Matagorda Bay Mitigation Trust 2022-2023 Funding Cycle
RFP # 2022-2023-1

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

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Y3 Q4 May 2026 Progress Report

Y3 Q4 Update:

This quarter represents the final quarterly progress reporting period for this project. Our primary accomplishments centered on (1) completing a second round of the adult PFOS + PFNA co-exposure using commercially sources sheepshead minnow (SHM) and successfully breeding the exposed adults to generate an F1 larval cohort, (2) conducting morphometric analysis of those F1 larvae to assess transgenerational or developmental effects of parental PFAS exposure, and (3) completing analytical water chemistry verification of tank water from the adult/larval exposures. No morphological differences were detected in F1 larvae born to PFAS-exposed parents relative to controls across all measured parameters, providing an important complement in findings from our first-generation larval exposures.

Phase 1: Completed

Phase 1, completed in Y2, characterized PFAS concentrations in paired sediment and water samples across four Matagorda Bay sites. PFOS and PFNA emerged as the most consistently elevated PFAS across matrices, guiding mixture selection for both adult and larval exposures.

Phase 2: Ongoing

Refinement of PFAS extraction methods for fish tissue using EPA Method 1633 as a baseline continues. Because this method was designed for biosolids rather than biological tissues, protocol adjustments remain necessary to achieve consistent recoveries in homogenized marine fish tissues. Current efforts focus on:

- Optimization of homogenization parameters for fish muscle and liver
- Testing modified solvent combinations to improve recovery consistency
- Identifying cleanup steps that minimize matrix interference

Seatrout muscle and liver tissues continue to serve as a surrogate matrix for optimization before applying the finalized method to SHM tissues. Our most recent tissue trial resulted in very consistent recoveries across both solvent and tissue samples indicating that we have successfully modified to account for the high lipid content of tissue when compared to a solvent like water; however, recoveries (around 30%) still remain too low to be approved under the EPA method. Focus will now be on fine tuning the modified method to reduce the loss of PFAS during extraction.

Phase 3 Adult SHM: Completed

A second 21-day adult co-exposure to 10 ppb PFOS + 10 ppb PFNA was conducted using commercially sourced, reproductively active SHM, replicating the conditions of the Y3 Q1 exposure. Following completion of the exposure, PFAS-exposed and control adults were evaluated for changes in weight and length; no differences were observed comparing across treatments within sex. Fish were maintained in their tanks and successfully bred. Spawned embryos were retained in the same treatment water as their parent generation (PFAS-exposed

embryos in PFAS-tank water; control embryos in control-tank water), allowing assessment of developmental outcomes in F1 larvae with continuous PFAS exposure from fertilization through 1 day post hatch.

Phase 3 Embryo-larval SHM: Completed Second Generation Exposure

Embryos spawned by PFAS-exposed and control adults were maintained in their respective treatment waters and reared under identical conditions to the first-generation larval exposure (25˚C, daily 50% water changes using tank water from the respective parental tank). Each adult tank was spawned for one week and spawns were collected daily if available and kept separate based on spawn day and parental tank. Two replicate control adult tanks were able to successfully spawn, and three PFAS-exposed adult tanks were able to successfully spawn. The other three control tanks (a total of 5 replicates were exposed per treatment) were unable to be spawned due to the aggressive nature of SHM spawning behaviors resulting in mortalities amongst tank mates. The same occurred for two PFAS adult tanks; this is a common occurrence in lab reared SHM in small volumes/densities (3 females/1 male per 5-gallon tank) as was per our study design to reduce PFAS wastewater. Embryo rearing ran through hatch (~7dpf) and until 1 day post hatch, at which larvae were humanely euthanized and imaged for morphometric analysis. Morphological measurements were taken in the same manner as our direct embryonic PFAS exposures (Figure 1).

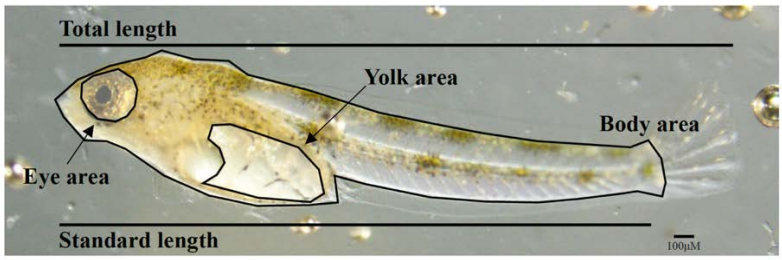

Figure 1: Morphological measurements of SHM larvae.

<u>Key Findings</u>:

Morphometric analysis of F1 larvae revealed no statistically significant differences between PFAS-exposed and control larvae across all measured parameters, including total length, standard length, body area, body area relative to standard length, eye area relative to standard length, and eye area relative to body area (Figure 2).

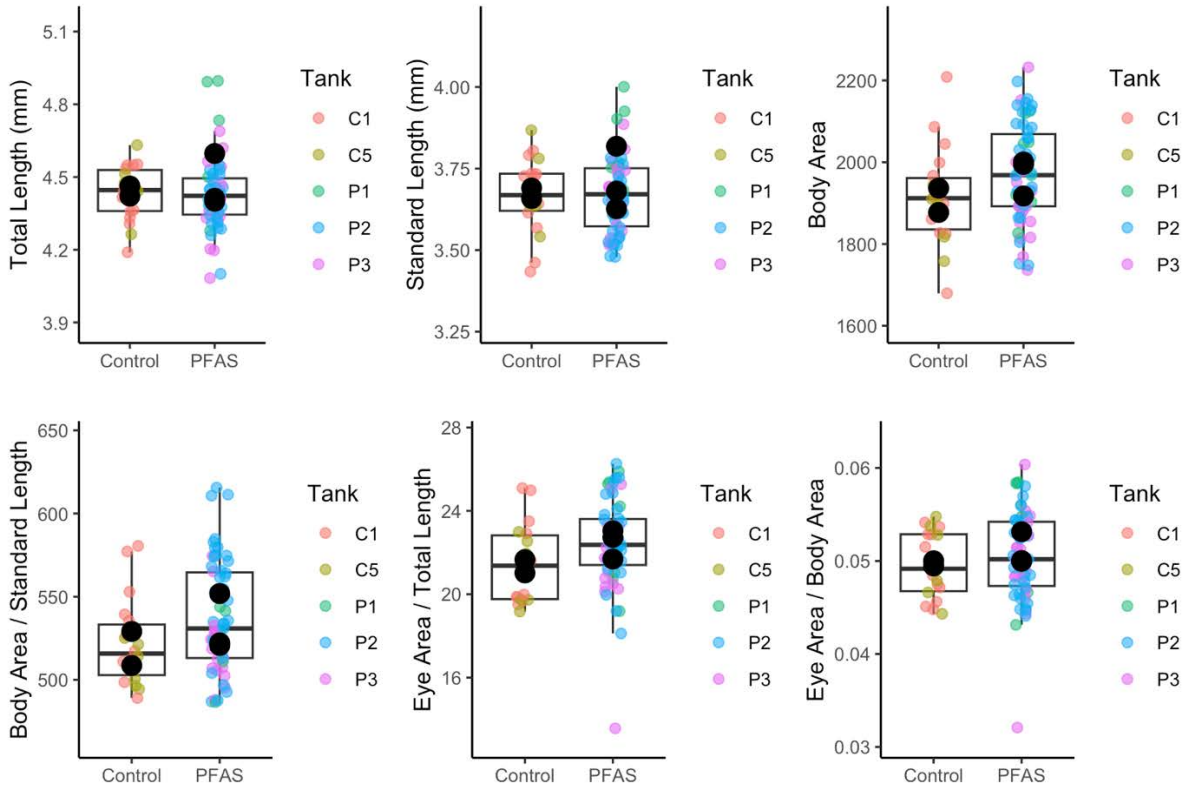

Figure 2: Morphometric parameters measured in F1 sheepshead minnow (SHM) larvae born to PFAS-exposed (10 ppb PFOS + 10 ppb PFNA) or control parents. No significant differences were observed between treatment groups for any parameter. Points represent individual larvae colored by replicate tank (C1, C5 = control; P1, P2, P3 = PFAS). Large black points represent tank means. n = 15 Control larvae, n = 52 PFAS larvae. R statistical analyses included a linear mixed effects model, ANOVA, and Wilcox test; $p < 0.05$ considered significant.

Analytical Water Chemistry Verification

Analytical chemistry was completed on archived tank water samples from the adult and larval PFOS + PFNA co-exposures using a modified EPA Method 537.1 approach. Results confirm the pattern of PFAS mixture interaction previously observed, with measured concentrations consistently below nominal spike concentrations of 10 ppb for both PFOS and PFNA (Table 1). When both compounds were present in seawater (SW), PFOS recoveries ranged from approximately 5.5–6.0 ppb and PFNA recoveries from approximately 1.8–2.0 ppb, substantially lower than when each compound was spiked individually. This concentration suppression in the co-exposure condition is consistent with competitive partitioning or matrix interactions between PFOS and PFNA in seawater, corroborating observations from both the adult and first-generation larval exposures reported in prior quarters. Blank (SPE_Blank) samples returned zero detections for both analytes, confirming no background contamination.

Table 1. Measured PFOS and PFNA concentrations (ppb) in tank water samples from SHM adult and larval co-exposures. Samples analyzed using modified EPA Method 537.1. NA = not analyzed for this compound; — = no data.

Sample ID	Sample Contents	Spike Conc. (ppb)	PFOS Conc. (ppb)	PFNA Conc. (ppb)
PFOS_STD_1_SW	PFOS	10 PFOS	7.755	NA
PFOS_STD_2_SW	PFOS	10 PFOS	6.876	NA
PFNA_STD_1_SW	PFNA	10 PFNA	NA	3.415
PFNA_STD_2_SW	PFNA	10 PFNA	NA	3.071
PFNA/PFOS_STD_1_SW	PFNA + PFOS	10 PFNA + PFOS	6.021	1.990
PFNA/PFOS_STD_2_SW	PFNA + PFOS	10 PFNA + PFOS	5.521	1.774
SPE_Blank	Blank	0	0	0
PFOS_STD_3	PFOS Stock in LCMS Water	10 PFOS	2.473	—
PFNA_STD_3	PFNA Stock in LCMS Water	10 PFOS	NA	1.214
PFNA/PFOS_STD_3	PFOS + PFNA stocks in LCMS Water	10 PFNA + PFOS	2.278	1.189

Project Summary

This final quarterly report completes the active experimental phase of the Matagorda Bay Mitigation Trust grant cycle. Across three years, this project has characterized environmental PFAS concentrations in Matagorda Bay (Phase 1), conducted single-compound and mixture toxicity exposures in adult and larval SHM (Phase 3), generated a first-of-its-kind F1 transgenerational dataset for PFAS mixtures in a Gulf Coast prey fish species, and advanced a tissue extraction workflow for PFAS bioaccumulation quantification in biological matrices (Phase 2). In addition, a publication is currently in the submission phase based on embryonic SHM exposures to individual PFAS including data from Phase 1 sediment PFAS measurements. A comprehensive final report summarizing all phases, findings, and broader implications for Matagorda Bay ecosystem health will follow.