

Y1Q4 Progress Report

Assessing the risks of lithium pollution on estuarine fishes

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i. Summary: During the reporting period, we continued to make progress on fish toxicity testing efforts. These efforts focused primarily on lethality sheepshead minnow and red drum, as well as behavioral testing for sheepshead minnow. We plan for southern flounder studies to commence in spring 2025. Field sampling for lithium occurred during the quarter as planned.

ii. Staffing and Procurement: No major staffing changes were made during the reporting period, although I can report that we successfully filled the summer undergraduate position. This undergraduate was primarily responsible for completing the sheepshead minnow lethality testing. No major procurement was undertaken during the reporting period, with expenditures owing to consumables related to larval rearing and toxicity testing.

iii. Toxicity Testing: During the reporting period we have made substantial headway in our lethality toxicity testing. This includes completion of the early life stage sheepshead minnow testing and 50% completion of the lethal red drum testing. Sheepshead minnow testing was undertaken using a standard embryonic survival and teratogenicity protocol approved by the Environmental Protection Agency, as described in the Y1Q3 report. Note that because this testing occurs in early development, it is considered a chronic exposure test. We can now report the toxicity of lithium to sheepshead minnow across the entire planned salinity gradient (Figure 1), which confirms the preliminary results from our previous report. More specifically, our findings suggest that lithium toxicity is higher in seawater salinities than what is found in local freshwater (i.e. Port Aransas tap water). However, it is interesting to note that there does appear to be protective effects of sodium within the hyperosmotic salinity treatments, whereby the 15 ppt has a lower EC50/EC20 than what is observed in the 45 ppt treatment. This may suggest that the underlying premise that sodium is protective against lithium toxicity is true in both freshwater and seawater, but the fundamental physiological differences between hypo-osmotic and hyperosmotic habitats means that lithium exposure is more harmful to freshwater fishes. Note that at present two of the four tests (15 and 45 ppt) are based on nominal concentrations, but thus far our measured concentrations have been very close to nominal doses when measured.

We have also made progress on red drum testing. In this case we are using a smaller salinity gradient owing to the fact that red drum are less tolerant of salinity deviations in early life. We are also using a modified test design for developmental toxicity of red drum, whereby we are allowing embryos to hatch prior to transfer to the test exposures. This is simply because red drum embryos exposed to reduced salinity will sink, and thus not hatch efficiently. As such, the red drum developmental test is effectively a 48-h exposure on post-hatch yolk sac larvae. At

present, we have completed two tests (15ppt and 35 ppt), although we may repeat the 15 ppt owing to a relatively steep dose response curve that results in large confidence intervals on effect estimates. Regardless, the data collected to date reveal EC20 values of 73.3 (50.6 – 106.3) mg/l and 80.9 (70.1 – 93.3) mg/l for the 35 ppt and 15 ppt treatment, respectively. These values are in line with observations in sheepshead minnow, which is unanticipated because sheepshead minnow are considered a “tough” species while red drum have been shown to be very sensitive to toxicants (e.g. polycyclic aromatic hydrocarbons). These data are very encouraging with respect to understanding the risks of lithium pollution to coastal ecosystems as these values are orders of magnitude higher than what would be expected from typical riverine input, and would likely only be observed in association with mining or fracking operations. Although it is important to recognize that the use of lithium in larger scale manufacturing (e.g. car batteries) remains an emerging area.

During the reporting period we have also continued our behavioral testing on sheepshead minnow. As previously reported, we first undertook a 24-h acute exposure of post-hatch larvae at four lithium doses (4, 16, 32 and 64 mg/L). Individuals were assessed for active time, percent time active, distance moved, acceleration and average speed, and lithium did not significantly affect any metric. During Y1Q4 we conducted a full embryonic exposure experiment at 85 mg/L (i.e. exposed from fertilization until 4 days post-hatch). Videos have been collected and will be analyzed for the metrics described above.

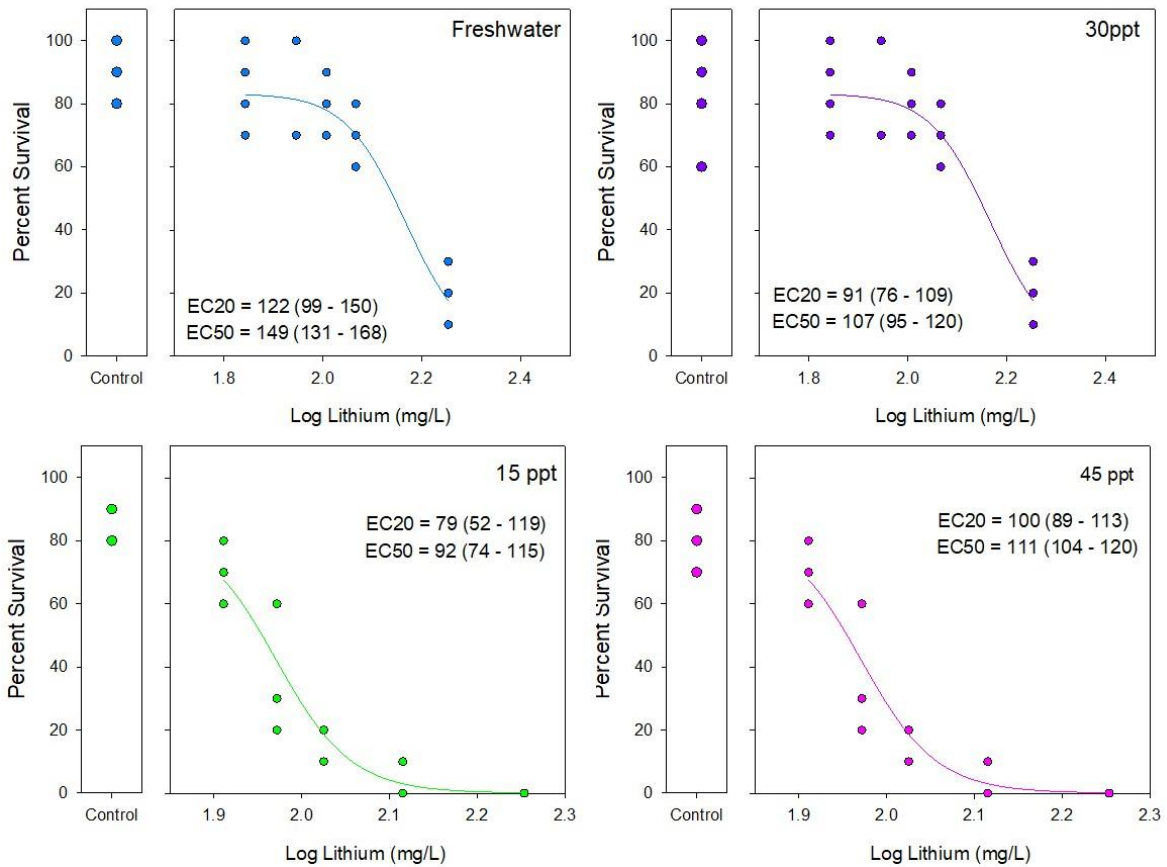


Figure 1: The complete survival dose response of embryonic sheephead minnows across a salinity gradient. In all cases the line represents a 3-parameter sigmoidal logistic curve with the effective concentration (EC) 20 and 50 values denoted in mg/L. The values in parenthesis are the lower and upper 95%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

iv. Analytical Testing and Field Sampling: Field sampling has continued throughout the reporting period. Note that our proposal calls for biannual sampling at three sites closely associated with wastewater treatment effluent, as well as one additional site that is associated with the barrier island and serves as a reference control. To date, we have collected samples from all four sites, including duplicate samples from several sites. At present, all samples are in the queue for analysis along with the toxicity testing samples described above.



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. Complications and Anticipated Changes: We have had no complications to report from Y1Q4, and we do not anticipate any changes in planned activities during the next quarter.