Matagorda Bay Mitigation Trust 2022-2023 Funding Cycle

RFP # 2022-2023-1 Contract #038

Title: Reproductive & Developmental Toxicity of "Forever Chemicals" to Matagorda Bay's prey fishes

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Y2 Q1 October 2024 Progress Report

Y2 Q1 Update:

We have continued to build the PFAS profile of Matagorda Bay as part of Phase 1, as we have received analyses for additional sediment samples. We have begun analyzing our morphology data for sheepshead minnow larval exposures as part of Phase 3. We also presented our data from this grant, including PFAS profiling in Matagorda Bay and the impacts of these PFAS on fishes important to the Gulf of Mexico, at an international conference.

Conference Presentations:

In October 2024, we gave two presentations at the North America Society of Environmental Toxicology and Chemistry (SETAC) annual meeting in Fort Worth, TX about our work under Contract #038. Dr. Ackerly (co-PI) gave an oral presentation titled "**Comparative Toxicity of PFAS on the Development of Lab-Reared and Wild-Caught Fishes in estuaries of the Gulf of Mexico**". Grace Walsh, an undergraduate researcher working in the Nielsen Lab, gave a poster presentation about our work titled "**Comparing the Impacts of 'Forever Chemicals' on the Development of Sheepshead Minnow and Red Drum**". Both presentations were part of a special session centered around "Environmental Issues in the Gulf of Mexico".

Phase 1:

During Y1 Q3, we collected additional samples from Matagorda Bay. Sediment samples were sent to an analytical chemistry lab – Eurofins Environmental Testing Northern California, LLC – for analysis using EPA Draft Method 1633. This is the same EPA Method that was used to analyze the sediment samples collected from our previous Matagorda Bay Reference Site and the site that is downstream of the Palacios Sewage Treatment Plant (as discussed in our Q3 Progress Report). This method will test for the 40 PFAS listed in Table 1.

During Y2 Q1, we received data back that showed the presence of PFOA and PFOS in sediment samples collected from Lavaca Bay (downstream of Point Comfort Wastewater Treatment Plant (WWTP), Formosa Plastics wastewater outfalls, and ALCOA wastewater outfalls) and in Chocolate Bay (downstream of the Port Lavaca WWTP). With these results, we now have data that shows PFAS contamination in all four of our sampling locations within the Bay (Figure 1).

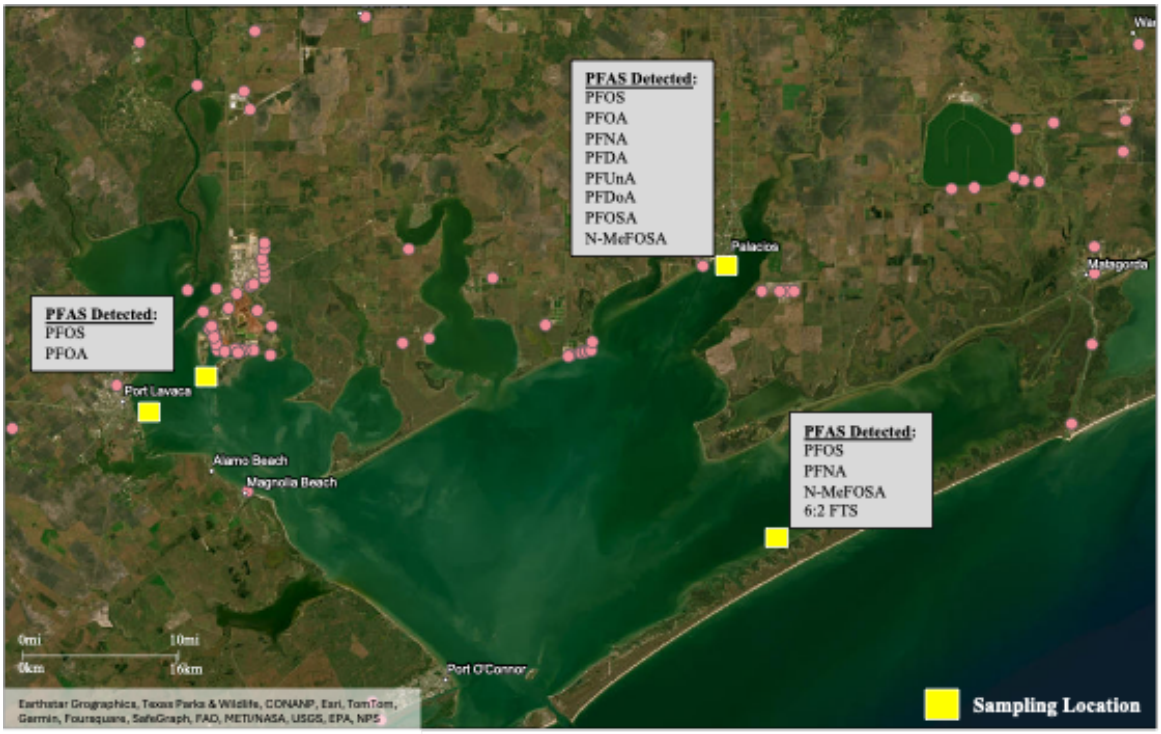

Figure 1. PFAS detected in sediment taken from the four indicated sampling locations.

Phase 3:

During Q4, we began PFAS testing on sheepshead minnow embryos and larvae in the lab as part of Phase 3. We are using commercially purchased embryos for these studies to establish concentrations of PFAS that impact the development of these embryonic and larval fish. To date, we have now collected data looking at the impacts of PFOS, PFOA, PFNA, PFDA, and PFOSA on the development of embryonic and larval sheepshead minnows. We chose these five compounds because our PFAS profiling detected them throughout the Matagorda Bay sediment samples (Figure 1). The concentrations used for this study (shown below in Figure 2) are anchored in the PFOS concentrations that the UT MSI Core Lab found of among our water

samples as well, with an average concentration of ~15 parts per billion (ppb). Therefore, we anchored our exposure concentrations for all 5 PFAS tested to these environmentally relevant data for Matagorda Bay.

To do this, we spawned commercially-purchased broodstock sheepshead minnows at the Fisheries and Mariculture Laboratory (FAML) at UT MSI. Sheepshead minnows were spawned first thing in the morning and collected embryos within 2 hours of spawning. Embryos were assessed for viability and left to develop for 24 hours. At ~24 hours post fertilization (hpf), embryos were staged and only embryos within the 16-17 embryonic stages[2] were used for the study. As previously mentioned, the PFAS doses for this study were anchored to the average concentration of PFAS detected in samples collected in Matagorda Bay (~15 ppt). We performed a graded dose-response using the following doses: 0 ppb (parts per billion; control), 2 ppb, 6 ppb, 16 ppb, and 44 ppb (Figure 2). Each experiment was set up individually, with embryos and larval fish being exposed to only one of the five PFAS mentioned above. At the start of the experiment, embryos (n=10/dish; 50 total per dose across dishes) were placed in the experimental treatments (Figure 2). Survival was assessed every 24 hours and embryos were checked for proper developmental milestones (e.g., eye pigment development). Following these checks, debris was removed from each dish and 50% water changes were made with water dosed with the relevant concentration. The experiments were performed in our lab's environmental chamber to ensure all dishes were maintained at the appropriate temperature (25°C) and were covered with transparent sheeting to prevent evaporation. Water quality parameters (temperature, pH, salinity, and dissolved oxygen) were measured daily prior to each dish's 50% water changes. Water samples were collected at the start of the experiment, from each dish during daily water changes, and at the end of the experiment for quantification of each PFAS. These samples are currently being prepared for analyses at the UT MSI Core Laboratory.

After the final assessment when all the fish had hatched (7dfp), half the fish (n=5/dish) were euthanized in buffered MS222, placed in a 3% methyl cellulose solution, and imaged using a Nikon SMZ800N fitted with a camera and accompanying software. We are currently in the process of analyzing the impacts of all five PFAS mentioned above. We have finished

measurements for PFOS and PFDA and have analyzed a suite of morphological parameters (Figure 3) to compare the impacts of each compound on the fish's development.

Our results indicate that, at the environmentally relevant concentrations tested, PFOS did not significantly impact the five morphological parameters measured. For example, we did not find significant differences in the length of the fish, or the amount of yolk used during development (i.e., relative yolk area) (Figure 4). However, we did find that at the environmentally relevant concentrations tested, PFDA *did* significantly impact the development of the fish tested. Our data (Figure 5) show that sheepshead minnow exposed to 44 PPB were significantly larger compared to other doses. Interestingly, we found no significant differences in relative yolk area (the area of the yolk sac divided by the total length of the fish). This indicates that, while fish at 44 PPB were significantly larger, they did not utilize more energy reserves compared to the smaller fish.

We will continue to measure and analyze the fish that were exposed to PFOA, PFNA, and PFOSA to compare them to the results discussed above.

Phase 2:

We will be collecting wild-caught sheepshead minnows to spawn in the lab to assess the impacts of the same 5 PFAS on the development of wild-caught sheepshead minnows. This will be an interesting comparison, as the lab-reared commercial fish are used as an important EPA model species – but may not be reflective of the fish found in the wild. We will also assess the PFAS body burdens of these fish, to assess their exposure and uptake of PFAS in the wild.

References:

[1]https://www.sgsaxys.com/2021/09/14/epa-announces-availability-of-epa-1633-draft-pfas-method-developed-by-sgs-axys-sgs-axys-continues-to-expand-range-of-pfas-testing-methods/

[2]Lencer, E. S., & McCune, A. R. (2018). An embryonic staging series up to hatching for Cyprinodon variegatus: An emerging fish model for developmental, evolutionary, and ecological research. *Journal of Morphology*, *279*(11), 1559-1578.

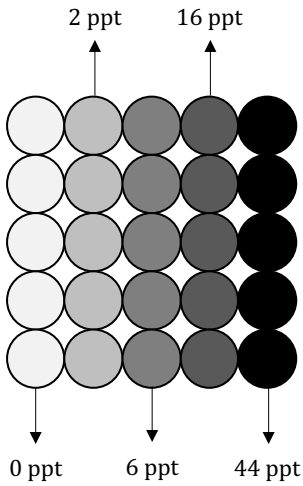

Figure 2. Depiction of the graded dose-response experiment performed in embryonic and larval sheepshead minnows. There was a total of five environmentally relevant doses in the study, ranging from 0 parts per billion (ppb) to 44 ppb. Each circle represents one replicate (i.e., dish), for a total of 5 replicates per dose. Briefly, fish (n=10/dish) were added at ~24 hours post-fertilization (dpf) and monitored through hatch (~ 6-7 dpf). At the end of the experiment (7 dpf), half the fish in each dish (n=5/dish) were imaged using a microscope for morphological analysis.

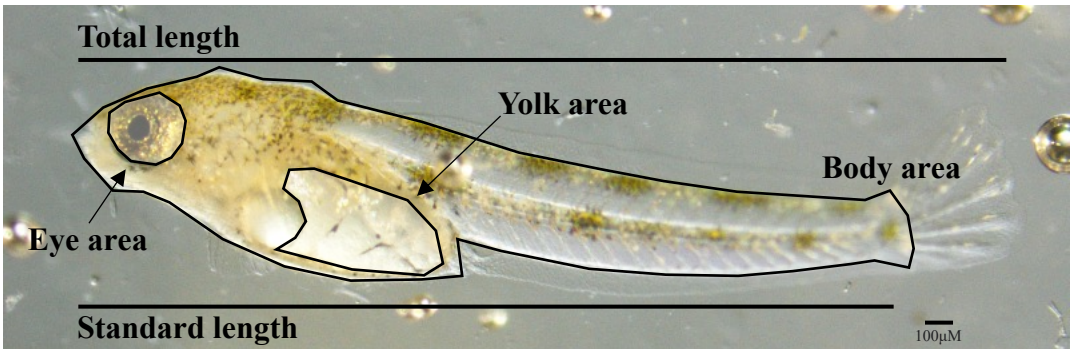

Figure 3. Morphological measures taken of each sheepshead minnow larva.

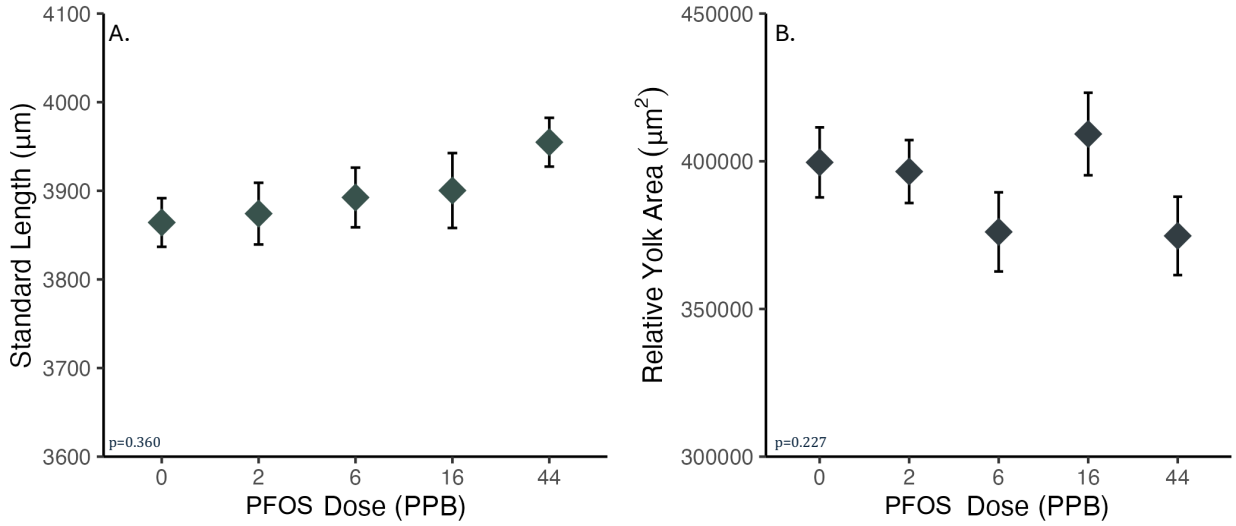

Figure 4. Impacts of PFOS on sheepshead minnow larvae exposure during embryonic life stages. (A) Standard length and (B) relative yolk area (see Figure 3 for morphological parameters) was not significantly affected by any PFOS dose tested.

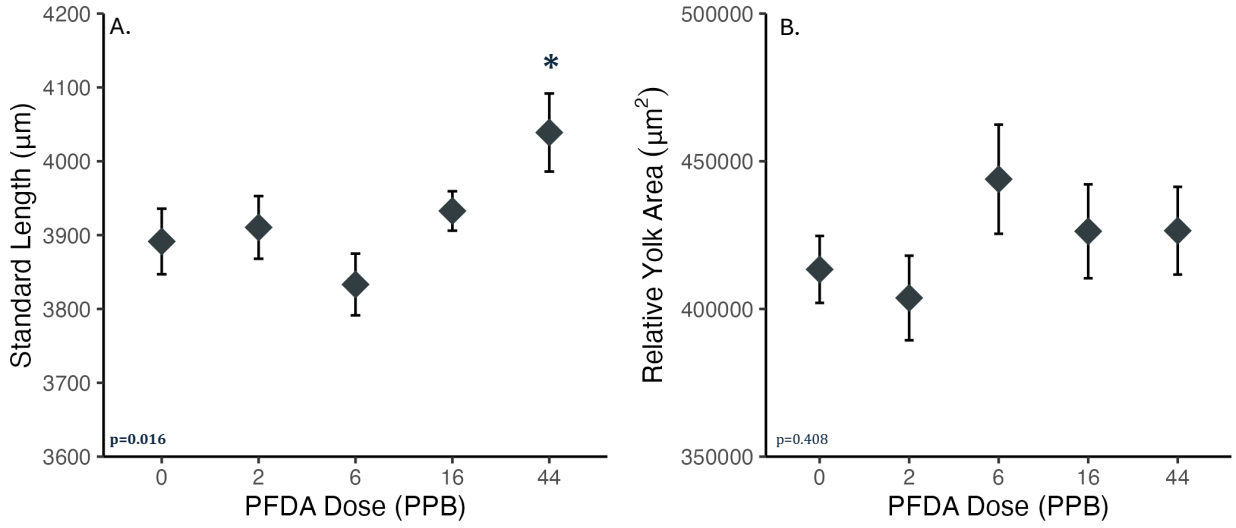

Figure 5. Impacts of PFDA on sheepshead minnow larvae exposure during embryonic life stages. (A) Standard length significantly increased at 44 PPB, although (B) relative yolk area was not significantly affected (see Figure 3 for morphological parameters). The asterisk (*) indicates significant differences.

Tables:

FAMILY	ANALYTE	TYPICAL REPORTING LIMITS
PFCA	PFBA, PFPeA, PFHxA, PFHpA, **PFOA**, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTetrDA	• 0.4-1.6 ng/L water • 0.04-0.16 ng/g solid • 0.1-0.4 ng/g tissue • 0.1-0.4 ng/mL serum • 10-40 ppb AFFF
PFSA	PFBS, PFPeS, PFHxS, PFHpS, **PFOS**, PFNS, PFDS, PFDoS	• 0.4 ng/L water • 0.04 ng/g solid • 0.1 ng/g tissue • 0.1 ng/mL serum • 10 ppb AFFF
FTS and FTCA	4:2, 6:2 and 8:2 FTS, 3:3, 5:3 and 7:3 FTCA	• 3.2- 10 ng/L water • 0.32 – 1 ng/g solid • 0.8 – 2.5 ng/g tissue • 40-250 ppb AFFF
Sulfonamides	EtFOSAA, MeFOSAA, PFOSA, EtFOSA, MeFOSA, EtFOSE and MeFOSE	• 0.4-4 ng/L water • 0.04-0.4 ng/g solid • 0.1-1 ng/g tissue • 10-100 ppb AFFF
Ether carboxylates and sulfonates	HFPO-DA (GEN-X), ADONA, F-53B, NFDHA, PFMBA, PFMPA, PFEESA	• 0.4-1.6 ng/L water • 0.04 – 0.16 ng/g solid • 0.1-0.4 ng/g tissue • 10-40 ppb AFFF

Table 1. PFAS to be analyzed using EPA Method 1633 for sediment (i.e., solid) samples taken from each sampling site in Matagorda Bay. Table provided by SGS AXYS Analytical Services, LTD[1].