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**

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Y3 Q1 August 2025 Progress Report

Y3 Q1 Update:

We have completed Phase 1, as described in the previous progress report, which characterized PFAS concentrations in paired sediment and water samples from four Matagorda Bay locations. Phase 2 is now underway and focuses on field collection of wild sheepshead minnows (SHM) and development of robust tissue extraction methods to quantify PFAS body burdens. In parallel, we have begun Phase 3; as part of this work, we have completed a 3-week exposure in commercial SHM to the two most abundant PFAS detected in Phase 1 (PFOS and PFNA) and are now analyzing tissue-specific PFAS burdens. Next, we will collect wild SHM for similar exposure which will inform comparisons between commercial and wild populations and guide upcoming fecundity and embryo-larval studies.

Phase 1:

This phase was completed and reported in Y2 Q2-4 progress report. In summary, paired sediment and water samples were evaluated for PFAS profiles (see Table 1 & 2). Utilizing this data, we prepared for Phase 3 adult sheepshead minnow (SHM) exposures to test the PFAS body burden in this estuarian fish model. The most abundant PFAS in water samples (PFOS) and the top two in sediment samples (PFOS and PFNA) were determined relevant and used for exposure in adult lab-cultured sheepshead minnows in Phase 3.

Phase 2:

The focus of Phase 2 is to quantify PFAS body burdens in SHM. We are currently developing and optimizing tissue extraction methods based on EPA Method 1633, which requires modification for our specific instrumentation and fish tissue. Red drum muscle tissue has been used for preliminary trials to improve extraction consistency and accuracy before applying the method to SHM. Development of extraction method involves modifications to homogenization and extraction solvents. Recommendations from EPA Method 1633 for extraction solvents were trialed and provided inconsistent recoveries. Further optimization efforts are being tested until an accurate and consistent recovery of PFAS in red drum muscle is reached. Once optimization is complete, liver, muscle, gonad, and other tissues collected from exposed SHM will be analyzed for PFAS content.

Phase 3:

Chronic PFAS Exposure in Adult Sheepshead Minnow

We conducted a 3-week adult SHM PFAS exposure to determine the impacts of chronic PFAS exposure on adult PFAS body burdens and to better understand PFAS interactions. These experiments are informed by our previously described SHM embryolarval individual PFAS exposures and our environmentally detected PFAS within the Matagorda Bay system (Tables 1 & 2). As we saw the most significant impacts of PFNA and PFOS on SHM larvae, we conducted a co-exposure of adult SHM to 10 ppb PFNA and 10 ppb PFOS. To do this, we established 5 control tanks and 5 co-exposure tanks, each containing one male and three females; SHM were

mature, reproductively active adults. Each tank contained 16 liters of filtered saltwater (25 parts per thousand (ppt)) at 20°C with a pH ~8.0 and 100% air saturation. Water quality parameters (including ammonia, nitrite, and nitrate levels) were checked daily in all tanks to ensure optimal water quality parameters. Fish were fed daily and lidibium and excess food and debris are removed daily. Prior to introduction into the tanks, all fish were weighed. Throughout the duration of the experiment, fish from each tank were again weighed weekly and on the last day of the experiment to assess impacts of PFAS exposure on body condition. Prior to PFAS dosing, fish acclimated to the tanks for 2 weeks. Following this acclimation period, the PFAS tanks were dosed with 10 ppb PFNA and 10 ppb PFOS, while control tanks received the same about of water as a solvent control. Immediately before dosing, water samples were collected from each tank to quantify any background PFAS levels present in the tanks. Water quality was recorded daily, and water samples were collected immediately prior to any necessary water changes. Water changes occurred as needed to account for water quality, and all water samples were saved to track PFNA and PFOS levels within each tank. Water changes were performed with PFAS spiked water to maintain PFAS levels or control water in control tanks. These exposures were run for 21 days. At the completion of the experiment, final water samples were collected. SHM were weighed and dissected for specific tissues. Brain, liver, gonad, gut, and muscle tissue were isolated. Each tissue was individually weighed. Tank-specific composite samples were made for female liver and muscle tissue samples to allow for adequate material (0.5 grams needed) to evaluate tissue-specific PFAS body burdens. Composites were not possible for male samples due to only one male per tank.

Water chemistry of spiked PFAS (PFOS & PFNA) samples were analyzed. PFOS and PFNA appear to interact and influence each other's behavior in the tank. While this was not unexpected, we are still working to analyze the movements of these PFAS to determine their bioavailability given these spike levels (10 ppb per PFAS). Tank-specific water samples across the 3 weeks demonstrate inconsistent water solubility of the PFAS indicating PFOS and PFNA partitioning (Figure 1).

Initial comparisons of whole-body weight and tissue masses revealed no significant differences between PFAS-exposed and control fish after the 3-week exposure to PFOS and PFNA (10 ppb each; Figure 2C). In females, neither whole-body weight nor tissue-specific weights differed between exposed and control groups (Figure 2A). In contrast, males exposed to PFOS/PFNA showed a significant reduction in gut mass compared to control males ($p = 0.0010$; Figure 2B). No other significant tissue weight differences were observed in males. Tissue-specific PFAS body burdens are currently being evaluated. We are actively optimizing EPA Method 1633, originally designed for biosolids, to reliably extract PFAS from fish tissues. Modifications are necessary to ensure accurate and consistent measurements in liver, muscle, and gonad samples. Once optimization achieves acceptable recovery and reproducibility, we will analyze SHM tissues collected from the adult exposures to quantify PFAS body burdens. We will repeat this co-exposure with wild-caught SHM to compare PFAS uptake, fecundity, and early-life stage effects between wild and commercial populations.

Embryolarval SHM PFAS exposures

We have completed the analyses of five individual PFAS on the development of SHM larvae. As reported in Y1 Q4, we began PFAS testing on SHM embryos and larvae in the lab. For this experiment, we used embryos from commercially purchased adult SHM to establish concentrations of PFAS that impact the development of these embryonic and larval fish. We collected data on the impacts of PFOS, PFOA, PFNA, PFDA, and PFOSA on the development of embryonic and larval SHM. We chose these five compounds because our PFAS profiling

detected them throughout the Matagorda Bay sediment samples (Table 1). We anchored our exposure concentrations for all five PFAS tested to these environmentally relevant data for PFOS in Matagorda Bay collected as part of Phase 1.

Building on these results, we will now conduct co-exposures in SHM embryos and larvae using PFOS and PFNA together, as we have done and will continue to do in adult exposures. These studies will be carried out in both wild-caught and commercially sourced SHM to evaluate potential differences in sensitivity and developmental outcomes between populations.

Tables & Figures:

Site ID	PFOA	PFOS	PFNA	PFDA	PFUnA	PFDoA	PFOSA	N-MeFOSA	6:2 FTS
Concentration	ppt	ppt	ppt	ppt	ppt	ppt	ppt	ppt	ppt
Palacios Site A	71.00	857	137.33	126.67	101.00	n.d.	49.00	47.00	n.d.
Palacios Site B	66	908	236.00	167.67	75.00	44.00	n.d.	n.d.	n.d.
Palacios Site C	97	765	116.67	148.00	77.00	n.d.	0.058	43.00	n.d.
Reference Site A	n.d	188	n.d	n.d	n.d	n.d	n.d	n.d	151.00
Reference Site B	n.d.	35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4960.00
Reference Site C	n.d.	200	39.00	n.d.	n.d.	n.d.	n.d.	39.00	n.d.
Point Comfort A	57	62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Point Comfort B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Point Comfort C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chocolate Bay A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chocolate Bay B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chocolate Bay C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 1: PFAS detected in sediment samples taken from all four sampling locations in Matagorda Bay. The average concentrations (reported in parts per trillion (ppt)) is shown among all sediments taken. Within each sampling site, we sampled three transects. Samples where a specific PFAS compound was not found above the limit of detection are indicated as n.d. (not detected). Only the compounds detected in samples are included here.

Site ID	PFDA	PFOS	PFHxS
Concentration	ppt	ppt	ppt
Chocolate Bay A	n.d.	2018.83	n.d.
Chocolate Bay B	n.d.	987.74	n.d.
Chocolate Bay C	n.d.	1792.86	n.d.
Palacios Site A	n.d.	77.44	n.d.
Palacios Site B	n.d.	958.46	n.d.
Palacios Site C	0.54	142.76	n.d.
Point Comfort A	n.d.	351.10	n.d.
Point Comfort B	n.d.	291.56	14.00
Point Comfort C	n.d.	416.08	n.d.
Reference Site A	n.d.	1403.14	n.d.
Reference Site B	n.d.	2591.10	n.d.
Reference Site C	n.d.	2252.07	n.d.

Table 2: PFAS detected in water samples taken from all four sampling locations in Matagorda Bay. The average concentration (reported in parts per trillion (ppt)) is shown among all water samples taken. Within each sampling site, we sampled three transects. Samples where a specific PFAS compound was not found above the limit of detection are indicated as n.d. (not detected). Only the compounds detected in samples are included here.

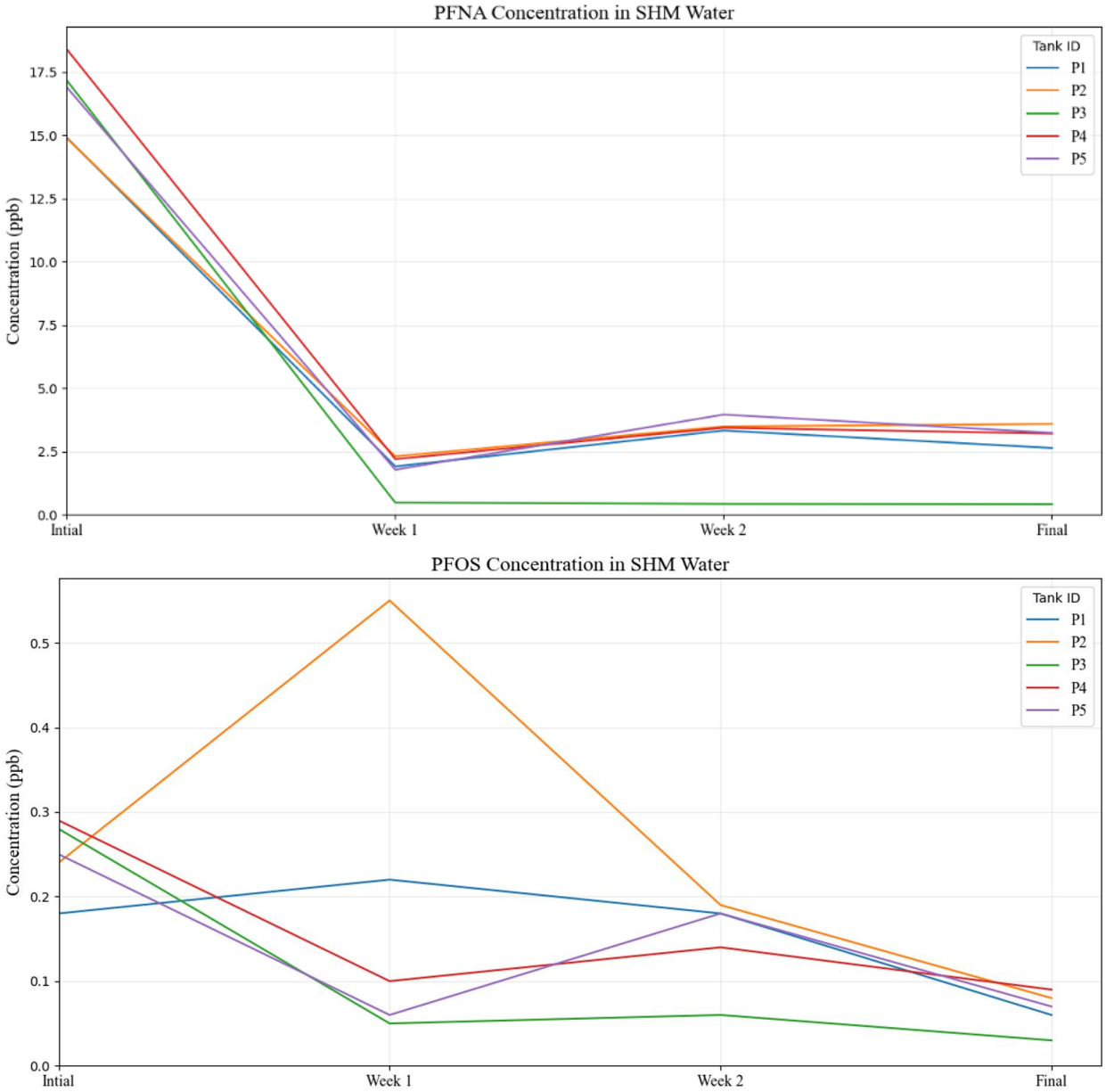
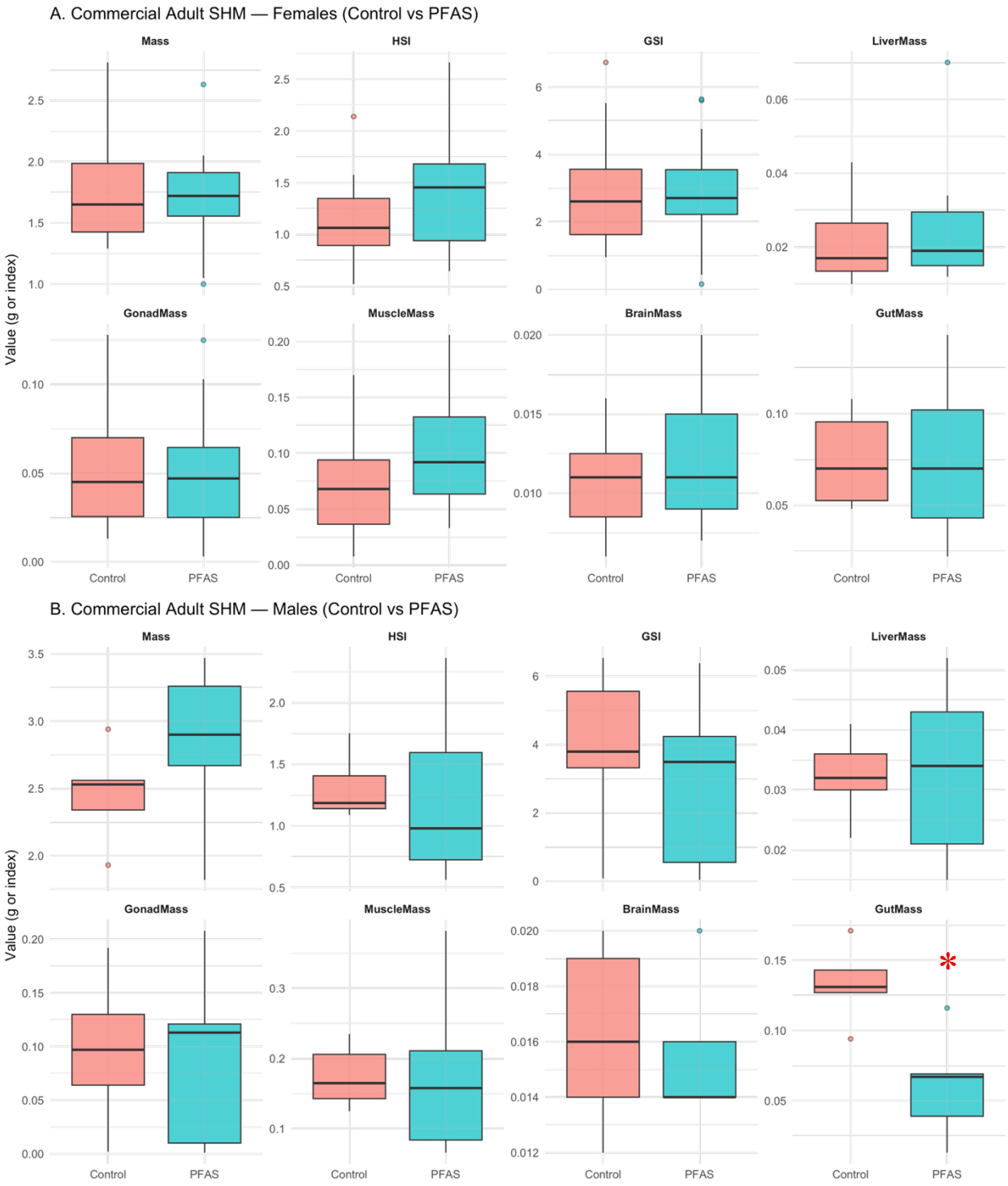


Figure 1: Concentrations of PFNA and PFOS in spiked co-exposure water for adult SHM exposure study. Each tank was spiked with 10 ppb of each PFAS (PFNA & PFOS). Invidiual tanks (P1-P5) acted as independent replicates (n=5). PFNA levels initially decline as PFNA is taken up by the fish and potentially other substrates in tank. PFOS levels indicated less clear understanding of the fate and destination of PFOS which could be influenced by salinity and co-presence of PFNA.


A. Commercial Adult SHM — Females (Control vs PFAS)
Mass
HSI
GSI
LiverMass
GonadMass
MuscleMass
BrainMass
GutMass
Value (g or index)
Control
PFAS
B. Commercial Adult SHM — Males (Control vs PFAS)
Mass
HSI
GSI
LiverMass
GonadMass
MuscleMass
BrainMass
GutMass
Value (g or index)
Control
PFAS
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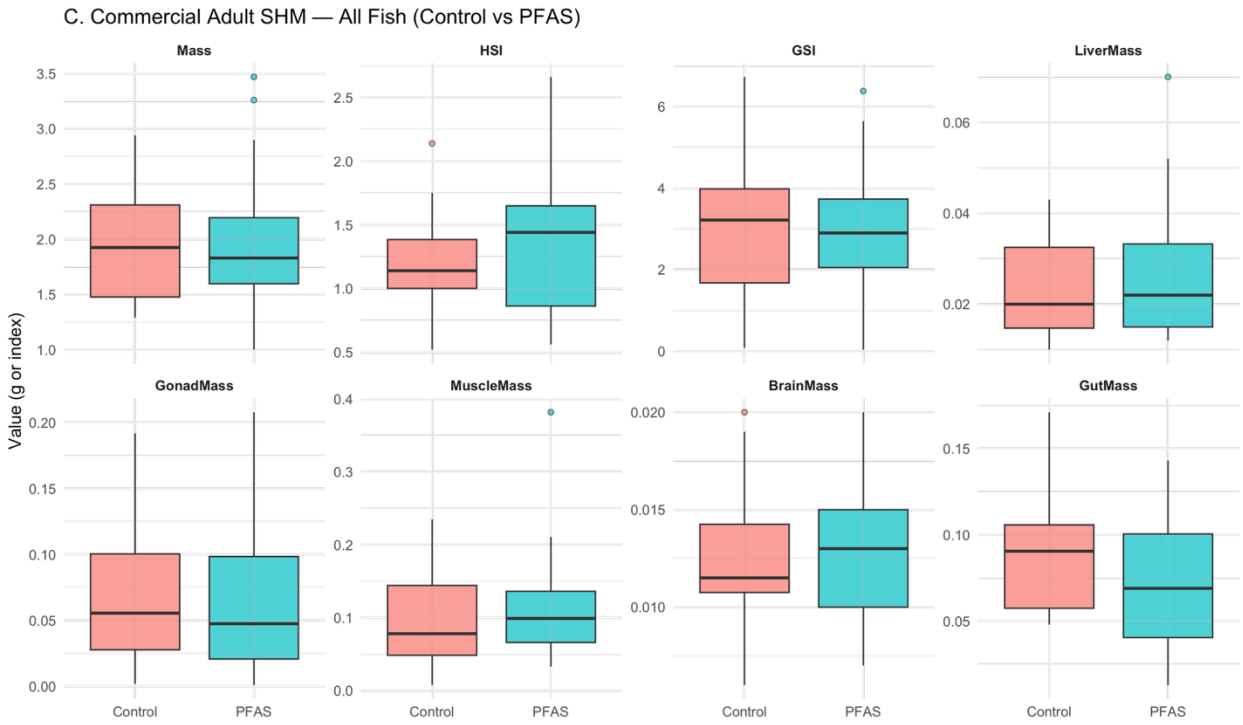

Figure 2: Tissue weights of adult sheepshead minnows after a 3-week exposure to PFOS and PFNA (10 ppb each) compared to controls. Boxplots show whole-body mass, hepatosomatic index (HSI), gonadosomatic index (GSI), and tissue weights (liver, gonad, muscle, brain, gut). (A) Females; (B) Males; (C) Combined sexes. Horizontal lines indicate medians; boxes represent the interquartile range; whiskers show minimum and maximum values. Significant difference in male gut mass is indicated by * ($p = 0.0010$). Data were analyzed using linear mixed-effects models (lmer) with TankID as a random effect, with post hoc comparisons via estimated marginal means (emmeans). P-values < 0.05 were considered significant ($n = 5$ tanks per treatment; females: 3 fish/tank; males: 1 fish/tank).