Y3Q1 Progress Report

Assessing the risks of lithium pollution on estuarine fishes

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i. Summary: Entering the final year of the project, we have made substantial progress on the all activities that were outlined in the original proposal, which includes: 1) an assessment of lithium toxicity across a salinity gradient in relevant estuarine fishes using standard toxicity endpoints (i.e. survival, development and growth); 2) an evaluation of lithium toxicity using non-standard toxicity endpoints (i.e. behavior, ionoregulatory disruption and mitochondrial dysfunction); and 3) to document lithium concentrations in Matagorda Bay to evaluate possible risk. At this point, we can confidently say that based on standard toxicity style outputs and environmental sampling, there does not appear to be any risk associated with lithium concentrations in Matagorda Bay; however, we are designing two additional studies to completely validate this conclusion.

We are now in the process of partitioning the data set collected to date into publishable units and planning auxiliary studies that ensure publication in high quality journals. This step is crucial to ensure that the data generated here are properly communicated to the scientific community, and therefore put to good use in the years after project completion. It is important to note that based on a recent review paper (Barbosa et al., 2023), there have only been 37 published work pertaining to lithium in marine and coastal environments, and only 48 sites have been measured for lithium worldwide. Only one of these sites was measured in the Gulf of Mexico, and it was sampled from the central Gulf region. As such, our work will represent a substantial step forward with respect to understanding the effects and risks of lithium on coastal marine fishes.

At present we are planning three separate submissions:

a) *Lithium toxicity to coastal marine fishes across a salinity gradient:* This study will include all developmental toxicity test data and associated acute no effect concentration data for all species. This study will also include the in-progress growth tests for sheepshead minnows and the planned morphological assessments following lithium exposure. Finally, this study will also be home to the environmental sampling data.

b) *Lithium is a behavioral modifier in coastal marine fishes*: This study will include all the behavioral and mitochondrial dysfunction data. We will also include data from a planned study that tracks tissue uptake and body burden in larval sheepshead minnow.

c) *The effects of lithium exposure on sodium uptake rates in euryhaline fishes*: This study will include the in-progress ionoregulatory data related to lithium exposure in hypo-osmotic environments.

ii. Progress to Date:

Standard Toxicity Endpoints:

At present we have completed all developmental toxicity assays for lithium across a salinity gradient in both sheepshead minnow (Table 1) and red drum (Table 2). These data show a generally similar trend whereby lithium toxicity is lower in hyperosmotic environments (i.e. seawater) than in hypo-osmotic environments. It is important to note that we have been attempting to repeat the red drum 5 ppt due to a sub-optimal dose response relationship. These developmental toxicity values would be considered chronic endpoints, and as such we also performed acute lethality tests in normal seawater to test whether acute lethality may represent a more sensitive endpoint. In accordance with the principles of reduce and replace with respect to animal use in toxicological research, these studies were performed using a no observable effect concentration design at the chronic EC20 concentration for each species. In both cases, 96 h lithium exposure had no significant effect on survival of larval fishes, which demonstrates that embryonic development is a more sensitive indicator of sensitivity. An additional finding from this work was that red drum were significantly more sensitive to lithium than red drum. This was somewhat surprising as sheepshead minnow are generally considered to be a very resilient species to toxicological agents, while red drum do not have this reputations.

Table 1: Developmental toxicity of lithium exposure across a salinity gradient as defined by 10-day embryonic survival in sheepshead minnow. Note the non-overlapping confidence intervals for EC50 values at 0 ppt and higher salinities, denoting statistically significant toxicity profiles.

	EC20	95% CI	EC50	95% CI
0 ppt	121.8	99.1-149.7	148.6	131.1-168.4
15 ppt	81.1	65.9-99.7	95.6	84.5-108.1
30 ppt	91.1	75.9-109.3	106.6	94.7-120.0
45 ppt	80.5	68.8-94.1	95.0	86.0-105.0

Table 2 Developmental toxicity of lithium exposure across a salinity gradient as defined by 48h embryonic survival in red drum. Note the non-overlapping confidence intervals for EC50 values at 0 ppt and higher salinities, denoting statistically significant toxicity profiles.

	EC20	95% CI	EC50	95% CI
5 ppt	*108.7*	*87.2-135.4*	*138.2*	*123.1-155.2*
15 ppt	72.8	71.9-73.6	75.6	74.9-76.4
35 ppt	64.1	49.5-83.0	85.8	75.2-97.9

Non-standard Toxicity Endpoints:

As reported previously, we have completed behavioral analysis following 96-h exposure to control, 7.5 and 75 mg/l lithium on larval sheepshead minnow. The 75 mg/l dose was selected to coincide with the lower confidence limit of the EC20 at 30 ppt (i.e. the highest dose that would be more sensitive than developmental toxicity), while the 7.5 mg/l was chosen as 10% of this value to provide a no effect anchor dose. We used an open field test design to assess for hypoactivity traits, as lithium is known as an anti-manic drug with respect to bipolar disorders in humans. Our measured lithium concentrations across all tests came in slightly below nominal at 58.3 ± 3.9 mg/l and 6.9 ± 0.2 mg/l (n=20 samples per dose). Interestingly, the 96-h exposure at 58 mg/l resulted in significantly reduced indices of activity relative to controls, including distance travelled, average speed, average acceleration and percent active time (Figure 1). The lower dose had no effect relative to control and was also significantly different from the high dose. These results suggest that sublethal hypoactivity is a more sensitive measure of lithium exposure than developmental toxicity in sheepshead minnow. Importantly, the low dose that exhibited no behavioral effects is still a lithium concentration well in excess of likely environmental concern in coastal estuaries.

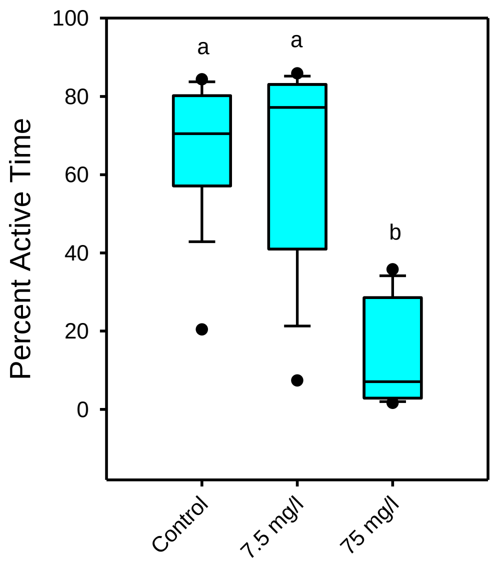

Figure 1: The effects of 96-h lithium exposure on larval sheepshead minnow activity behavior. Box plots denote the median, 25% and 75% quartiles, with the whiskers showing the minimum and maximum data spread. The black dots represent statistical outliers. N=16-19 per treatment.

We have since undertaken two additional behavioral tests: 1) scototaxis in sheepshead minnow, and 2) open field tests in red drum. The scototaxis test is designed to assess the anxiety aspect of lithium behavioral modification by assessing the proclivity of individuals to spend time on a white or dark background substrate. Sheepshead minnow are typically highly scototaxic, meaning they show strong preference for dark background. If lithium also modifies the anxiety-like behavior of fishes then we would hypothesize that animals will spend more time on white background. At present, we have completed two spawns worth of scototaxis tests using a similar dosing design as described above for the open field test in sheepshead minnow. We are in the process of performing the final rounds of exposure (3 spawns) after which video analysis will begin. Unfortunately, our attempts to perform behavioral testing in red drum failed owing to substantial cannibalism in control and lithium exposed doses. As such, our behavioral studies will focus exclusively on sheepshead minnow.

As mentioned in our previous report, we have also performed initial assessments of mitochondrial dysfunction, we chose to use brain tissue so results could be paired with the above stated findings on behavioral impairment. In this case, we assayed mitochondrial function in the presence or absence of 75 mg/l lithium (nominal dosing) using a paired design. At present, we have completed three assays with suitable QA/QC metrics, so data are not yet suitable for statistical analysis – we are aiming for a samples size of 8-10. Nonetheless, the available data are intriguing as there is evidence of an overall decline in oxidative phosphorylation (OXPHOS) when exposed to lithium (Figure 2).

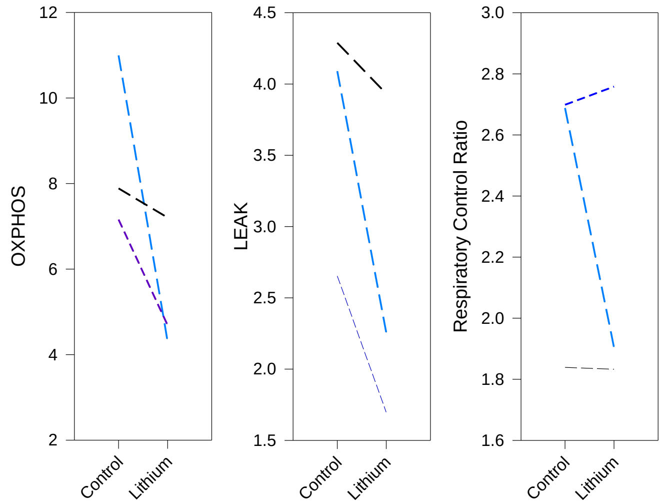

Figure 2: The effects of direct exposure of 75 mg/l lithium on brain mitochondria. Experiments were paired, with each line denoting the control and lithium exposed values for an individual. Note that preliminary findings (n=3) show a 35% decline in oxidative phosphorylation (OXPHOS) with little effect on efficiency as indicated by respiratory control ratios.

The final piece of data to report is the outcome of our environmental samples (Figure 3). At present, we have analyzed 9 samples collected from Matagorda Bay. The samples showed an average lithium concentration of 747 ± 43 µg/L. This value is well in excess of reported values for open ocean systems; however, other coastal systems have shown values in this range or higher (Barbosa et al., 2023). There does not appear to be a strong relationship with salinity, suggesting that riverine input is neither a source nor a diluting influence on coastal lithium. Most relevant is that while lithium values seem very high relative to expectation, the values are well below the risk thresholds established by our toxicity testing.

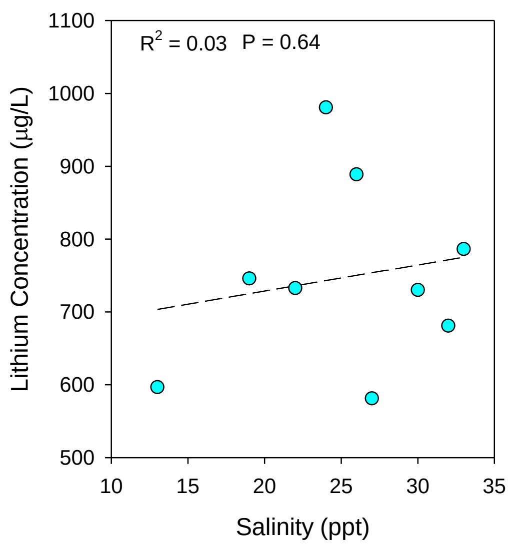

Figure 3: The relationship between water salinity and lithium concentration. There is no significant relationship, which suggests a source of lithium beyond freshwater input and baseline marine contributions.

iii. Plans for Y3Q1

#1) Due to the high concentrations of observed lithium, we have decided to perform some QA/QC analytical controls to ensure that the values are not being skewed by high ion content interference. We also plan to process additional water samples, and in fact, we have partnered with two UTMSI research teams that are sampling Matagorda Bay, which will provide us greater spatial coverage than we had initially planned.

#2) We are now prioritizing the completion of the sheepshead minnow growth studies, after which we will begin preparing our first manuscript. Growth studies will be performed at two salinities (freshwater and 30 ppt seawater) and consist of 6 concentrations of lithium per test. We anticipate these tests will be completed and fully analyzed this quarter.

#3) As mentioned above, we are in the process of running our final behavioral trial after which we will commence video analysis. We anticipate this will be completed this quarter. We have also planned an additional biological burden test as a component of the behavioral manuscript. This test will expose fish to the EC20 lithium dose for 7 days, and samples will be collected every day for analysis of total tissue lithium. This will allow us to ascertain whether a longer duration behavioral test might be necessary to get a better understanding of the environmental risk of lithium.

#4) This quarter we will begin our exploration of lithium as an ionoregulatory toxicant using sheepshead minnow. These studies will begin with a direct assessment of the effects of lithium on sodium uptake in larval fish as measured via scanning ion-selective micro-electrode methods. If lithium is shown to impair uptake, we will perform then perform a series of sodium rescue experiments whereby we will assess embryonic sensitivity in dilute waters with varying concentrations of sodium.

V. Complications and Anticipated Changes: During the last quarter we had notable challenges with the planned and undertaken red drum tests. As mentioned above, larval red drum exhibited substantial within replicate cannibalism, which means that behavioral and growth testing is not feasible for this species. While disappointing, this is ultimately not that significant owing to the fact that the sheepshead minnow and red drum showed relatively similar sensitivity profiles. As such, we believe the results from sheepshead minnow can be reasonably extended to red drum and other estuarine species. Similarly, we had poor control survival in several 5 ppt test re-run attempts in red drum. This is surprising given that we had prior success; however, this salinity does represent the lower bounds of their salinity tolerance. We will continue in our efforts on these tests until suitable control survival is obtained.

References:

Barbosa, H., Soares, A.M.V.M., Pereira, E., Freitas, R., 2023. Lithium: A review on concentrations and impacts in marine and coastal systems. Sci. Total Environ. 857, 159374.