Study Title

Acute Toxicity of Salinity
To the Inland Silverside (*Menidia beryllina*) and Mysid Shrimp (*Mysidopsis bahia*)
Under Static Test Conditions

Performed For

Parsons Environment & Infrastructure Group
9101 Burnet Road, Suite 210
Austin, TX 78758

Project Officer
Randy Palachek

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Study Period
15 July 2021 to 19 July 2021

Performing Laboratory

STILLMEADOW
INCORPORATED

Environmental Toxicology Laboratory
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Certificate Number T104704352-20-13

Project Number
21-607-007
21-607-008

STATEMENT OF PROCEDURAL COMPLIANCE

I certify that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. The information contained herein is accurate and complete.

Janelle Mikulas, M.S.

28 Jul 21
Date

STATEMENT OF QUALITY ASSURANCE

The report and study data were audited to assure that the study was performed in accordance with STILLMEADOW, Inc. Standard Operating Procedures and regulatory guidelines. This report is an accurate reflection of the raw data.

Quality Assurance Auditor

28 Jul 21
Date

EXECUTIVE SUMMARY

Objective	The objective of this study was to determine the acute toxicity of Salinity for Parsons Environment & Infrastructure Group to the inland silverside, *Menidia beryllina* and the Mysid shrimp, *Mysidopsis bahia.*			
Study Director	Janelle Mikulas, M.S.			
Test Type	2-Minute Static Acute			
Test Method	United States Environmental Protection Agency (EPA-821-R-02-012) (2002) Method 2006.0 and 2007.0.			
Test Dates (Times)	*M. beryllina*: 15 July 2021 (1512:00) to 15 July 2021 (1526:00) *M. bahia*: 19 July 2021 (1537:00) to 19 July 2021 (1551:00)			
Test Substance	Salt			
Dilution Water	Synthetic Seawater			
Test Concentrations	Control (35 ppt), 45 ppt, 50 ppt, 55 ppt			
Source of Organisms	STILLMEADOW Inc. Culture Laboratory			
Age of Test Organisms	*M. beryllina*: 7-11 days; *M. bahia*: 7 days			
Test Acceptability (Control Survival)	**Parameter**		**Test Data**	**EPA Criterion**
	Survival	*M. beryllina*	100%	≥90%
		M. bahia	100%	≥90%
Test Results	**Test Organism**		**Critical Concentration**	**NOEC[1] Test Solution**
	M. beryllina		Pass	55 ppt
	M. bahia		Pass	55 ppt

[1]NOEC = No Observed Effect Concentration

INTRODUCTION

The objective of this study was to determine the acute toxicity to *Menidia beryllina* and *Mysidopsis bahia* larvae of salinity for Parsons Environment & Infrastructure Group. This study is conducted in compliance with Texas Pollution Discharge Elimination System (TPDES) permit requirements; and in accordance with Texas Water Code Chapter 5, Subchapter R, Title 30 Texas Administrative Code Chapter 25 and the National Environmental Laboratory Accreditation Program (NELAP), Certificate Number T104704352-20-13. All original data, laboratory notebooks, and associated documentation are archived by the STILLMEADOW, Inc. Environmental Toxicology Laboratory.

METHODS AND MATERIALS

Test Substance/Dilution Water

Dilution water was synthetic seawater prepared according to USEPA (2002) guidelines. Initial characterization of the dilution and control water is given in Table 1. Dilution water was salted to the appropriate salinity for each test concentration.

Table 1. Chemical characterization of dilution water

Batch/Sample # Synthetic Seawater	Date Prepared	pH (SU)	Salinity (ppt)	Ammonia (mg/L NH_3N)	Total Residual Chlorine (mg/L)
QA21099	12 Jul 21	7.7	25	0.00	0.01
QA21103	16 Jul 21	7.6	25	0.00	0.01

TEST CONDITIONS

Procedures

Testing was carried out according to procedures specified in *Methods for Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms* (EPA, 2002), and STILLMEADOW, Inc. Standard Operating Procedures. The test organisms were 7-11-day-old *M. beryllina* and 7-day-old *M. bahia* cultured at STILLMEADOW, Inc. In each test, 40 organisms (5 replicates of 8 organisms each) were exposed to each test concentration in polystyrene cups of appropriate volume. Tests were not fed.

The tests were performed using synthetic seawater as a control salted to 35 ppt. The test concentrations were synthetic seawater salted to 45, 50 and 55 ppt. Temperature, pH, salinity, and dissolved oxygen were measured before the addition of the organisms. Aeration was not employed.

The tests were terminated after 2 minutes and a count of the surviving organisms was recorded at 15, 30, 60, 90 and 120 seconds.

RESULTS

Survival in the synthetic seawater control met EPA test acceptance criterion for acute toxicity test (≥90%).

Table 2. Percent survival of *Menidia beryllina* in the 2-minute toxicity test.*

		Percent Survival			
Time	Replicate	Control (35 ppt)	45 ppt	50 ppt	55 ppt
2 Minutes	A	100	100	100	100
	B	100	100	100	100
	C	100	100	100	100
	D	100	100	100	100
	E	100	100	100	100
2-Minute Mean		100	100	100	100
Standard Deviation		0.0	0.0	0.0	0.0
Coefficient of Variation		0.00	0.00	0.00	0.00

Mysidopsis bahia

Survival in the synthetic seawater control met EPA test acceptance criterion for acute toxicity test (≥90%).

Table 3. Percent survival of *Mysidopsis bahia* in the 2-minute toxicity test.*

		Percent Survival			
Time	Replicate	Control (35 ppt)	45 ppt	50 ppt	55 ppt
2 Minutes	A	100	100	100	100
	B	100	100	100	100
	C	100	100	100	100
	D	100	100	100	100
	E	100	100	100	100
2-Minute Mean		100	100	100	100
Standard Deviation		0.0	0.0	0.0	0.0
Coefficient of Variation		0.00	0.00	0.00	0.00

*Note that observations were made of general survival approximately 1 hour and 24 hours after testing with no significant mortality in either species in any salinity.

Table 4. Summary of Statistical Endpoints.

Endpoint NOEC (No Observed Effect Concentration)	**Value (ppt)**
Menidia beryllina	55
Mysidopsis bahia	55

REFERENCE TOXICANT TEST RESULTS

STILLMEADOW, Inc. conducts routine standard reference toxicant testing using *Menidia beryllina* and *Mysidopsis bahia* obtained from their respective cultures. Sodium Dodecyl Sulfate is used respectively as the reference toxicant with synthetic seawater as the dilution water; the test method followed is USEPA (2002). A copy of STILLMEADOW, Inc. most recent standard reference toxicant control chart for each species is presented in Appendix B.

STUDY DEVIATIONS

No deviations from the prescribed guidelines or standard operating procedures were identified during the study.

REFERENCES

U.S. Environmental Protection Agency (USEPA). 2002. *Short-term Methods for Estimating the Acute Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*. Third Edition, October 2002. EPA-821-R-02-012.

Ives, Michael A. TOXCALC™ Version 5.0. 1994. TidePool Scientific Software. McKinleyville, California.

APPENDIX A

Statistical Analysis

Larval Fish Growth and Survival Test-2 Minute Survival

Start Date:	7/15/2021	Test ID:	21-607-007	Sample ID:	Salt
End Date:	7/15/2021	Lab ID:		Sample Type:	
Sample Date:		Protocol:	EPA-821-R-012	Test Species:	MB-Menidia beryllina
Comments:					

Conc-ppt	1	2	3	4	5
ontrol (35 ppt)	1.0000	1.0000	1.0000	1.0000	1.0000
45	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
55	1.0000	1.0000	1.0000	1.0000	1.0000

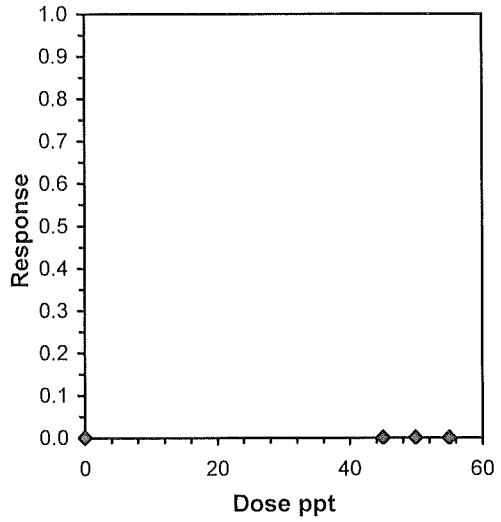
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-ppt	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
ontrol (35 ppt)	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5			1.0000	1.0000
45	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000
50	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000
55	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	1	0.868		
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	55	>55		

Linear Interpolation (200 Resamples)

Point	ppt	SD	95% CL(Exp)	Skew
IC05	>55			
IC10	>55			
IC15	>55			
IC20	>55			
IC25	>55			
IC40	>55			
IC50	>55			



Mysid Survival, Growth and Fecundity Test-2 Minute Survival

Start Date:	7/19/2021	Test ID:	21-607-008	Sample ID:	Salt
End Date:	7/19/2021	Lab ID:		Sample Type:	
Sample Date:		Protocol:	EPA-821-R-012	Test Species:	MY-Mysidopsis bahia
Comments:					

Conc-ppt	1	2	3	4	5
ontrol (35 ppt)	1.0000	1.0000	1.0000	1.0000	1.0000
45	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
55	1.0000	1.0000	1.0000	1.0000	1.0000

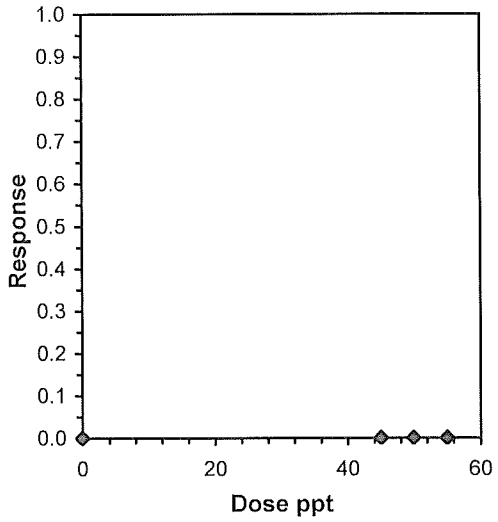
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-ppt	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
ontrol (35 ppt)	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5			1.0000	1.0000
45	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000
50	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000
55	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	1	0.868		
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	55	>55		

Linear Interpolation (200 Resamples)

Point	ppt	SD	95% CL(Exp)	Skew
IC05	>55			
IC10	>55			
IC15	>55			
IC20	>55			
IC25	>55			
IC40	>55			
IC50	>55			



Reviewed by:

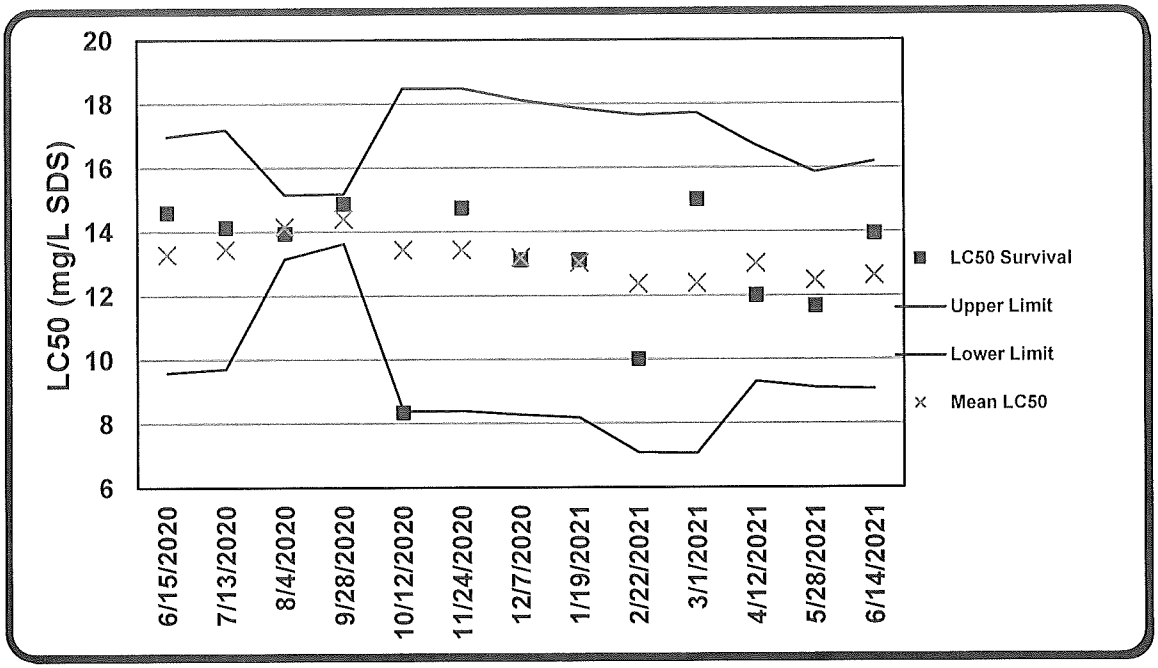
Port Authority 024933

APPENDIX B

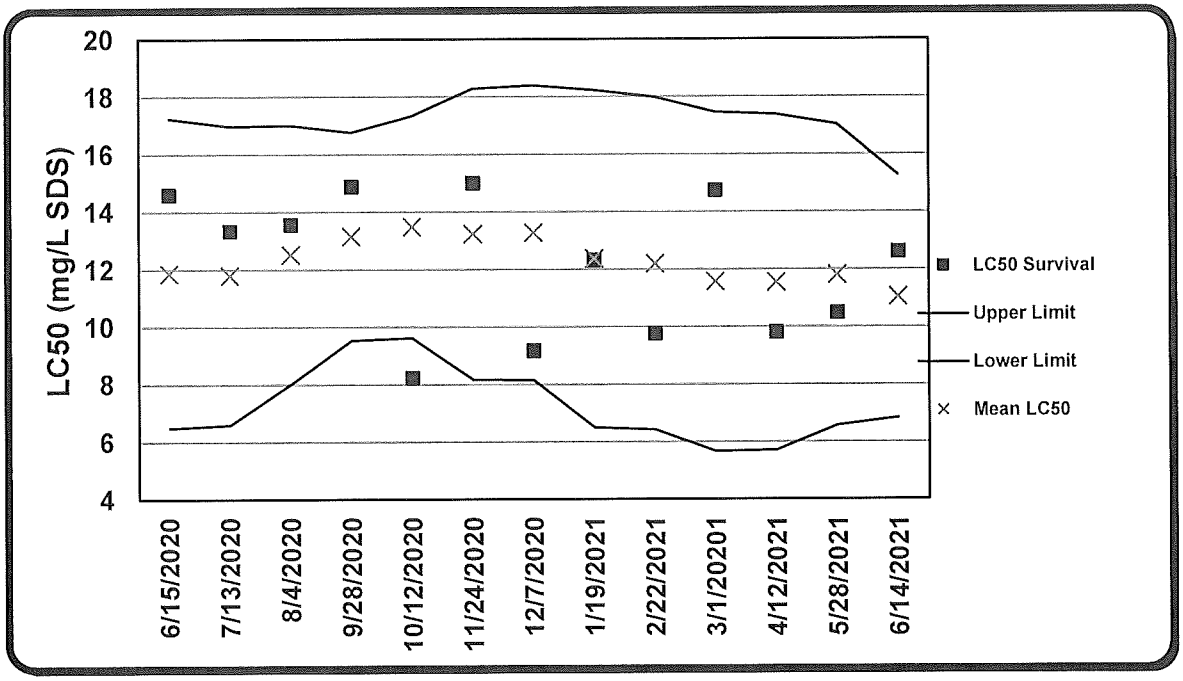
Standard Reference Toxicant Control Charts

Menidia beryllina Acute Standard Reference Toxicant Control Charts

24-hr Acute LC50 (mg/L SDS) Survival



48-hr Acute LC50 (mg/L SDS) Survival



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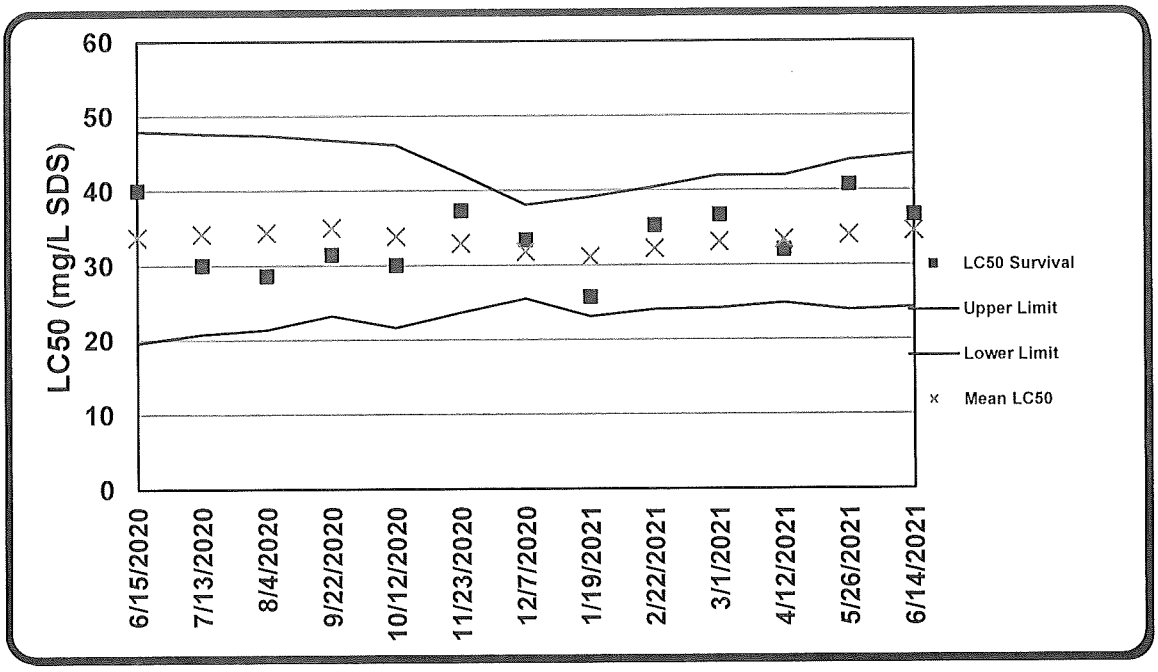
Mysidopsis bahia Acute Standard Reference Toxicant Control Charts

24-hr Acute LC50 (mg/L SDS) Survival



48-hr Acute LC50 (mg/L SDS) Survival