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

Kristin Nielsen (PI) & Kerri Lynn Ackerly (co-PI)

Y3 Q2 November 2025 Progress Report

Y3 Q2 Update:

This quarter, our primary accomplishments centered on (1) completing the first embryo-larval PFOS + PFNA co-exposure, (2) advancing the optimization of the PFAS extraction method required for all tissue analyses, and (3) preparing adult SHM tissues from the completed Phase 3 exposure for future extraction following EPA Method 1633. Phase 2 method development remains ongoing. The embryo-larval exposure yielded clear morphological responses to the PFAS mixture, providing important early-life toxicity evidence that complements our existing single-compound larval dataset.

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

Work this quarter continued the refinement of PFAS extraction methods for fish tissues using EPA Method 1633 as a baseline. Because this method was designed for biosolids rather than biological tissues, protocol adjustments remain necessary to achieve consistent recoveries in homogenized fish tissue. 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

Red drum muscle and liver tissues continue to serve as a surrogate matrix for optimization before applying the finalized method to sheepshead minnow (SHM) tissues.

Phase 3 Adult SHM: Ongoing

The 21-day adult co-exposure to 10 ppb PFOS + 10 ppb PFNA, completed in Y3 Q1, provided mature, reproductively active SHM with environmentally relevant PFAS mixture exposures. All dissections are complete, and tissues (liver, muscle, gonad, gut, and brain) are catalogued and stored for future PFAS quantification once the extraction workflow (Phase 2) is finalized.
As previously mentioned, no significant differences were observed in whole-body weight or most tissue weights in females. PFAS-exposed males exhibited a significant reduction in gut mass, suggesting possible disruptions to nutrient assimilation or feeding physiology. Water chemistry measurements across the exposure revealed interactions between PFOS and PFNA

resulting in inconsistent water-column concentrations and evidence of partitioning. These biological observations support the hypothesis that PFAS mixtures may influence metabolic and digestive processes in adult SHM.

Phase 3 Embryo-larval SHM: Ongoing

Commercially sourced SHM embryos were collected within two hours of spawning, rinsed, and held for 24 hours to reach consistent developmental stages. Embryos were then transferred into exposure dishes for:

- Control seawater
- 10 ppb PFOS + 10 ppb PFNA co-exposure

Five replicate dishes per treatment were used, with 10 embryos per 250 mL. Dishes were maintained at 25°C with daily 50% water changes using pre-spiked water for exposed groups. Daily measurements included survival and developmental progression, and complete water quality in control dishes. Water removed during renewals was archived as composite samples for future PFAS analysis. The exposure ran through hatch (~7 dpf) and until ~10 dpf. At termination, larvae were humanely euthanized following approved protocols and imaged for morphometric analysis (Figure 1).

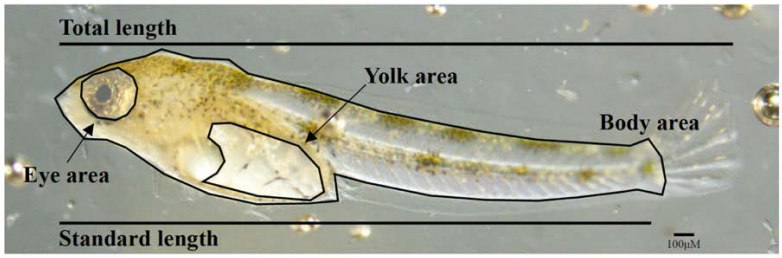

Figure 1: Morphological measurements of SHM larvae.

Key Findings: Morphometric measurements revealed no significant effects of PFOS+PFNA on standard length, relative body area, or relative eye area relative to control fish. However, a clear increase in relative yolk sac area in PFOS+PFNA-exposed larvae was observed, indicating reduced yolk utilization (Figure 2).

This response suggests that the PFAS mixture may alter energetic allocation or interfere with yolk mobilization during early development. These findings complement our individual PFAS larval exposures, where PFNA and PFOS also produced strong energetic and developmental disturbances. These findings

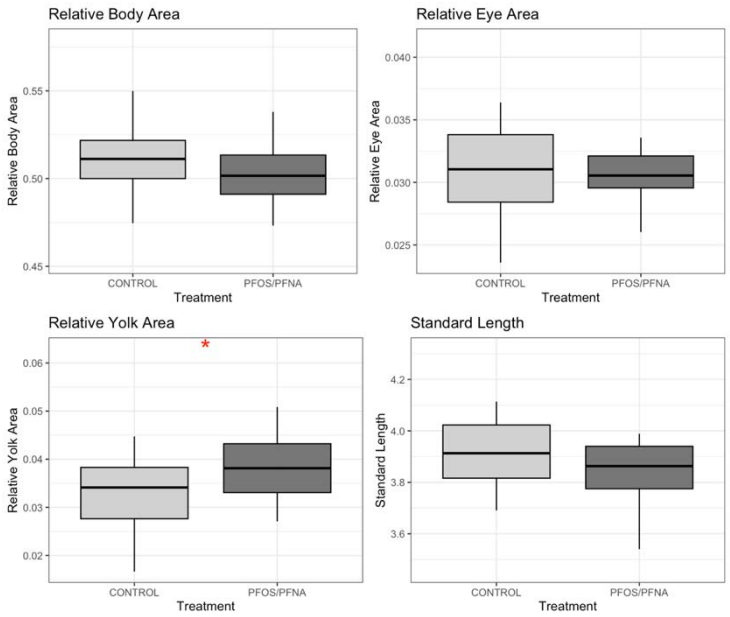

Figure 2: Morphological measurements of SHM larvae after PFOS+PFNA co-exposure for 9 days.

also complement our finding in adult SHM where PFAS similarly likely effected metabolism and nutrient uptake visualized by reduced male gut mass.
Preliminary composite water chemistry shows mixture PFAS are interactive during these larval exposures (Table 1). These findings are similar to those seen from adult SHM co-exposure water chemistry.

Table 1: PFAS levels in composite water samples from SHM larval PFOS+PFNA co-exposure. Samples analyzed using modified EPA method 537.1.

Sample	PFNA Conc. (ppb)	PFOS Conc. (ppb)
PFOS+PFNA Day 0	1.8268	0.0464
Control Day 0	0.0000	0.0000
PFOS+PFNA Day 8	3.8397	0.1952
Control Day 8	0.0000	0.0000

Next Steps:
Our next steps include completing PFAS verification of larval exposure water, beginning parallel PFOS+PFNA co-exposures using wild-caught SHM adults and embryos, and processing adult fish tissues for PFAS bioaccumulation.