Matagorda Bay Mitigation Trust 2023-2024 Funding Cycle <u>Title</u>: **Evaluating Ecological and Human Health Risk of PFAS in Matagorda Bay**

Contract #067 Kerri Lynn Ackerly (PI) & Kristin Nielsen (co-PI) Y2 Q3 November 2025 Progress Report

Y2 Q3 Update:

During this reporting period, we continued advancing Phase 1 through method development and optimization to support PFAS body-burden analyses in eastern oyster tissues collected from Matagorda Bay. This work is centered on modifying EPA Method 1633 for use with complex oyster tissue matrices to ensure robust and reproducible measurements across a broad suite of PFAS compounds. Concurrently, we expanded Phase 2 planning activities and finalized experimental design elements for upcoming oyster gamete, embryo, and larval PFAS exposure studies. These exposures are scheduled to begin in Spring and Summer 2026, coinciding with seasonal availability of ripe broodstock and larval production at regional hatcheries. Phase 3 remains dependent on data produced in Phases 1 and 2 and will progress once tissue extraction methods are validated, and experimental exposures generate PFAS concentration and effect data.

Phase 1:

Phase 1 efforts this quarter focused on continuing the development and refinement of PFAS extraction methods tailored to eastern oyster (*Crassostrea virginica*) tissues. Oyster tissues present unique analytical challenges due to their high organic content and heterogeneous lipid composition, requiring modifications to standard EPA Method 1633 to improve extraction efficiency and reproducibility. Current method optimization includes testing multiple homogenization procedures, evaluating solvent systems with varying acid strengths, and assessing recovery of target PFAS analytes across repeated extractions. Tissue homogenization trials have incorporated both stainless-steel grinding and manual mortar-and-pestle processing to determine the most efficient way to generate a uniform matrix. Sequential acid-based extractions are being refined to balance PFAS liberation from tissue while minimizing matrix-associated interferences during subsequent SPE cleanup.

Preliminary trials have demonstrated variable recoveries across PFOS, PFNA, PFHxS, PFOA, and PFDA, highlighting the necessity of continued optimization before tissue quantification of field-collected oysters can begin. These efforts are essential to ensuring that the final extraction protocol produces consistent recoveries and accurate PFAS concentrations relevant to seafood safety assessments. Once the method is finalized, we will apply the optimized protocol to commercial-sized oysters collected from multiple Matagorda Bay sites to characterize PFAS bioaccumulation in edible tissues.

Phase 2:

Phase 2 activities this quarter focused on detailed experimental planning for the PFAS exposure studies that will evaluate impacts across key eastern oyster life stages. We have been coordinating closely with collaborators at the Texas A&M University AgriLife Mariculture facility (Dr. Chris Hollenbeck) to prepare for Spring 2026 gamete and embryo exposures, which require access to reproductively ripe broodstock for strip spawning. These experiments will evaluate the effects of environmentally relevant PFAS mixtures—including PFOS, PFOA, PFHxS, PFNA, and PFDA—on gamete morphology, viability, fertilization success, and early embryogenesis (Figure 1).

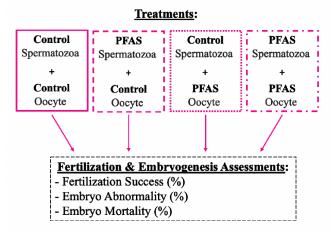


Figure 1: Experimental design and assessment endpoints for fertilization success and embryogenesis for Phase 2.

Experimental conditions will follow established eastern oyster culture parameters and toxicology guidelines, with oysters maintained in 20°C, 20 ppt seawater, and fed cultured algae prior to and during experimentation. Strip-spawned oocytes and spermatozoa will be exposed separately to PFAS mixtures for 20 minutes, assessed for viability via fluorescence staining, and used in fertilization assays designed to quantify abnormality rates and early embryo development.

Planning is also underway for the larval exposure phase, scheduled for Summer 2026, which will assess PFAS impacts on larval survival, shell deposition, growth, and bioaccumulation following EPA Method 850.1025. Juvenile Eastern oysters (30–50 mm; 3-5 months old) supplied by the TAMU AgriLife Mariculture facility will be maintained under standard larval culture conditions and exposed to a five-point concentration series anchored to PFAS levels previously detected in Matagorda Bay (Table 1).

Table 1: Experimental design for larval oyster PFAS exposures in Phase 2.

Treatment	# Replicates	# Oysers / Replicate	Total # Oysters
Contrtol (No PFAS)	4	n=8	n=32
PFAS Mixture Dose #1	4	n=8	n=32
PFAS Mixture Dose #2	4	n=8	n=32
PFAS Mixture Dose #3	4	n=8	n=32
PFAS Mixture Dose #4	4	n=8	n=32
PFAS Mixture Dose #5	4	n=8	n=32
All	-	-	192

The experimental design includes daily-renewal exposures with daily water quality monitoring, replicate tanks per treatment, and final measurements of survival and shell growth after 96 hours. Post-exposure, oyster tissues will be collected to quantify PFAS uptake using the method being developed in Phase 1. These combined experiments will generate the data necessary to characterize PFAS sensitivity across multiple oyster life stages and evaluate potential human health risks associated with oyster consumption.

Phase 3:

Phase 3 quantitative ecological and human health risk assessments will proceed once PFAS occurrence, body-burden, and toxicity data are available from Phases 1 and 2. Current efforts are focused on ensuring the analytical and experimental components are well aligned with the data requirements of Phase 3. Model inputs will include PFAS concentrations in oyster tissues, oyster-specific bioaccumulation factors, PFAS levels in Matagorda Bay water, and toxicity endpoints generated from gamete, embryo, and larval exposures. Integration of these components will allow for development of exposure models that evaluate risks to both oyster populations and human consumers.

Next steps:

Our next steps are to complete the gamete/embryo oyster PFAS exposures, continue to finalize tissue PFAS method in Phase 1, evaluate oyster tissues for PFAS bioaccumulation, and prepare for larval oyster exposures.