Matagorda Bay Mitigation Trust 2023-2024 Funding Cycle

Title: **Evaluating Ecological and Human Health Risk of PFAS in Matagorda Bay**

Contract #067

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Y3 Q1 May 2026 Progress Report

Y3 Q1 Update:

During this reporting period, we continued advancing Phase 1 through ongoing PFAS tissue method refinement, conducted Phase 2 oyster exposure studies on gamete and embryo experiments, and continued preparation and finalization for Phase 2 oyster exposure studies on juvenile oysters. Gamete and embryo exposure experiments were conducted at the Texas AgriLife Facility in April. In parallel, we continue to plan for juvenile oyster experiments at the hatchery scheduled for late June/early July. Phase 3 remains contingent upon the generation of tissue body-burden and toxicity endpoint data from Phases 1 and 2.

Phase 1:

Phase 1 efforts this quarter focused on continued refinement of PFAS extraction methods for eastern oyster (*Crassostrea virginica*) tissues, with particular emphasis on adapting and expanding EPA Method 1633 for complex oyster matrices. While earlier efforts were based on elements of EPA Method 537.1, we are now transitioning fully to Method 1633 to enable quantification of a broader suite of PFAS compounds relevant to Matagorda Bay. In addition, water samples from Matagorda Bay were also collected by collaborators during oyster farm seeding that will supply our juvenile oysters for Phase 2. These water samples will be analyzed for PFAS to confirm the continued relevance of our exposure design in Phase 2 experiments.

Tissue method development continues to address challenges associated with high organic content and heterogeneous lipid composition in oyster tissues. Recent publications on similar oyster tissue PFAS extraction methods are being used to troubleshoot our current protocol to ensure roust quantification across all compounds in the EPA 1633 method framework. Optimization trials have focused on balancing extraction efficiency with matrix cleanup, including refinement of homogenization procedures, solvent systems, and Quenchers SPE methods that have been commonly employed in published PFAS extraction methods from marine matrices.

Phase 2:

Phase 2 activities this quarter focused on gamete and embryo exposure experiments and finalizing experimental logistics and exposure design in coordination with hatchery collaborators for juvenile oyster experiments.

Hatchery Coordination and Pre-Exposure Logistics

A final coordination meeting was held with hatchery staff to confirm experimental logistics prior to initiating gamete and embryo exposures. Key decisions finalized at this meeting included the use of 1 L plastic beakers with an 800 mL working volume and no aeration, based on a successful trial run conducted by hatchery staff the previous week that demonstrated normal fertilization and survival rates under these conditions. Experimental design and steps were finalized for the gamete embryo exposure (Figure 1).

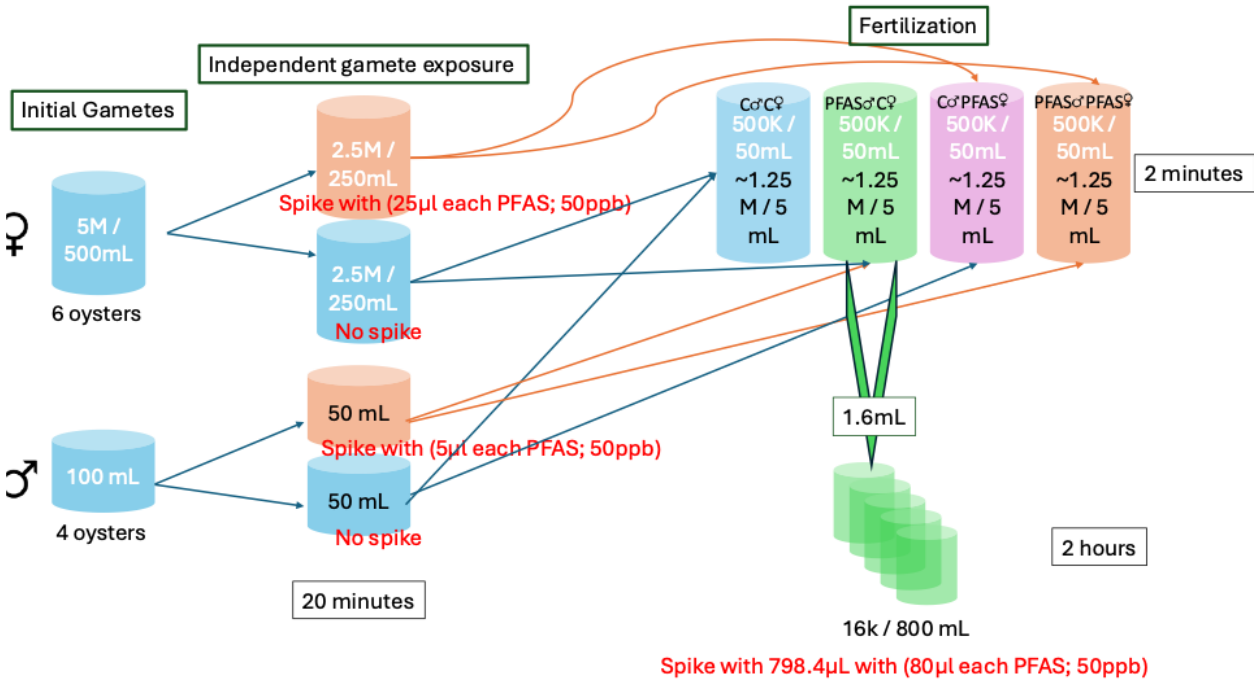

Figure 1: Outline of oyster gamete/embryo PFAS exposure experiment.

Stock Water Quality Evaluation

Prior to initiating exposures, stock water quality was assessed to confirm suitability for experimental use. The stock water consisted of filtered bay water set aside by the hatchery in a large, dedicated holding tank. The following parameters were measured:

- Ammonia: 0 mg/L
- pH: 7–8 (measured via pH paper)
- Salinity: 28 ppt

Two 45 mL water samples were collected from the stock water tank and will be analyzed for background PFAS analysis to characterize pre-exposure contamination levels.

Broodstock Collection and Gamete Assessment

Six ripe female and five ripe male oysters were evaluated individually for reproductive condition. Of the five males assessed, four demonstrated acceptable sperm motility and were retained for the experiment; one male was excluded.

Gametes from retained individuals were processed as follows:

- Females: Eggs from all six females were pooled to achieve a density of 5 million eggs per 500 mL.
- Males: Sperm from the four retained males were pooled into a total volume of 100 mL.

Gamete Exposure Design and PFAS Spiking

Egg and sperm pools were each divided evenly into PFAS-spiked and control fractions:

- Eggs: Split into two aliquots of 2.5 million eggs in 250 mL each. The PFAS fraction was spiked with 25 µL of each of the five PFAS compounds (PFOS, PFNA, PFOA, PFHxS,

PFDA) to achieve a total target concentration of 50 ppb ∑PFAS. The control fraction received no addition.

- Sperm: Split into two aliquots of 50 mL each. The PFAS fraction was spiked with 5 µL of each PFAS compound (50 ppb ∑PFAS target). The control fraction received no addition.

Gametes were incubated in their respective PFAS or control conditions for 20 minutes prior to fertilization.

Fertilization and Transfer to Exposure Beakers

Following the 20-minute gamete independent gamete exposures, fertilization was conducted using a 2 × 2 factorial design (PFAS-exposed vs. control females crossed with PFAS-exposed vs. control males), yielding four treatment groups:

- Control females × Control males (C♀C♂)
- PFAS females × Control males (PFAS♀C♂)
- Control females × PFAS males (C♀PFAS♂)
- PFAS females × PFAS males (PFAS♀PFAS♂)

Each fertilization mixture consisted of approximately 500,000 eggs in 50 mL combined with 1.25 million sperm in 5 mL (total volume ~55 mL). Fertilization was allowed to proceed for 2–3 minutes at this concentrated volume.

Following the brief fertilization window, 1.6 mL of each treatment mixture was transferred into designated 800 mL exposure beakers (pre-filled with stock water). Each treatment group was replicated five times (n = 5 replicates per treatment). Beakers receiving at least one PFAS-exposed gamete fraction (PFAS♀C♂, C♀PFAS♂, and PFAS♀PFAS♂ groups) were re-spiked with 80 µL of each PFAS compound per beaker to restore and maintain the 50 ppb ∑PFAS target concentration throughout the developmental exposure period (fertilization to 48 hours).

Embryo Developmental Sampling Timeline

Day 1: ~2 Hours Post-Fertilization (Early Cleavage)

Approximately two hours after fertilization, post-spike water samples were collected (one 40 mL sample per replicate beaker) for PFAS chemistry verification. Beakers were gently mixed to resuspend settled eggs/embryos prior to sampling. A subsample (2 mL) was taken from the control replicates and examined under a microscope to confirm normal early cleavage before proceeding with preservation of all treatment groups. A 25 mL sample of embryos from each replicate was preserved in 10% buffered formalin (25 mL embryos + 20 mL formalin in 50 mL falcon tubes) for fertilization success assessment.

Day 2: 24 Hours Post-Fertilization (D-Stage Larval Assessment)

At 24 hours post-fertilization, a composite water sample was collected from each treatment group by pooling 25 mL from each of the five replicate beakers into a single 250 mL HDPE bottle; samples were stored frozen in the hatchery freezer for subsequent PFAS analysis. Beakers were mixed to resuspend larvae, and a control subsample was assessed microscopically before preservation. A 25 mL larval sample from each replicate was preserved in 10% buffered formalin for D-stage larval normality and hatching success assessment. Larvae were fed 1 mL of TISO algae per replicate (approximately 750 mL volume at time of feeding) and the remaining beaker contents were retained for the 48-hour assessment.

Day 3: 48 Hours Post-Fertilization (Final Assessment and Experiment Takedown)

At 48 hours post-fertilization, a 2 mL subsample from one replicate per treatment was examined under the microscope to assess larval survival and morphology. A 25 mL larval sample from each replicate was preserved in 10% buffered formalin (in 50 mL falcon tubes) for continued larval development and morphological assessment. Following sample collection, the experiment was concluded. PFAS-containing water from all relevant beakers was poured over activated carbon into a collection bucket, then diluted prior to drain disposal. All beakers and materials that had contact with PFAS solutions were collected in a designated contaminated equipment bucket for transport back to UTMSI for proper decontamination and cleaning.

Juvenile Oyster Planning

Planning for the juvenile exposure phase is ongoing. Juvenile oysters are expected to be grown in the hatchery to approximately 3–5 mm before the experiment. This method will follow the EPA Method 850.1025 to evaluate PFAS toxicity on oyster shell deposition. Hatchery collaborators anticipate juvenile oysters will be of size in late June or early July. A final logistics planning meeting is anticipated to take place in early June to finalize experimental layout and timeline.

Ongoing Work and Phase 3 Planning

Efforts continue toward refining oyster tissue PFAS extraction protocols under EPA Method 1633. Phase 3 ecological and human health risk assessments remain contingent on quantitative outputs from Phases 1 and 2. Model inputs will ultimately include:

- PFAS concentrations in wild and experimentally exposed oyster tissues
- Bioaccumulation factors
- Matagorda Bay water PFAS concentrations
- Life-stage–specific toxicity endpoints

Current efforts continue to ensure that analytical outputs (Phase 1) and exposure designs (Phase 2) align directly with the data needs required for quantitative risk modeling.

Next Steps

- Process formalin-fixed larval samples to assess fertilization success, D-stage normality, and developmental endpoints across all treatment groups.
- Analyze PFAS water chemistry samples (post-spike and 24-hour composites) to verify exposure concentrations
- Continue refinement and validation of oyster tissue PFAS extraction under EPA Method 1633
- Prepare for Phase 2 juvenile oyster growth experiments and subsequent analyses.