Y1Q3 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 made significant progress on fish toxicity testing efforts. These efforts focused primarily on sheepshead minnow, as the results from this model species work will inform on how we design the later red drum and southern flounder studies. Interestingly, our embryonic lethality work from sheepshead minnow suggests that prevailing views of lithium toxicity in aquatic environments do not extend to the marine environments, namely that sodium concentrations do not offer significant protection when compared to freshwater. Our initial work in behavioral studies suggest that lithium may not be a behavioral modifier in fishes; however, more study is required before this can be definitively concluded. Field sampling for lithium occurred during the quarter as planned.

ii. Staffing and Procurement: Some minor change to staffing are scheduled beginning in the upcoming quarter. The current technician is transitioning to a graduate student position in the lab, which required me to hire a new technician. A candidate has been identified and will start May 15th. Note that the new staff has worked in my lab last summer and was responsible for the first round of preliminary toxicity testing, and they will also overlap with the current technician through much of the summer. The summer undergraduate researcher has also been identified and agreed to the position. The candidate was responsible for the lethal toxicity testing that will be described in the subsequent section, and will continue the work on sheepshead minnow as well as contributing the work on larval red drum that will begin in the coming weeks.

iii. Toxicity Testing: During the reporting period we have made substantial headway on toxicity testing of the model organism, sheepshead minnow. This includes lethal and sub-lethal testing. The lethal testing was undertaken using a standard embryonic survival and teratogenicity protocol approved by the Environmental Protection Agency. In short, embryos (≤16 hours post-fertilization) are collected and subjected to a 1-hour formalin wash to eliminate any potential parasites. The embryos are then assessed for viability via a stereoscope and randomly sorted into test replicates. Each replicate contained 10 embryos. A total of five lithium concentrations were tested along with a control, and each concentration had four test replicates for a total of 240 embryos per test. During the reporting period our goal was to assess the impacts of hypo-osmotic versus hyperosmotic salinity, and as such, we performed tests at 0 parts per thousand (ppt) salinity (i.e. freshwater) and at 15 ppt (brackish water). The plotted data is shown in Figure 1 and effective concentration estimates shown in Table 1. Interestingly, the data reveal that lithium is more toxic at 15 ppt than in freshwater, which runs counter to the prevailing theories related to lithium toxicity in aquatic habitats. In freshwater, higher sodium concentrations have been shown to be protective, likely owing to the fact that lithium and sodium compete for entry into the animal. While more work needs to be done, we believe that these results relate to the differing osmoregulatory strategies of freshwater seawater fishes, with the former taking ions from the environment while the latter must excrete them into the environment. We hypothesize that lithium exposures is disrupting gill mitochondrial function, thereby disrupting ion excretion in seawater. This mode of toxicity would also further explain the protective nature of sodium in freshwater environments, as higher sodium reduces the demands on gill mitochondria for ion uptake.

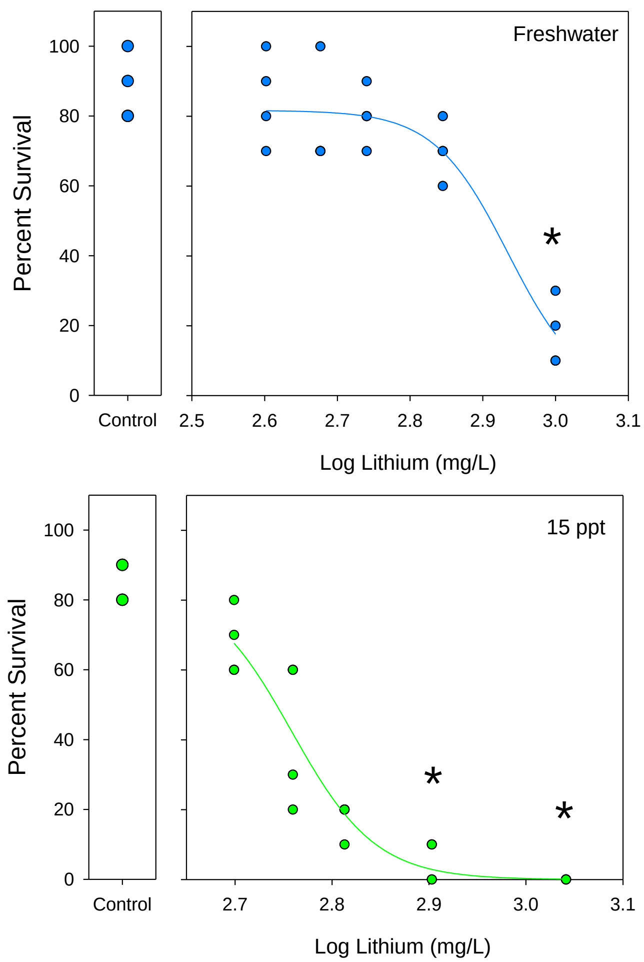

Figure 1: The dose response curves for sheepshead minnow embryos exposed to lithium through 10-days post-fertilization. The upper panel (blue) shows experiments in freshwater while the lower panel (green) shows experiments in 15 ppt water. The asterisks denote a significant difference in survival relative to control (ANOVA; P ≤ 0.05).

It is important to note two important caveats to the data presented here. First, these results are based on nominal lithium concentrations (i.e. the anticipated concentrations), and we still must validate the doses through analytical chemistry. This will be done in the coming weeks. The second major caveat is that the lithium values presented here are well above environmentally relevant concentrations in the Matagorda Bay system, and would only apply to more extreme settings, such as fracking fluid or mining effluent. This was always expected for sheepshead minnow, as they are an incredibly hardy species that toxicity thresholds that are orders of magnitude above those of other fishes. We chose to start with sheepshead because they are an excellent mechanistic model, and the data presented here will help plan the experiments on the more sensitive species, such as red drum and southern flounder.

During the reporting period we also undertook our initial behavioral testing, which was performed on sheepshead minnow. In this first trial we exposed post-hatch larvae for 24 h to increasing concentrations of lithium. Animals were exposed as described above for the acute testing, with five individuals per replicate and four replicates per concentration. A total of twenty individuals were tested for each lithium dose, and controls were

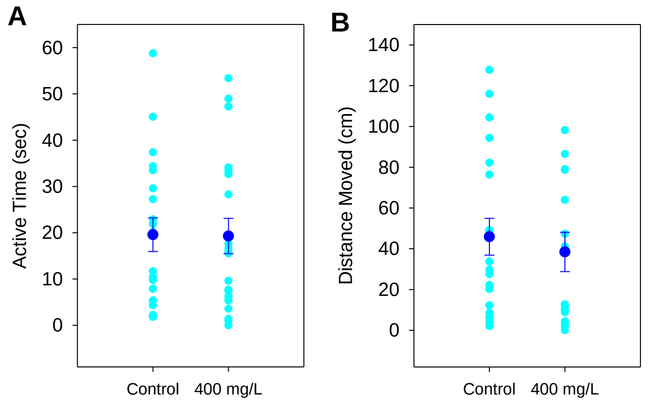

Figure 2: The effects of 24 h lithium exposure on two indices of yolk-sac larval behavior. The light blue points represent individual fish and the dark blue points represent the mean ± S.E.M.

run concurrently with each dose. A total of four lithium doses have been tested (25, 100, 200 and 400 mg/L). Each individual was placed in a testing arena and movements were recorded for 5 minutes, and the videos were subsequently analyzed for active time, percent time active, distance moved, acceleration and average speed. Lithium did not significantly affect any of these metrics in any of our tested doses. The collected videos will still be analyzed for thigmotaxis (i.e. wall-hugging) behavior, which is an indicator of anxiety. On the basis of the results collected to date, we have developed three additional sheepshead minnow studies that will ultimately inform on our work on red drum and southern flounder. First, we will conduct a 600 mg/l lithium dose that will be added to the data set collected above. Second, we will perform a full embryonic exposure experiment at 600 mg/L (i.e. exposed from fertilization until 4 days post-hatch), which will account for the possibility that exposure duration is an important variable. Third, we will perform a larval behavioral test at 14 days post-hatch. If all of these tests return no effect results, we can reasonably conclude that lithium is not a behavioral modifier in fishes.

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 three of four sites with the final site near Point Comfort scheduled for sampling in the coming weeks. 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 Y1Q3. Our red drum brood stocks are entering spawning season, so we anticipate the start of red drum toxicity testing to occur in Y1Q4. The only noteworthy change from the initially proposed experiments pertains to the behavioral testing. Because no behavioral modifications were noted in our first set of studies, we must perform some follow-up studies (noted in the Section III) that explore the effects of life stage and exposure duration. This is a minor change and will not impact our ability to meet the project objectives as outlined in the original proposal.