

Y2Q1 Progress Report

Assessing the risks of lithium pollution on estuarine fishes

Andrew Esbaugh, University of Texas at Austin

i. Summary: During the reporting period, we completed the developmental fish toxicity testing for the primary species, red drum and sheepshead minnow. Similar experiments are planned for southern flounder will commence in spring 2025. No field sampling for lithium occurred during the quarter, as expected, but field sampling is planned for early 2025.

ii. Staffing and Procurement: No major staffing changes or major procurement was undertaken during the reporting period; however, we successfully recruited two undergraduates to perform behavioral and mitochondrial studies on sheepshead minnow. These activities relate to Task #3, which will focus on chronic lithium exposure.

iii. Progress to Date: During the reporting period we completed our developmental toxicity testing for red drum across a salinity gradient. Note that we completed these studies for sheepshead minnow in Y1Q4. Interestingly, both species show relatively similar sensitivities and both show a similar trend of lower sensitivity at lower estuarine salinities. While trend was only significant for sheepshead minnow, it is important to acknowledge that the lowest salinity for red drum exposures was 5 ppt while sheepshead could be tested at 0 ppt. Nonetheless, the relative toxicity of lithium to these species is quite low with EC20 values ranging from 79 – 122 mg/L. For a point of comparison, freshwater toxicity of fathead minnows in water containing 0.5mM Na⁺ is approximately 1.5 mg/L; however, this value increases as sodium levels increase. Our value of 122 mg/L was obtained from freshwater contained approximately 7mM Na⁺.

The tests reported here are developmental tests, and thus serve as a chronic testing endpoint. As described in Y1Q1, our Institutional Animal Care and Use Committee recommended replacing acute larval test procedures with embryonic testing in accordance with the replace, reduce and refine guidelines to limit animal harm in toxicological studies. As such, the tests to date place us well ahead of schedule. We initially planned to begin chronic testing in year 2, but instead have already have completed standard chronic testing for the primary target species. That being said, we are currently performing “no observable effect concentration (NOEC)” tests for acute larval survival and chronic larval growth. These tests will all be performed at only one salinity (30 ppt) as they are intended to demonstrate that the sensitivity of larval growth and survivability is not greater than that of chronic development. At present tests have already been completed for larval (21 dpf) red drum and sheepshead minnow with no noted difference in survival between controls and the EC20 concentrations of the respective species. Growth tests will be performed in Y2Q2.

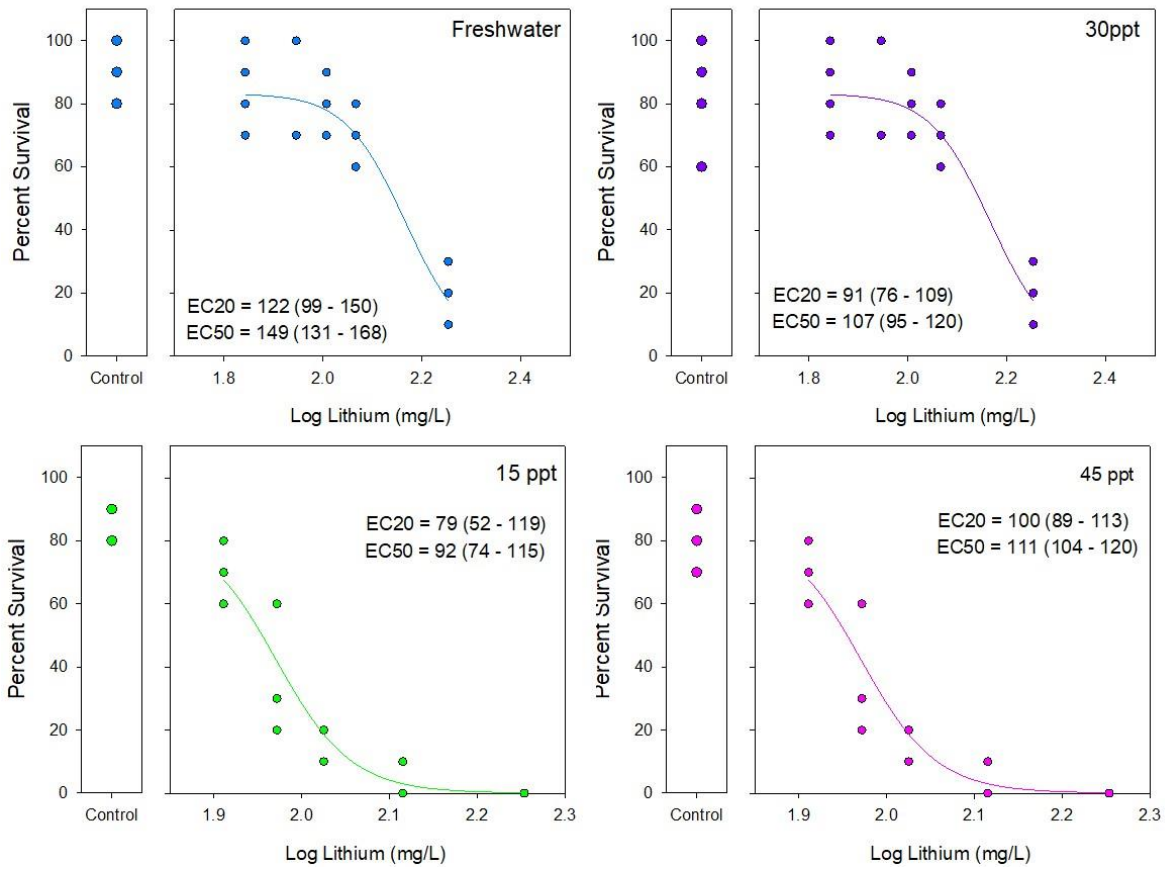


Figure 1: The complete survival dose response of embryonic sheephead minnows across a salinity gradient. In all cases the line represents a 3-parameter sigmoidal logistic curve with the effective concentration (EC) 20 and 50 values denoted in mg/L. The values in parenthesis are the lower and upper 95% confidence interval of the estimate.

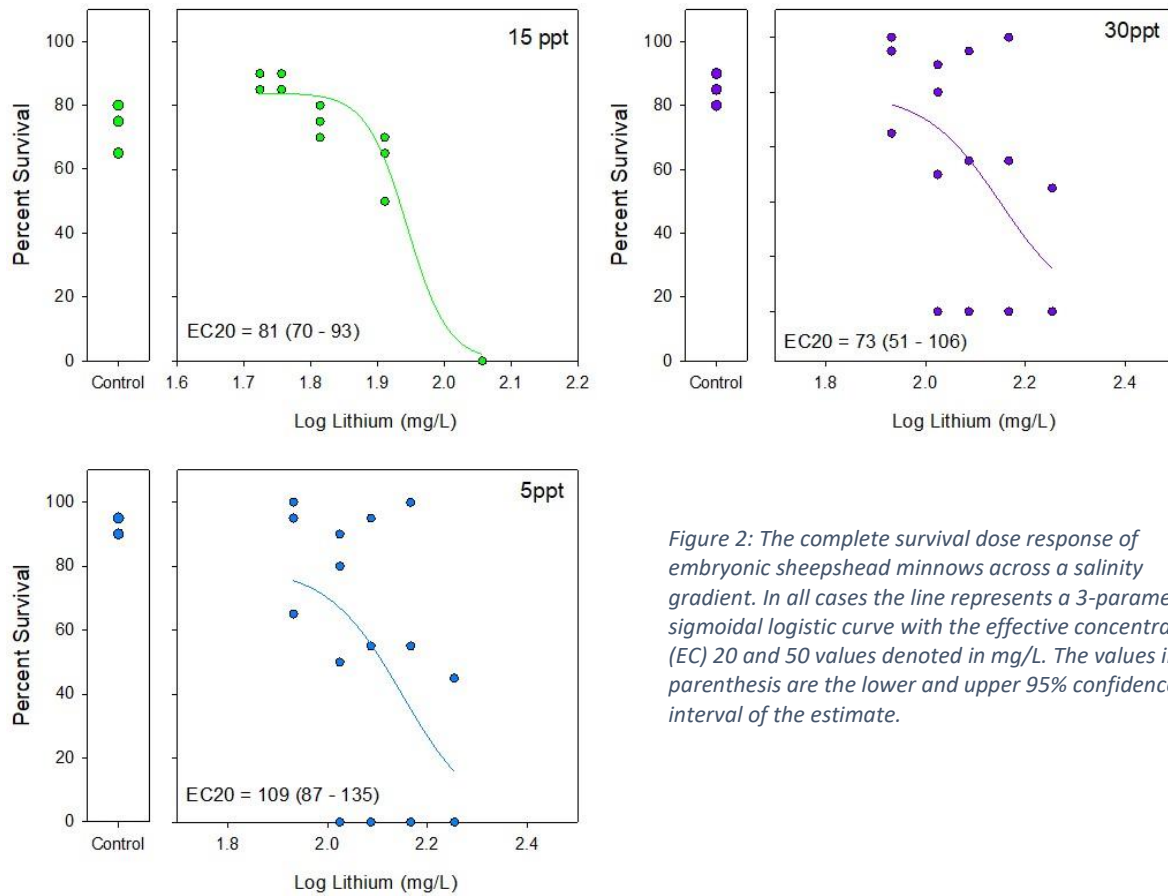


Figure 2: The complete survival dose response of embryonic sheepshead minnows across a salinity gradient. In all cases the line represents a 3-parameter sigmoidal logistic curve with the effective concentration (EC) 20 and 50 values denoted in mg/L. The values in parenthesis are the lower and upper 95% confidence interval of the estimate.

iv. Analytical Testing and Field Sampling: There was no planned field sampling for Y2Q1, as our proposal calls for biannual sampling. As stated in the prior report, we have collected samples from all four sites, including duplicate samples from several sites. Analytical determinations will be completed during the Y2Q2 period.



Figure 3: Proposed field sampling sites for the determination of lithium input into Matagorda Bay. All sites are associated with effluent inputs via wastewater treatment plants, while the reference site is intended as a non-effluent input site for the purposes of background values.

v. Activities Planned for Y2Q2: We have four main goals that will be undertaken in the upcoming quarter.

- 1) We will complete winter biannual field sampling at all four sites.
- 2) We will complete larval growth NOEC tests for both red drum and sheepshead minnow. Both tests will be performed at the species EC20, as determined by developmental toxicity testing.
- 3) We will complete sheepshead minnow and red drum behavioral testing. These tests will be performed at two lithium concentrations (EC20, 10x dilution of EC20) as well as at control concentrations. Behavior will be assessed using an open field test that specifically highlights activity-based endpoints, which is ideal because lithium is a known anti-manic drug in mammals.
- 4) We will complete mitochondrial impairment tests in sheepshead minnow. These tests will explore the inhibitory and stimulatory impacts of lithium exposure on mitochondria through direct exposure (i.e. homogenate exposure) and via organism uptake routes (i.e. expose the whole animal).

vi. Complications and Anticipated Changes: We have had no complications to report from Y2Q1, and studies are proceeding on schedule. The only minor change is that we have decided to perform an additional sheepshead minnow embryonic exposure at their lowest tolerable salinity (2 mM Na⁺; 3.5x dilution of dechlorinated tap water). This is largely to place our data within the existing literature framework of lithium toxicity and aquatic sodium concentrations.