Y2Q4 Progress Report

Assessing the risks of lithium pollution on estuarine fishes

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i. Summary: At the conclusion of Y2, we have made substantial progress on the activities listed in Task #3. This includes completion of all sub-lethal toxicity testing for red drum and sheepshead minnow, completion of behavioral analyses for sheepshead minnow, and preliminary analysis for mitochondrial impairment. On-going and planned work for Y3 includes exploration of ion regulatory impairments caused by lithium using sheepshead minnow, southern flounder developmental toxicity testing, growth impairment studies and the planned freshwater anchoring studies. The biannual field sampling described in Task #1 will also continue, while the acute toxicity testing described in Task #2 is complete.

ii. Staffing and Procurement: No major staffing changes or major procurement was undertaken during the reporting period. Spring undergraduate research has been completed and no undergraduate researchers were hired for summer 2025.

iii. Progress to Date:

During the most recent quarter, our work focused on three major areas: 1) completing the chemical analysis on developmental toxicity tests to validate nominal concentrations; 2) 96-h exposure behavioral testing; 3) assessing mitochondrial dysfunction in association with lithium exposure. With respect to the first area, we are happy to report that the chemistry for all but one test has been completed, with values generally supporting the prior nominal dosing and conclusion (Table 1 and 2). As such we can now confidently say that developmental toxicity from lithium is well outside the range likely to coincide with routine ground water sources. We can also say that developmental toxicity is more sensitive than larval acute toxicity, and that red drum are not statistically more sensitive than sheepshead minnow.

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

With respect to behavioral analysis, we performed 96-h exposure tests on 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.

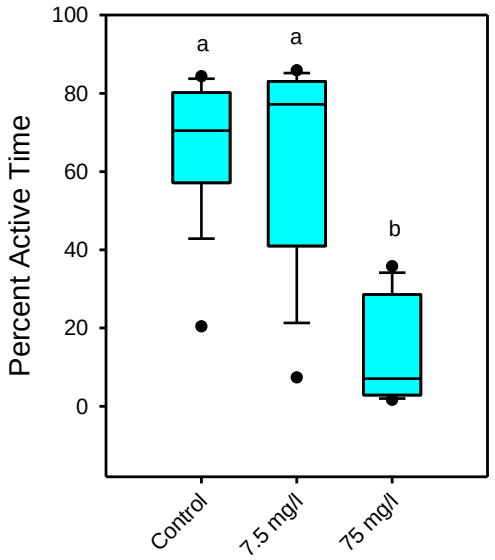

Figure 1: The effects of 96-h lithium exposure on larval sheepshead minnow activity behavior. Box plots denote the median, 25% and 75% quartiles, with the whiskers showing the minimum and maximum data spread. The black dots represent statistical outliers. N=16-19 per treatment.

For 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

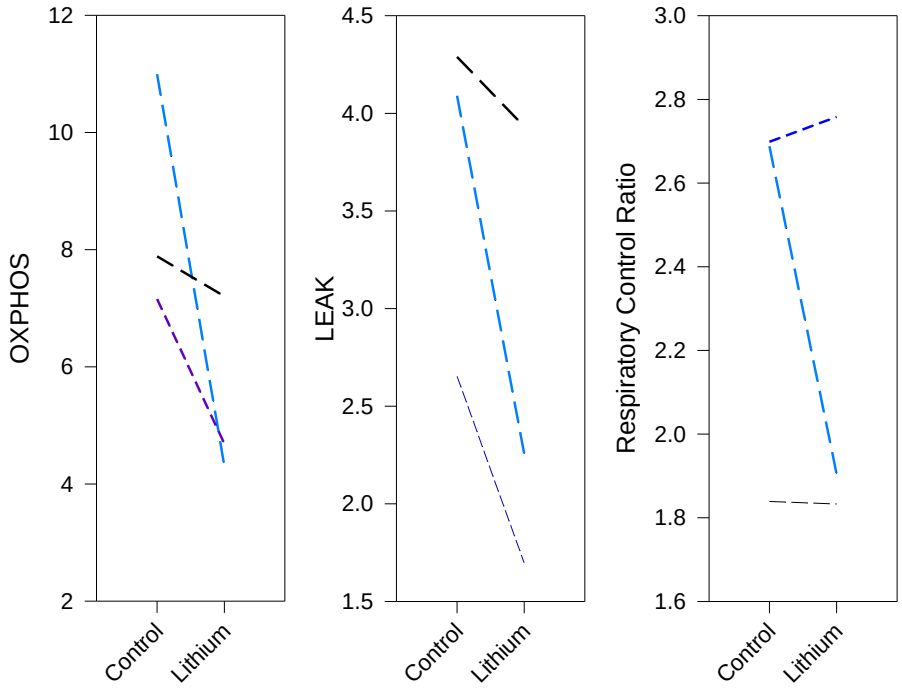

Figure 2: The effects of direct exposure of 75 mg/l lithium on brain mitochondria. Experiments were paired, with each line denoting the control and lithium exposed values for an individual. Note that preliminary findings (n=3) show a 35% decline in oxidative phosphorylation (OXPHOS) with little effect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

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).

IV. Plans for Y3Q1:

#1) Now that our chemical analysis is operating at high efficiency we will prioritize field sample analysis, which will also include a summer sampling period from all four sample sites (Figure 3).

#2) During the summer of 2025, we will prioritize red drum work owing to the fact that TPWD spawning operations are available to augment on-site larval production. To this end, we will assess larval behavioral effects in red drum following lithium exposure. In this case, we will likely perform an open field test that includes measures of sociability by testing the activity of five fish simultaneously. This will provide similar measures of activity, while also allowing assessment of social cohesion. This is required for red drum testing owing to the fact that preliminary work in red drum has shown them to be almost relatively inactive when tested in isolation, which represents a confounding factor when exploring hypoactivity. We will also attempt to perform

larval growth studies at 30 ppt and 5 ppt, although there is a strong possibility that conspecific cannibalism may invalidate these tests.

#3) Sheepshead minnow work will continue, with priority placed on completing mitochondrial dysfunction experiments, as well as adding an additional dilute freshwater developmental test as an anchor to the freshwater literature. We also plan to begin osmoregulatory dysfunction studies by assessing the impairment of sodium uptake in hypo-osmotic salinities. These studies will likely include a freshwater test, a 5-fold dilution of freshwater, and a 5 ppt test. We also intend to begin sheepshead growth tests, which will be performed in freshwater and seawater.

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 decided on four minor changes to original activities to coincide with data collected thus far. The first is that we are now planning 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 to replace the originally planned fathead minnow studies that were planned to provide a comparison to the literature. Use of the fathead minnow is no longer useful, as the animals do not allow for a developmental toxicity test, only a larval test. We are also planning to perform a second behavior test (light dark preference) to explore the anxiety behavior axis. Finally, we are reducing the growth tests from 4 salinities to 2, which is being done owing to specific effects in developmental toxicity observed between hypo- and hyperosmotic environments. We will now only test between freshwater and normal strength

seawater. This change will allow us to add an additional test to more specifically explore anatomical deformities that make occur in conjunction with embryonic developmental toxicity.