Matagorda Bay Mitigation Trust 2022-2023 Funding Cycle
RFP # 2022-2023-1 Contract #038
Title: Reproductive & Developmental Toxicity of "Forever Chemicals" to Matagorda Bay's prey fishes
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Y2 Q2-4 2025 Progress Report

Y2 Q2-4 Update:
We have now completed Phase 1 of our proposed work, and here report both sediment and water PFAS profiles and concentrations for four locations throughout the Matagorda Bay system. We have also completed the morphological analyses of commercial sheepshead minnow larval exposures as part of Phase 3. Based on these data, we have also begun our long-term, chronic exposure of adult commercial sheepshead minnow to PFAS in the lab to determine the impacts of PFAS on fecundity of these species. We are continuing to develop methods for tissue extraction of PFAS to assess PFAS body burdens of sheepshead minnow from the Matagorda Bay system for Phase 2.

Phase 1:
As reported in previous progress reports, we collected paired water and sediment samples (n=9/site per media) from four locations in the Matagorda Bay system (Figure 1). At each location, samples were taken in triplicate from 3 sites along a 20 meter transect (an example of this for the Palacios Wastewater Treatment Plant is shown in Figure 2). At each sampling site along the transects, a total of three water samples were collected in PFAS-free bottles. Following water collection, five 5cm sediment cores were taken at the same location and placed in a PFAS-free bottle. In addition to sample collection, each site had the following recorded: latitude + longitude, salinity, dissolved oxygen, pH, and temperature. The data we present here are the average of three samples analyzed for each transect for both water and sediment.

We have previously reported all sediment PFAS profiles and PFAS compound loads (see Table 1), which were analyzed by two EPA certified commercial labs (SGS AXYS Analytical Services, LTD and Eurofins Environmental Testing Northern California, LLC). These sediment samples were analyzed using EPA Method 1633, which tests for 40 unique PFAS compounds. Only the compounds detected in samples are included in Table 1.

During Q2-4, we analyzed all the paired water samples for each location for 13 PFAS compounds (Table 2) and can now report PFAS profiles for water collected at each of the four sampling locations shown in Figure 1. These samples were analyzed using modified EPA Method 537.1 to test for the presence of 13 PFAS (Table 2).

Prior to solid phase extraction (SPE), all water samples were passed through a 0.7-micron glass micro fibre Whatman filters to remove suspended particulate matter. Samples are filtered to determine the PFAS that are dissolved in water samples and not partitioned to the suspended particulate matter. Following filtering, samples were passed through polystyrenedivinylbenzene (SDVB) containing cartridges (Agilent: Catalog No.1225-5021) to extract method analytes, which were then eluted from the solid phase sorbent with methanol (ThermoFisher; Catalog No. 325740010). After being concentrated to dryness, extract was adjusted to a 90:10% (vol/vol) water/methanol in 100uL. Analysis of PFAS was performed at the Mass Spectrometry

Facility of the Department of Chemistry at the University of Texas at Austin in Austin, Texas using a Shimadzu 8060 LC MS/MS Triple Quadrupole. Briefly, 10uL of sample (and standards) were injected and a Purospher Star RP-18 endcapped (3micron) Hibar TR 50-4 delay column was utilized to delay elution, preventing interference during separation by the analytical column. Analytes were then separated using a Waters Crop Acquity UPLC BEH C18 (2.1x50mm, 1.7micron) column. A mobile phase LC gradient consisted of Bottle A [acetonitrile] and Bottle B [ammonium acetate (2mM) in 95:5 water/acetonitrile] beginning at 2%-A and 98%-B, reaching 95%-A and 5%-B at 10 minutes, and ending at 2%-A and 98%-B over 12 minutes. An in house-PFAS standard mix containing 13 reagent grade PFAS stocks was used to generate calibration curves for quantification. Post-analysis data processing (identification and quantification) was performed using LabSolutions Insight software.

As shown in Table 3, the most found PFAS among the 13 tested in each water samples is PFOS at each of our four sampling locations. These data also show higher concentrations of PFOS in water samples compared to sediment samples. This is interesting, as PFOS is predicted to quickly partition to and be bound by sediment at the salinities measured at each of the sampling locations (~30 ppt). Partitioning and binding to sediment would remove the PFOS from the water column, suggesting that the compound is less mobile within the water column and may, therefore, be less biologically available. However, these data suggest that PFOS is staying mobile within the Matagorda Bay system at these four sampling locations and could be more biologically available to aquatic organisms within the Bay. Another interesting aspect of these data are the detection of two PFAS that were not detected in sediment samples in the Bay: PFHxS and PFDA (Table 3). This suggests that these two PFAS are not partitioning to the sediment, and instead, stay suspended in the water column and may be more biologically available to aquatic organisms.

Overall, these data do show different PFAS profiles within the paired water and sediment samples. Samples from our Palacios and Reference Sites showed a more varied PFAS profile within the sediment compared to the samples from Port Comfort and Chocolate Bay (Table 1). In fact, our sediment data do not indicate the detection of the 40 PFAS tested for in the sediment at our Chocolate Bay location. Interestingly, of the 13 PFAS tested for in water samples, we only detected three PFAS compounds: PFOS, PFDA, and PFHxS. One important finding is that we did detect PFOS in every water sample analyzed, and at significantly higher concentrations relative to the paired sediment tested – including at the Chocolate Bay site where there was no PFOS detected in our sediment samples. This suggests that at these sites, PFOS is remaining mobile within the water column instead of partitioning to the sediment. To determine factors that could be affecting the behaviour of PFOS at each of these sites, we are currently analyzing environmental factors that influence partitioning behaviour. We are currently quantifying dissolved organic carbon (DOC) in each of these water samples tested to determine if there is a relationship with DOC and PFOS concentrations, as DOC is known to influence the behaviour of PFAS in the aquatic environment. We are also currently looking at the effects that salinity could have on the partitioning behaviour of a variety of PFAS.

Phase 2:
We are actively working to optimize methods to test body burdens of adult sheepshead minnows (SHM) to assess the PFAS body burdens of wild-caught SHM. We have been working to modify

EPA Method 1633 and additional methods published in peer reviewed literature to optimize the extraction protocol for muscle, liver, and gonad tissues collected from adult fish. We continue to modify the protocol to improve our percent recovery of PFAS from each tissue type and can run samples on both the IM Q-TOF LC-MS and Shimadzu 8060 LC MS/MS Triple Quadrupole. Once our method is optimized and has a sufficient percent recovery, we will be able to analyze liver, muscle, and gonad tissues from wild-caught adult SHM for PFAS body burdens.

Phase 3:

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 (Figure 1). We anchored our exposure concentrations for all 5 PFAS tested to these environmentally relevant data for PFOS in Matagorda Bay collected as part of Phase 1.

To collect these data, commercially purchased broodstock adult SHM at the Fisheries and Mariculture Laboratory (FAML) at UT MSI. SHM were spawned first thing in the morning and collected embryos within 2 hours of spawning. Embryos were assessed for viability and left to develop for 24 hours. At ~24 hours post fertilization (hpf), embryos were staged and only embryos within the 16-17 embryonic stages were used for the study. We performed a graded dose-response using the following doses: 0 ppb (parts per billion; control), 2 ppb, 6 ppb, 16 ppb, and 44 ppb (Figure 4). Each experiment was set up individually, with embryos and larval fish being exposed to only one of the five PFAS mentioned above. At the start of the experiment, embryos (n=10/dish; 50 total per dose across dishes) were placed in the experimental treatments (Figure 2). Survival was assessed every 24 hours and embryos were checked for proper developmental milestones (e.g., eye pigment development). Following these checks, debris was removed from each dish and 50% water changes were made with water dosed with the relevant concentration. The experiments were performed in our lab's environmental chamber to ensure all dishes were maintained at the appropriate temperature (25°C) and were covered with transparent sheeting to prevent evaporation. Water quality parameters (temperature, pH, salinity, and dissolved oxygen) were measured daily prior to each dish's 50% water changes. Water samples were collected at the start of the experiment, from each dish during daily water changes, and at the end of the experiment for quantification of each PFAS. After the final assessment when all the fish had hatched (7dfp), half the fish (n=5/dish) were euthanized in buffered MS222, placed in a 3% methyl cellulose solution, and imaged using a Nikon SMZ800N fitted with a camera and accompanying software. We have analyzed a suite of morphological parameters (Figure 5) to compare the impacts of each compound on the fish's development.

Overall, our data show differential effects of each PFAS compound tested on SHM larval development. All the statistics for this study were analyzed in JMP Pro using Mixed Models and Student's T All Pairwise post hoc tests. We found significant impacts of both PFAS compound and dose on the length of SHM larvae (Dose [Compound]: $F_{20,4}$=1.79; **p=0.0186**; Compound:

$F_{4,4}$=12.50; **p<0.0001**; Figure 6). Individuals exposed to 16 and 44 ppb PFNA were significantly longer compared to the individuals exposed at lower doses. We also found that individuals exposed to 44 ppb PFOS were also significantly larger compared to the other doses; and that individuals exposed to PFOSA showed an increase in length with increasing concentrations. On the other hand, exposure to 16 and 44 ppb PFOA resulted in significantly shorter individuals compared to controls. When assessing the relative body area of these individuals (i.e., the total body area divided by the length, see Figure 5), we again saw differential effects of each PFAS (Dose [Compound]: $F_{20,4}$=3.07; **p<0.0001**; Compound: $F_{4,4}$=18.91; **p<0.0001**; Figure 7). Exposure to 16 and 44 ppb PFDA resulted in fish with significantly larger relative body areas compared to control, while we saw decreasing relative body areas with increasing concentrations of PFOA (Figure 7). We also found impacts of both PFOS and PFOSA on the relative body areas of larvae, but not in a dose-dependent manner (Figure 7). We also assessed the impact of each compound on relative eye area (i.e., eye area divided by body area) and saw differential results depending on the compound (Dose [Compound]: $F_{20,4}$=1.80; **p=0.018**; Compound: $F_{4,4}$=6.51; **p<0.0001**; Figure 8). Here, we found that individuals exposed to 16 and 44 ppb PFDA had larger relative eye areas; while PFNA also resulted in individuals with bigger eyes at 2, 16, and 44 ppb (Figure 8). When assessing the relative yolk area (i.e., total yolk area divided by body area) among individuals, we also saw differential effects of each PFAS (Dose [Compound]: $F_{20,4}$=1.93; **p=0.0091**; Compound: $F_{4,4}$=13.24; **p<0.0001**; Figure 9). Individuals exposed increasing doses of PFNA and PFOSA has decreasing relative yolk areas compared to control individuals, suggesting these PFAS affected energy utilization (Figure 9). This could be due to a variety of reasons, including increased metabolic demands at higher exposure concentrations or changes to the fish's ability to utilize lipids. Further work is required to parse out the driving causes of these effects.

Our next steps are the repeat these same five individual PFAS on embryos collected from wild-caught adult SHM. We will be beginning collections of these wild-caught fish in the coming weeks. These adults SHM will be collected under Texas Parks & Wildlife Scientific Permit Number SPR-0822-116 issued to PI Nielsen. Following collection, the fish will be given a 2-week acclimation period in the lab and then will be spawned. Upon collection, a subset of males and females will be humanely euthanized and dissected to quantify PFAS body burdens for the 13 PFAS measured previously in the Matagorda Bay system (Table 2). We will assess PFAS burdens of pooled gonads, livers, and muscle between sexes. Once spawning begins, we will also collect a subset of embryos and resulting larvae to pool and quantify PFAS burdens. These data will inform of background level PFAS burdens among individuals and may offer insight into potential depuration of PFAS upon entering the lab.

These experiments with wild-caught SHM will offer an important comparison on the impacts of PFAS on wild-caught versus commercially available SHM. These data are important, as SHM are an important EPA model fish species used to perform toxicity testing of a wide variety of toxicants. Previous work in other EPA model fish species, including fathead minnows, has shown that wild-caught fish have significantly different responses to toxicants and pharmaceuticals compared to the commercially purchased fish of the same species used for EPA testing. Therefore, we are interested in this comparison to be able to perform the most accurate risk assessment of PFAS on fishes local to the Matagorda Bay system and the Coastal Bend.

Adult SHM PFAS exposures

We are currently conducting adult SHM PFAS exposures to determine the impacts of chronic PFAS exposure on adult fecundity and embryolarval development. These experiments are informed by our previously described SHM embryolarval individual PFAS exposures and our environmentally detected PFAS within the Matagorda Bay system (Figures 1, 3). As we saw the most significant impacts of PFNA and PFOS on SHM larvae (Figures 6-9), we are currently conducting a co-exposure of adult SHM to 10 ppb PFNA and 10 ppb PFOS. To do this, we have established 5 control tanks and 5 co-exposure tanks, each containing one male and three female reproductively active adult SHM. Each tank contains 16 litres 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) are checked daily in all tanks to ensure optimal water quality parameters. Fish are fed daily *ad lidibium* and excess food and debris are removed daily. Prior to introduction into the tanks, all fish were measured and weighed. Throughout the duration of the experiment, fish from each tank are again weighed and measured 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 is recorded daily, and water samples are collected immediately prior to any water changes. Currently, water changes occur one time weekly, and all water samples are saved to track PFNA and PFOS levels within each tank. Water changes are performed with PFAS spiked water to maintain PFAS levels or control water in control tanks. These exposures will run for 28 days before we initiate spawning in each tank. At the completion of the experiment, final water samples will be collected.

Once spawning begins, eggs will be immediately collected and isolated within their home tank to develop to the same stage of larva discussed above in Phase 3: *Embryolarval SHM PFAS exposures*. Embryos will be checked daily to quantify survival and stage development. Once larvae reach the sampling stage where all the fish hatch (~7dfp), larvae will be euthanized in buffered MS222, placed in a 3% methyl cellulose solution, and imaged using a Nikon SMZ800N fitted with a camera and accompanying software. We will then analyze a suite of morphological parameters (Figure 5) to compare the impacts on the fish's development. Immediately following imaging, embryos will be placed at -20°C for measurement of PFAS body burdens. Once tanks have spawned and larvae have been collected, adults will also be euthanized in buffered MS222 and dissected to isolate gonad, liver, and muscle tissue to assess PFAS body burdens.

In addition to these adults PFNA + PFOS exposures, we will also be collecting SHM minnow eggs from the same SHM broodstock populations used to quantify the effects of individual PFAS on SHM embryolarval development (described above in Phase 3: *Embryolarval SHM PFAS exposures*) for additional comparative experiments. These embryos will be exposed to 10 ppb PFNA + 10 ppb PFOS, as previously described above in Phase 3: *Embryolarval SHM PFAS exposures* to compare the combined effects of these compounds on SHM embryolarval development relative to the individual PFAS exposures and the embryos resulting from adults in a chronic PFNA + PFOS exposure.

Figures:

Figure 1. PFAS detected in sediment taken from the four indicated sampling locations.

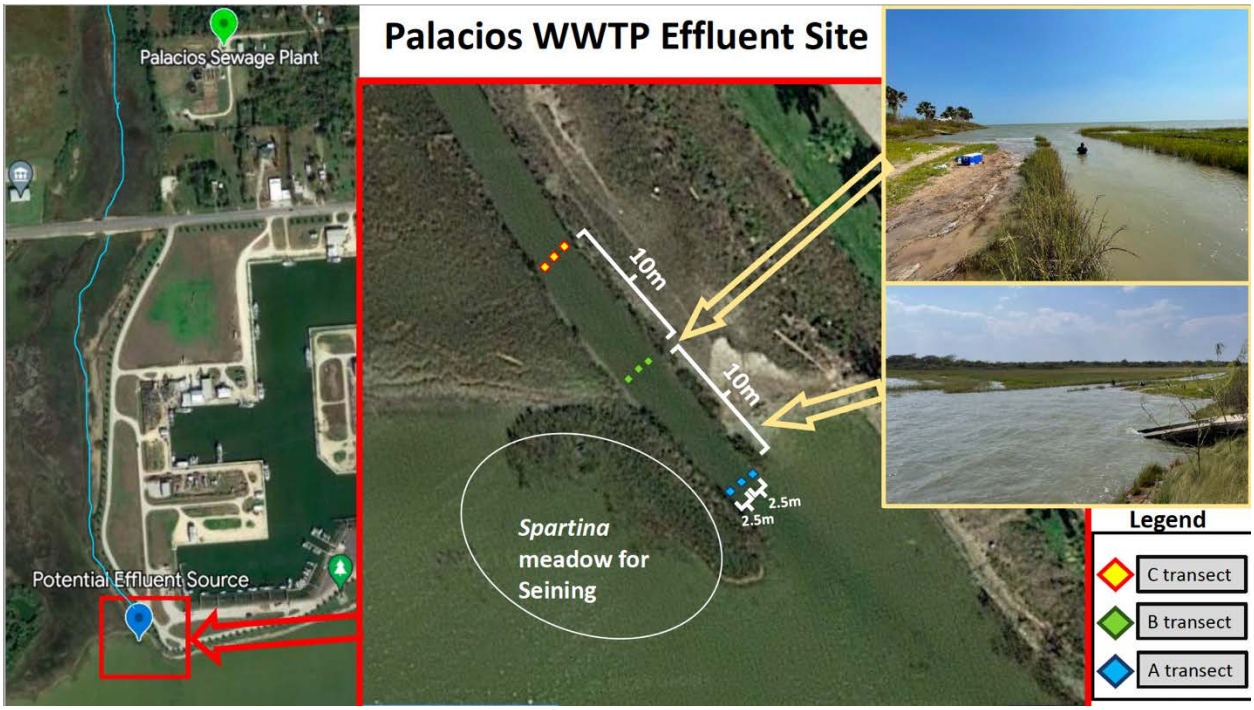

Figure 2. Site sampled for PFAS characterization of Matagorda Bay near the Palacios Wastewater Treatment Plant. Here, you can see the three transects that were sampled.

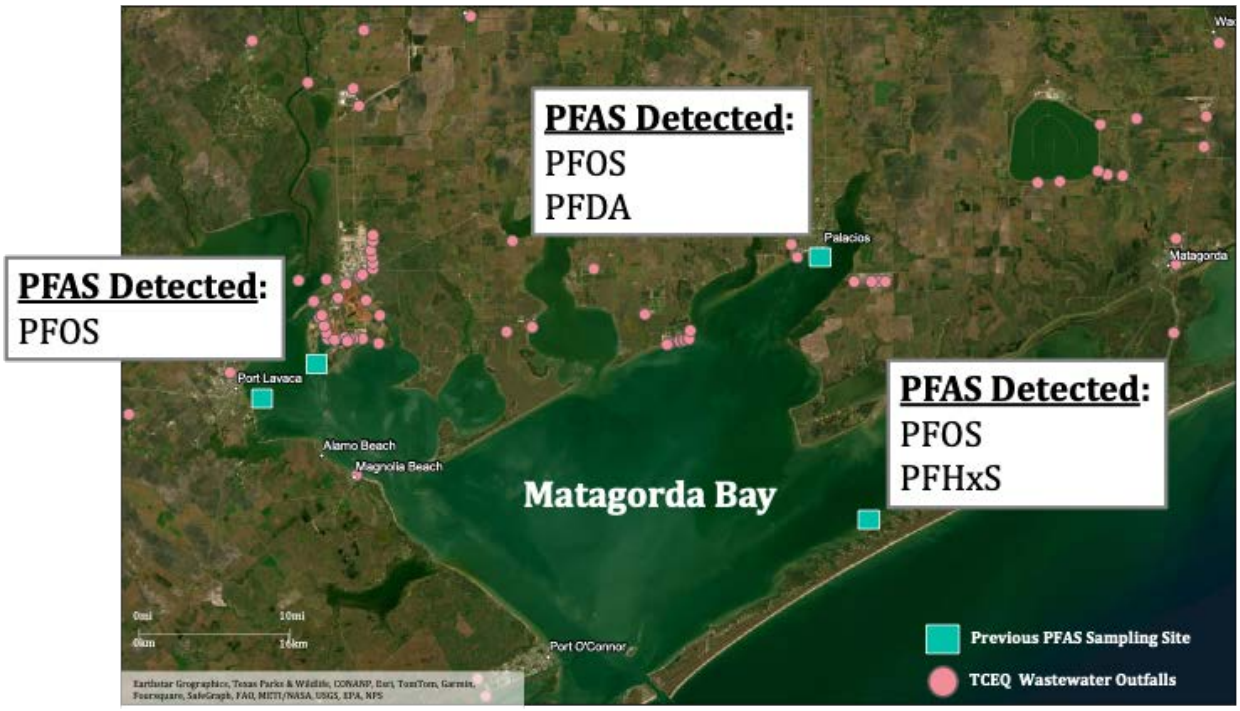

Figure 3. PFAS detected in water samples taken from the four indicated sampling locations.

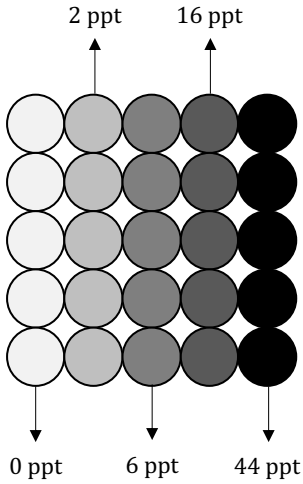

Figure 4. Depiction of the graded dose-response experiment performed in embryonic and larval sheepshead minnows. There was a total of five environmentally relevant doses in the study, ranging from 0 parts per billion (ppb) to 44 ppb. Each circle represents one replicate (i.e., dish), for a total of 5 replicates per dose. Briefly, fish (n=10/dish) were added at ~24 hours post-fertilization (dpf) and monitored through hatch (~ 6-7 dpf). At the end of the experiment (7 dpf), half the fish in each dish (n=5/dish) were imaged using a microscope for morphological analysis.

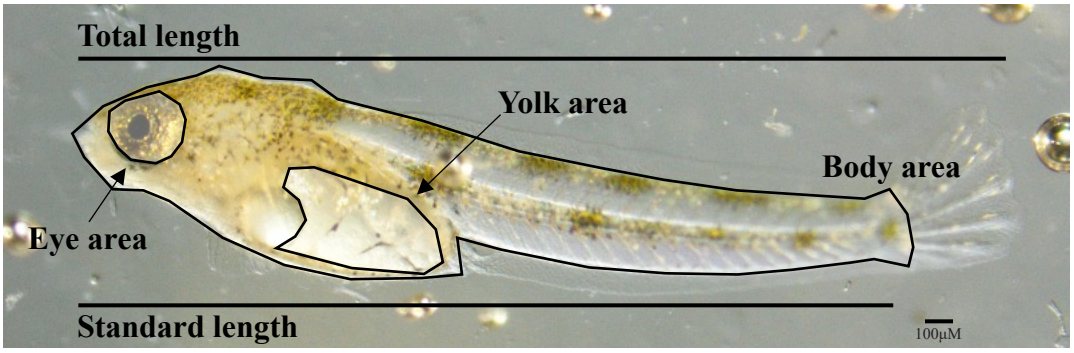

Figure 5. Morphological measures taken of each sheepshead minnow larva.

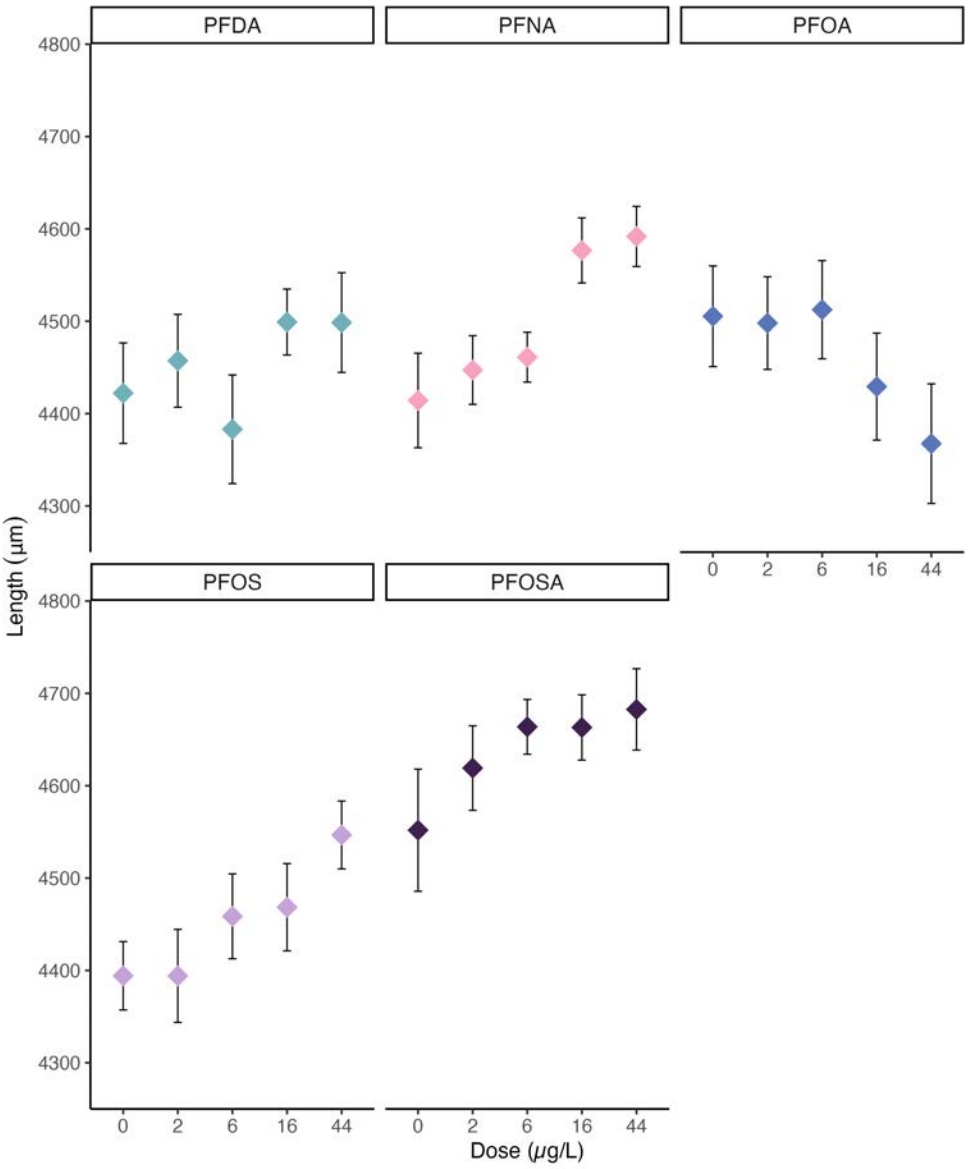

Figure 6. Impacts of five individual environmentally relevant PFAS on sheepshead minnow larvae length on individuals exposed during embryonic and larval life stages. Each point shows average; error bars show the standard error of the mean. Results of statistical analyses are included in the text. Doses are shown in the equivalent of parts per billion (ppb).

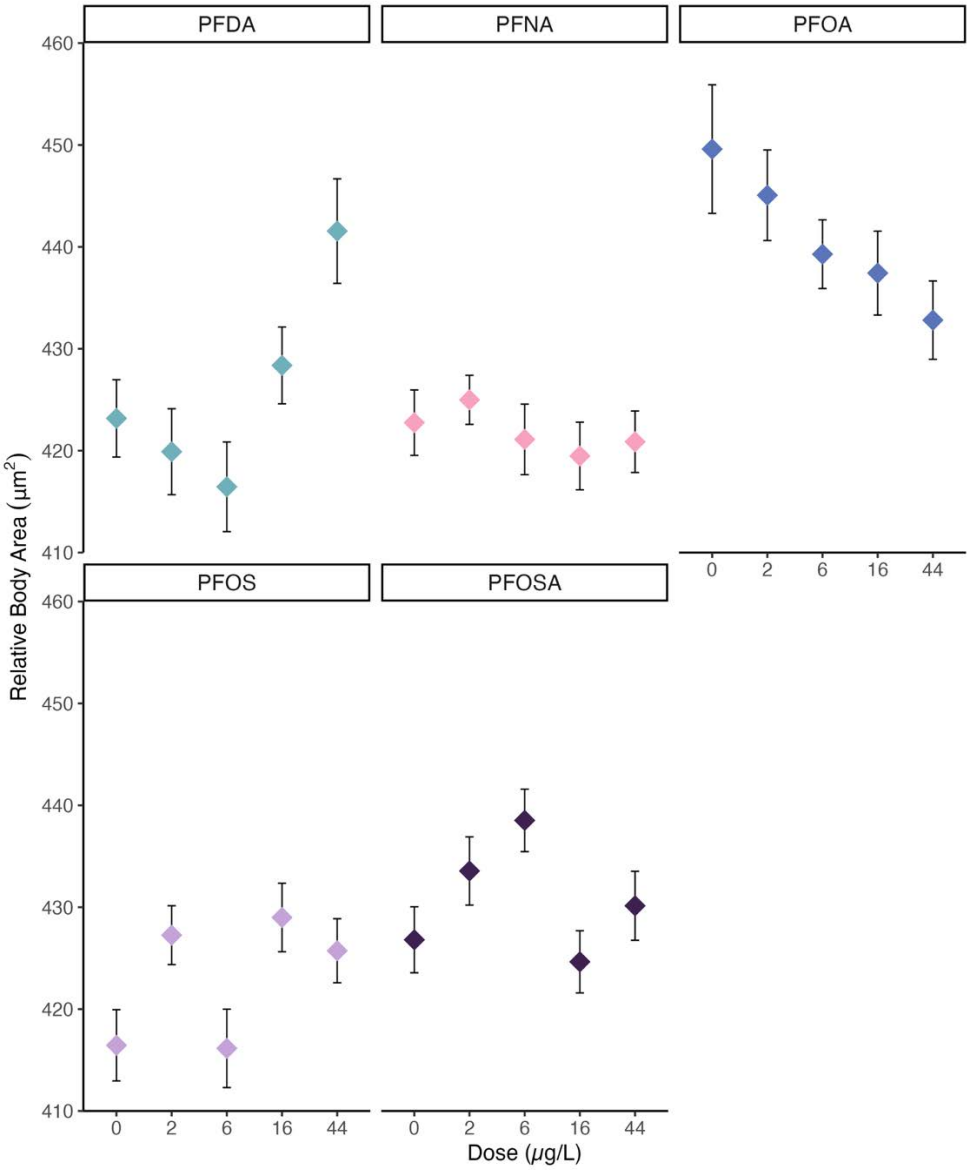

Figure 7. Impacts of five individual environmentally relevant PFAS on sheepshead minnow larvae relative body area (i.e., total body area divided by length) on individuals exposed during embryonic and larval life stages. Each point shows average; error bars show the standard error of

the mean. Results of statistical analyses are included in the text. Doses are shown in the equivalent of parts per billion (ppb).

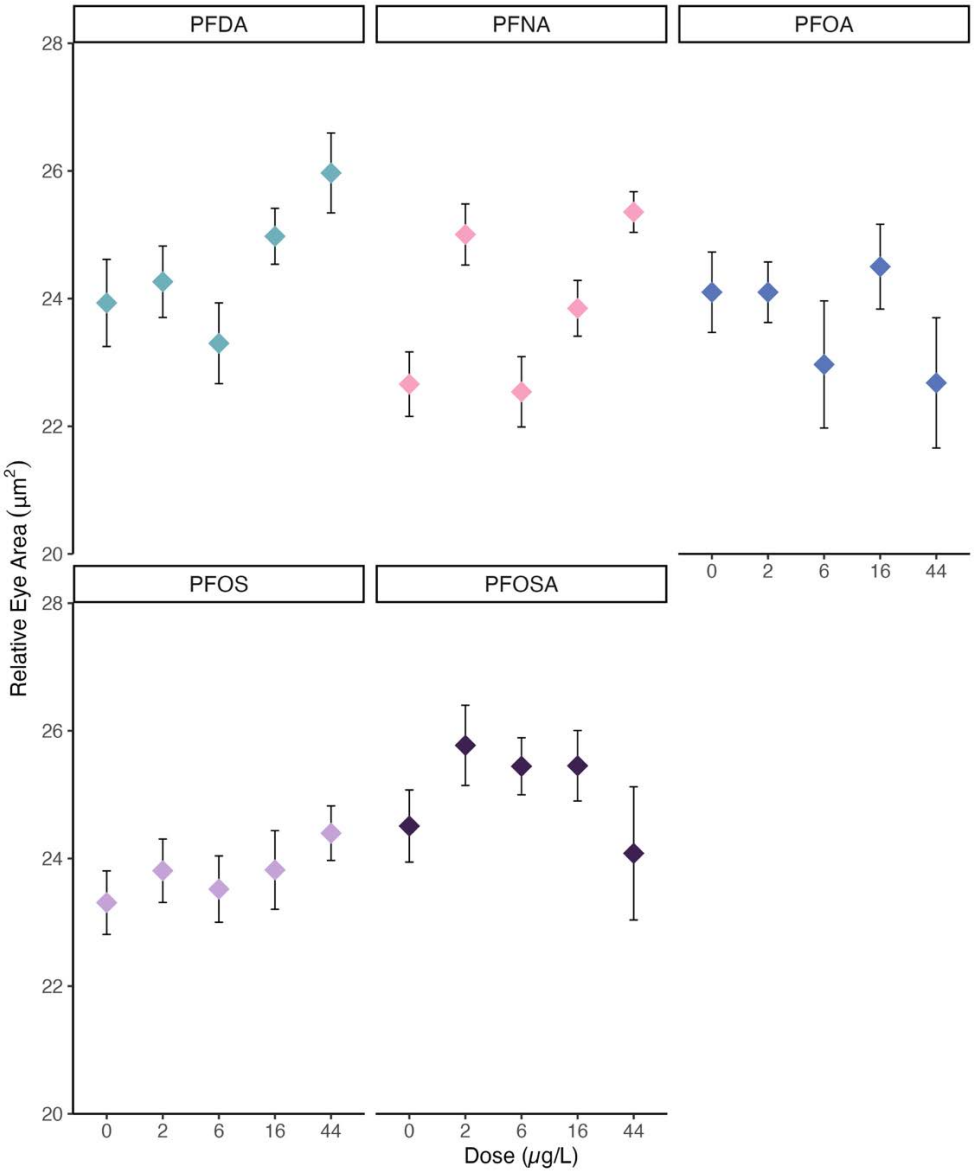

Figure 8. Impacts of five individual environmentally relevant PFAS on sheepshead minnow larvae relative eye area (i.e., total eye area divided by body area) on individuals exposed during embryonic and larval life stages. Each point shows average; error bars show the standard error of the mean. Results of statistical analyses are included in the text. Doses are shown in the equivalent of parts per billion (ppb).

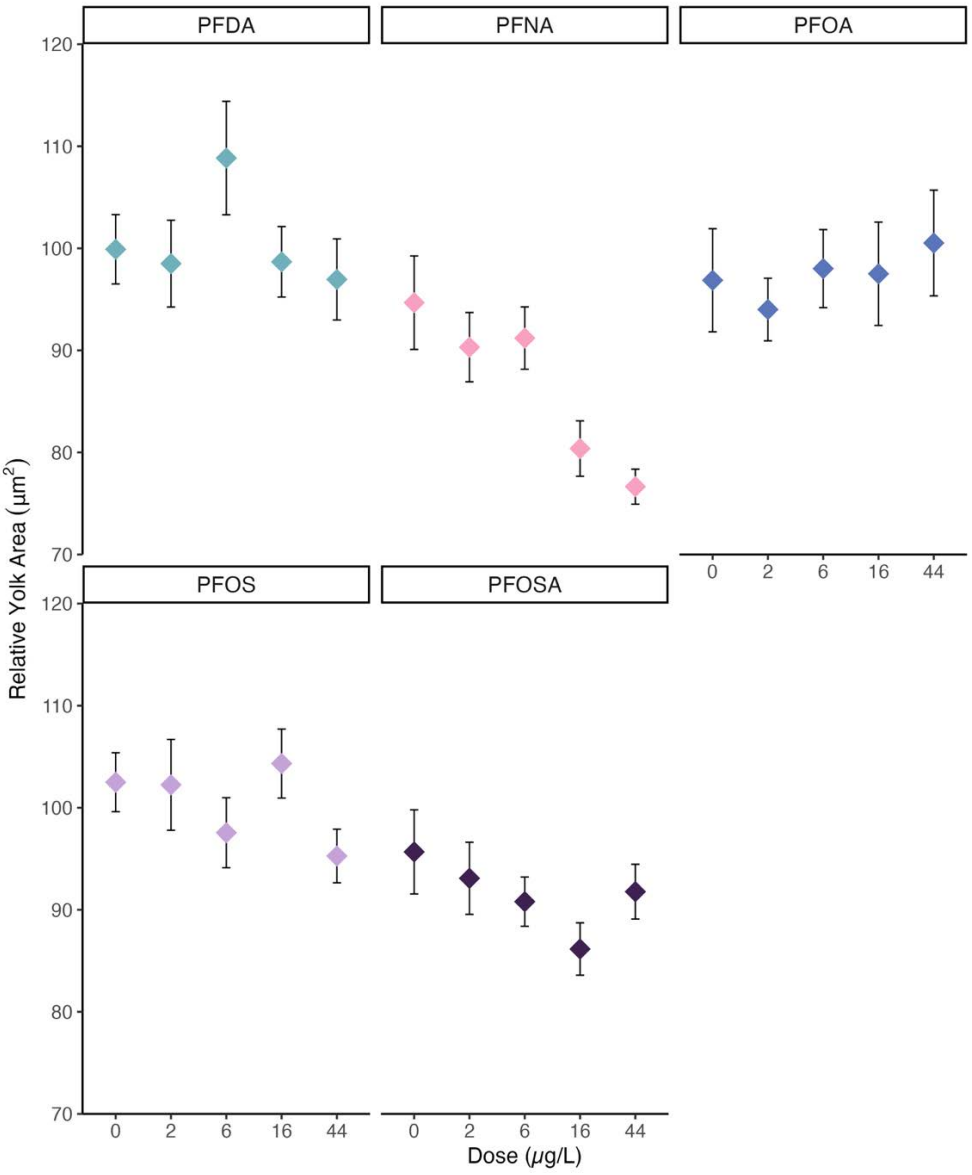

Figure 9. Impacts of five individual environmentally relevant PFAS on sheepshead minnow larvae relative yolk area (i.e., total yolk area divided by body area) on individuals exposed during embryonic and larval life stages. Each point shows average; error bars show the standard error of the mean. Results of statistical analyses are included in the text. Doses are shown in the equivalent of parts per billion (ppb).

Tables:

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 as shown in Figure 1. The average concentration (reported in parts per trillion (ppt)) is shown among all sediment samples taken. Within each sampling site, we sampled three transects (indicated here by A-C, shown in Figure 2). 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.

Compound	Limit of Detection (ppt)
PFDA	0.3
PFDoA	30
PFTrDA	30
PFBA	10
PFOA	7.5
PFOS	10
PFHpA	4
PFNA	5
PFHxS	10
PFTeDA	300
PFHxA	17
PFUnA	3

Table 2. PFAS analyzed using a modified EPA Method 537.1 for filtered water samples taken from each sampling site in Matagorda Bay. The limit of detection for each compound is shown here in parts per trillion (ppt).

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 3. PFAS detected in water samples taken from all four sampling locations in Matagorda Bay shown in Figure 1. The average concentration (reported in parts per trillion (ppt)) is shown among all water samples taken. Within each sampling site, we sampled three transects (indicated here by A-C, shown in Figure 2). 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.