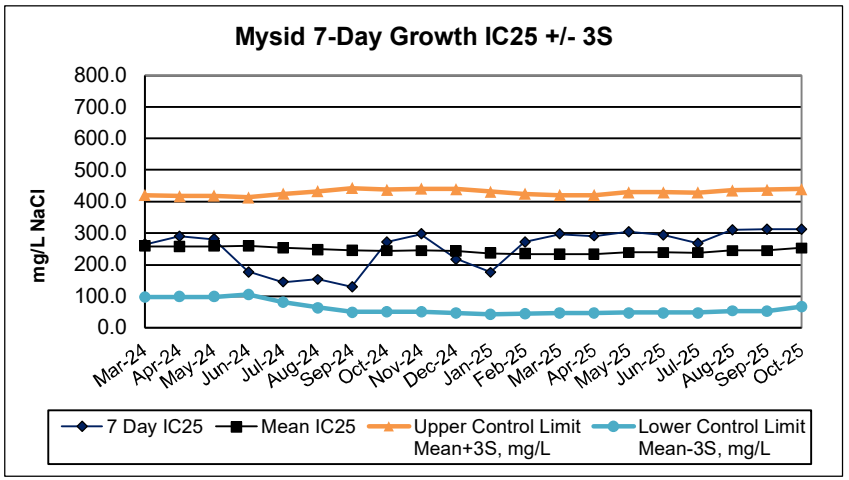

Mysid Chronic Survival NOEC Control Chart
mg/L NaCl
One Above Mean NOEC
NOEC mg/L
Mean NOEC
One Below Mean NOEC


Mysid Chronic Growth NOEC Control Chart
mg/L NaCl
One Above Mean NOEC
NOEC mg/L
Mean NOEC
One Below Mean NOEC


Mysid 7-Day Survival LC50 +/- 2S
mg/L NaCl
7 Day LC50
Mean LC50
Upper Control Limit Mean+2S, mg/L
Lower Control Limit Mean-2S, mg/L


Mysid 7-Day Survival LC50 +/- 3S
mg/L NaCl
7 Day LC50
Mean LC50
Upper Control Limit Mean+3S, mg/L
Lower Control Limit Mean-3S, mg/L


Mysid 7-Day Growth IC25 +/- 2S
mg/L NaCl
7 Day IC25
Mean IC25
Upper Control Limit Mean+2S, mg/L
Lower Control Limit Mean-2S, mg/L


Mysid 7-Day Growth IC25 +/- 3S
mg/L NaCl
7 Day IC25
Mean IC25
Upper Control Limit Mean+3S, mg/L
Lower Control Limit Mean-3S, mg/L

Appendix B

Menidia beryllina

EUROFINS ENVIRONMENT TESTING BIO-AQUATICS

Carrollton, TX

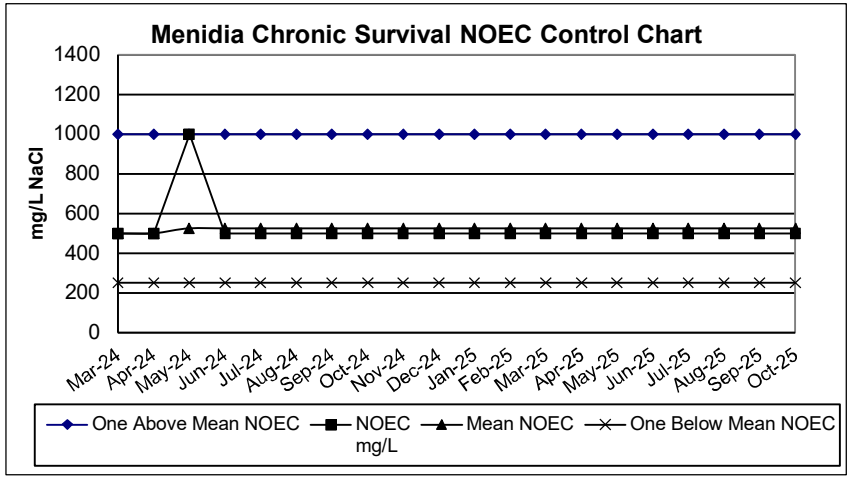
REFERENCE TOXICANTS

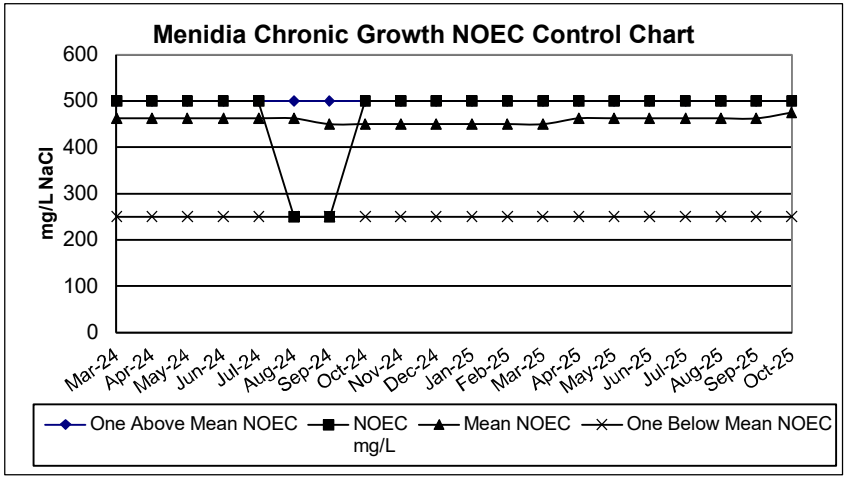
Eurofins Environment Testing Bio-Aquatics conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent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

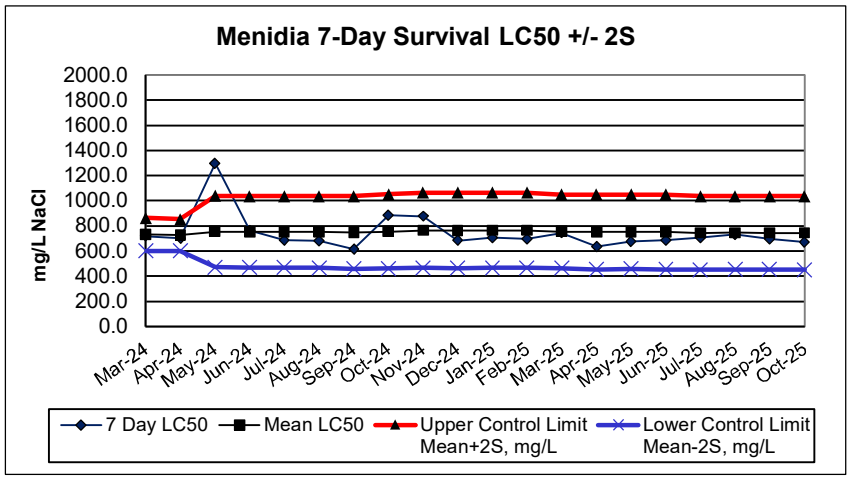
CHRONIC REFERENCE TOXICANT TEST RESULTS

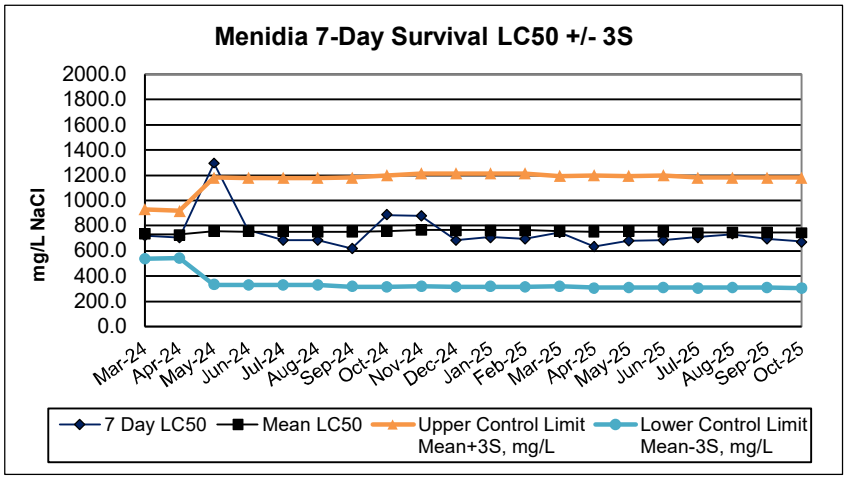
DILUTION WATER:	Standard Synthetic Saltwater						
CHEMICAL:	Potassium Chloride						
DURATION:	7 Days						
TEST NUMBER:	172						
PROJECT NUMBER:	98699						
START DATE:	10/29/2025						
START TIME:	11:10						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	125	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						

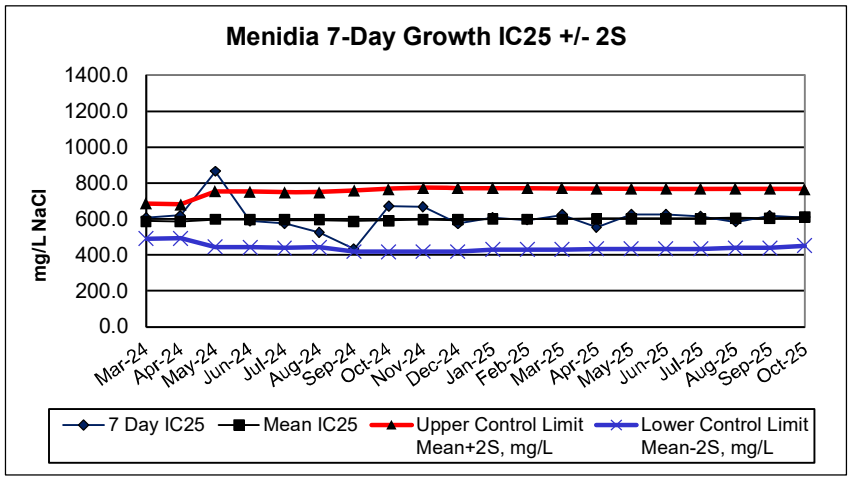
PMSD: 16.1

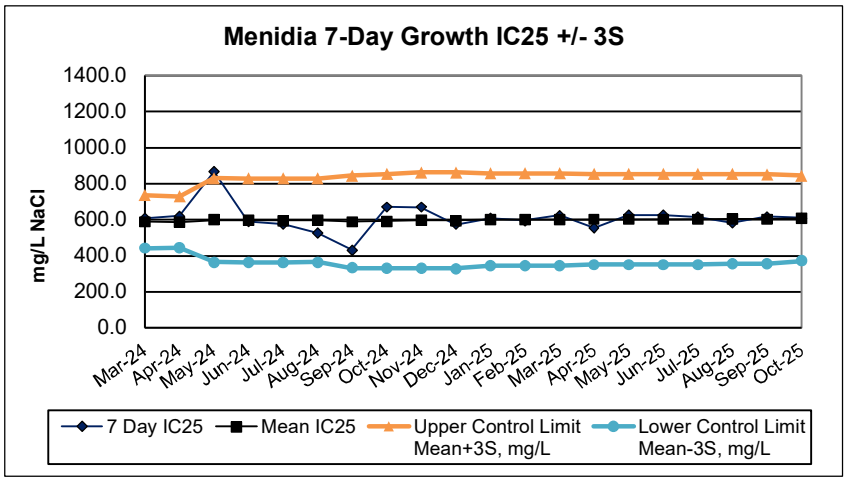

Menidia Chronic Survival NOEC Control Chart
mg/L NaCl
One Above Mean NOEC
NOEC mg/L
Mean NOEC
One Below Mean NOEC


Menidia Chronic Growth NOEC Control Chart
mg/L NaCl
One Above Mean NOEC
NOEC mg/L
Mean NOEC
One Below Mean NOEC


Menidia 7-Day Survival LC50 +/- 2S
mg/L NaCl
7 Day LC50
Mean LC50
Upper Control Limit Mean+2S, mg/L
Lower Control Limit Mean-2S, mg/L


Menidia 7-Day Survival LC50 +/- 3S
mg/L NaCl
7 Day LC50
Mean LC50
Upper Control Limit Mean+3S, mg/L
Lower Control Limit Mean-3S, mg/L


Menidia 7-Day Growth IC25 +/- 2S
mg/L NaCl
7 Day IC25
Mean IC25
Upper Control Limit Mean+2S, mg/L
Lower Control Limit Mean-2S, mg/L


Menidia 7-Day Growth IC25 +/- 3S
mg/L NaCl
7 Day IC25
Mean IC25
Upper Control Limit Mean+3S, mg/L
Lower Control Limit Mean-3S, mg/L

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

eurofins | Environment Testing Bio-Aquatics

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

CHAIN OF CUSTODY

☐ Bio Only: No Sample Left

Lab Id : 98986

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

Sample No: 98986 – ____

Revision 2 Effective Date 9/25/2017

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

P.O. No:

Report Date 01/06/2026 Revision 0

Client: Natural Energy Laboratory of Hawaii

Facility: Hawaii Ocean Science and Technology Park

Permit N/A

Outfall Fish Farm

Client Contact: Pam Madden

Client Phone: 808-327-9504

A. REVIEW SCHEDULED TEST(s):

Chronic	Americamysis bahia
Chronic	Menidia beryllina

Concentration: 100

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

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

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

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

Notes: Non-Routine/Specialty Testing for Information purpose

C.

Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1 Fish farm	other	12/15/25	–	0833	–	G	Pam Madden	1
2								
3								

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	Pam Madden	12/15/25	1230	Fedex		
2				DT	12.18.25	1356
3						

Bio-Aquatic Lab ID: 98986

Bio-Aquatic Sample Login

BAT sample personnel: ● Yes ● No

Dechlorinate Sample: ○ Yes ○ No

Dilution Water: ○ Receiving Stream ● Synthetic Lab

Date: 12-18-25 Time: 1356 By: DT Temperature: 2.8 (C) IR#: 0.6

Chlorine: 0.1 mg/l Ammonia: 0.25 mg/l Int. Sal\Cond: 39.6 ppt/uS Adj. Salinity ppt

pH: 8.2 Hardness: mg/l Other

DO: 7.6 mg/l Alkalinity: mg/l Condition: Good