Y1Q1 Progress Report

Assessing the risks of lithium pollution on estuarine fishes

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i. Summary: Activities over this first quarter have largely focused on the on-boarding aspects of the described activities, which includes identifying personnel, procuring necessary equipment and performing needed preliminary experiments that will ultimately define the protocols utilized throughout the remaining project period. We have made sufficient progress on all of these fronts during the reporting period.

ii. Staffing and Procurement: Upon notice of the award and finalizing of the contract I began advertising for a postdoctoral scientist to join my team. My originally intended target staff was fortunate enough to get her own grant through the Matagorda Mitigation Trust (Kerri Ackerly) and thus I had to find someone new. Unfortunately, 4-months of searching has not yielded a suitable candidate, and since I do not want to delay the project I have decided to change the personnel make-up of the staffing to technicians and students. Specifically, I was able to re-work the budget to fund a technician, graduate student and summer undergraduate students. Suitable candidates have been identified for all positions with exception of the summer undergraduates, which will operate on a rotating basis. Note that the technician will be responsible for acute testing and field sampling, while the graduate student will take over sub-lethal testing of behavior and mitochondrial efficiency.

The second major project start-up hurdle was procurement of necessary equipment. In our case, the two major items that were need related to modifying our cation analytical equipment for lithium – which required new lamps with relatively long delivery times – as well as scaling up our sheepshead minnow colony to supply sufficient embryo numbers for toxicity testing. Both of these things have been completed, and I can verify that we are now producing sufficient sheepshead minnow embryos on a daily basis for to meet the demands for toxicity testing.

iii. Toxicity Testing: After consultation with the University of Texas Institutional Animal Care and Use Committee, it was requested that we attempt to meet the goals of the proposal while also trying to reduce animal harm in research. To do this, we have slightly modified our original design to first incorporate developmental toxicity as a chronic toxicity endpoint. We will then proceed on the assumption that developmental toxicity is the most sensitive endpoint, and thus employ acute survival and chronic growth assessments only at the identified developmental toxicity threshold concentrations (EC20 and EC50). This approach will yield more data while using fewer animals. To accommodate this new design, we were required to develop early life developmental assays for our target species. Suitable tests approved by the EPA have already been developed

for sheepshead minnow and zebrafish – the latter we have adopted as a freshwater surrogate. The shorter developmental life cycle of red drum required modification of the standard developmental design, particularly with respect to incorporating the effects of salinity. To this end, we first performed a basic salinity survival curve across developmental time to assess the impact of changing salinity one developing embryos (Figure 1). The results were very encouraging as they demonstrated that we could effectively transfer post-hatch yolk-sac larvae to salinities as low as 2 ppt for 24 h while maintaining suitable survival. However, survival at 2ppt began to decline in later life stages. Nonetheless, the efficacy of the developmental test design was validated for two of the three proposed marine test species. Note that southern flounder spawning occurs in the winter, at which time we will undertake species specific test design procedures.

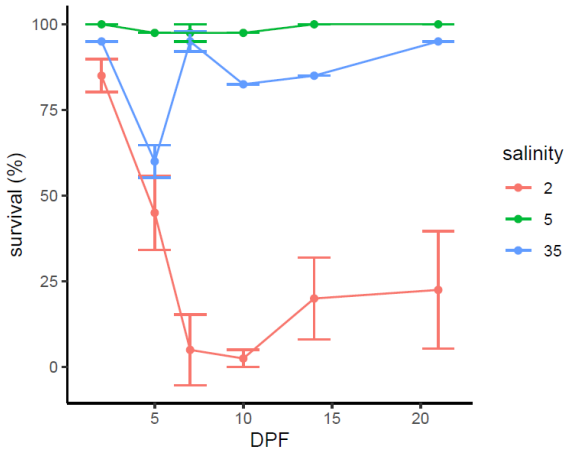

Figure 1: The effects of salinity on embryonic survival of red drum (Sciaenops ocellatus). Each data point represents the outcomes of a 24 h survival assay at the noted salinity and developmental time point (days post-fertilization).

The next step was to perform preliminary dose-response curves using both red drum and sheepshead minnow. All listed experiments are only nominal concentrations, and thus the data have no risk assessment value at present because nominal values for metals can be substantially different from measured owing to water chemistry-based speciation and precipitation. However, these are informative in the context of experimental design as they will define the nominal dosing regimen that will be employed for definitive experiments (i.e. those that use measured concentrations). Preliminary experiments suggest that the nominal effective concentration 50 for developmental toxicity for lithium in hypo-osmotic waters (i.e. 0 to 5 ppt) ranges from 300 to 600 mg/L, which is well in excess of levels found in the environment. But I would stress that these data should not be over-interpreted until full definitive testing and statistical analysis is complete.

iv. Analytical Testing and Field Sampling: Our proposal called for biannual field sampling at several locations in Matagorda Bay. These activities will commence beginning in early November with a spring sampling period scheduled for May. We have coordinated with several other projects at the University of Texas Marine Science Institute that are funded through the Matagorda Mitigation Trust to develop a single sampling site plan (Figure 2). This will allow information related to a wealth of different chemicals to be collected in a unified way in relation to likely effluent inputs (e.g. water treatments plants). Based on the nominal dosing described above and the expected environmental concentrations, we anticipate that our originally described analytical procedures will be sufficient. We have also worked out a secondary

analytical plan in the event that a more sensitive (i.e. lower concentration) procedure is required. This plan will be to outsource analytical measurements to the University of Texas at Austin's Jackson School analytical lab for ICP-MS determination.

Figure 2: Proposed field sampling sites for the determination of lithium input into Matagorda Bay. Note that the reference site is intended as a non-effluent input site for the purposes of background values.

v. Complications and Anticipated Changes: At this time, the only complication that has arisen have related to requirement of staff, which is a common challenge. Importantly, this project was originally designed to be flexible with regard to staffing, and as such, when a suitable postdoctoral candidate to not emerge soon after the award was granted, I was able to pivot to a back-up plan to maintain our timeline. We have no other anticipated changes.