Texas A&M University at Galveston
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FOURTH INTERIM PERFORMANCE REPORT

MAY 31ST, 2022

Project Title: The Fate and Toxicity of Microplastics and Persistent Pollutants in the Shellfish and Fish of Matagorda Bay

Submitted To:

Matagorda Bay Mitigation Trust

Performing Laboratory:

Texas A&M University on behalf of Texas A&M University at Galveston

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The Fate and Toxicity of Microplastics and Persistent Pollutants in the Shellfish and Fish of Matagorda Bay

Personnel

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Location(s):

Texas A&M University at Galveston

Project Duration:

01 June 2021 – 31 August 2024

Objectives:

Objective 1: Quantify the extent of microplastics pollution in the surface waters and biota of Matagorda Bay.

Objective 2: Measure levels of persistent pollutants in surface waters, adsorbed to microplastics, and bioaccumulated in the biota of Matagorda Bay.

Objective 3: Study the toxicity of microplastics and adsorbed pollutants using embryo-larval life stages of sheepshead minnow.

Objective 4: Public educational outreach to local high school students on the science of ecosystem health monitoring.

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1. INTRODUCTION

1.1 Background

This project is studying the extent of microplastics and persistent pollutant exposure of resident biota (shellfish and fish) sampled from Matagorda Bay, and also assessing any likely toxicity effects due to exposure. The *new knowledge* gained from the successful completion of this project will contribute to an understanding of the long-term fate and toxicity of microplastics (and adsorbed pollutants) in the Matagorda Bay system.

In this fourth interim report (March 1st, 2022 – May 31st, 2022) we provide a list of key accomplishments as per the end of Year 1 of the project.

2. Key Updates

As of the period encompassing the fourth interim report (March 1st, 2022 – May 31st, 2022), the key achievements associated with each stated objective are detailed below.

Objective 1: Quantify the extent of microplastics pollution in the surface waters and biota of Matagorda Bay.

- The collection of biotas (oysters, fish) has been completed. However, the collection and analysis of water samples from Matagorda Bay, which has already commenced in Year 1, will continue into Year 2. The biota collection duration has encompassed a time frame from May – December 2021. As of current evaluation, the total numbers of organisms sampled from Matagorda Bay are listed in **Table 1**, and for each total we indicate in parenthesis the numbers of biota samples already processed and analyzed for pollutant body-burdens.

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Table 1. Summary of the total numbers of fish (muscle, liver, and digestive tract) and oysters (gill and mantle) sampled from Matagorda Bay (May – December 2021). The number in parenthesis listed besides the Total Numbers Sampled indicates the numbers of fish processed for the analysis of pollutant body-burdens (PAHs and PCBs).

Common Name	Scientific Name	Total Numbers Sampled
Gulf menhaden	*Brevoortia patronus*	44
Red drum	*Sciaenops ocellatus*	7 (7)
Black drum	*Pogonias cromis*	9
Hardhead catfish	*Ariopsis felis*	77 (10)
Flathead grey mullet	*Mugil cephalus*	78 (14)
Gafftopsail catfish	*Bagre marinus*	5
Bluefish	*Pomatomus saltatrix*	3
Atlantic croaker	*Micropogonias undulatus*	16
Spot	*Leiostomus xanthurus*	1
Lady fish	*Elops saurus*	8
Spotted seatrout	*Cynoscion nebulosus*	9 (9)
Pinfish	*Lagodon rhomboides*	6
Southern kingfish	*Menticirrhus americanus*	2
Atlantic spadefish	*Chaetodipterus faber*	1
American gizzard shad	*Dorosoma cepedianum*	11
Crevalle jack	*Caranx hippos*	2
Eastern Oyster	*Crassostrea virginica*	20
	Total biota sampled =	**301**

- The GCMS/MS-pyrolysis method has been optimized for the analysis of microplastics from biota samples. The high interference of organic carbon detected from the samples has been removed by pre-digesting tissue samples in 10% potassium hydroxide (KOH) for 48 hours at 60^{o}C for water samples, or enzymatic digestion for biota samples. After digestion, samples are filtered onto 25 mm glass fiber filters (GFF, Whatman), the filters are dried and then packed into steel cups for pyrolysis.

- The updated GCMS/MS-pyrolysis analytical method can monitor all common plastics (**Fig. 1**) and phthalate plasticizers (**Fig. 2**). Phthalate plasticisers are detected and quantified based on their characteristic pyrolyzate product phthalic anhydride (**Fig. 2**).

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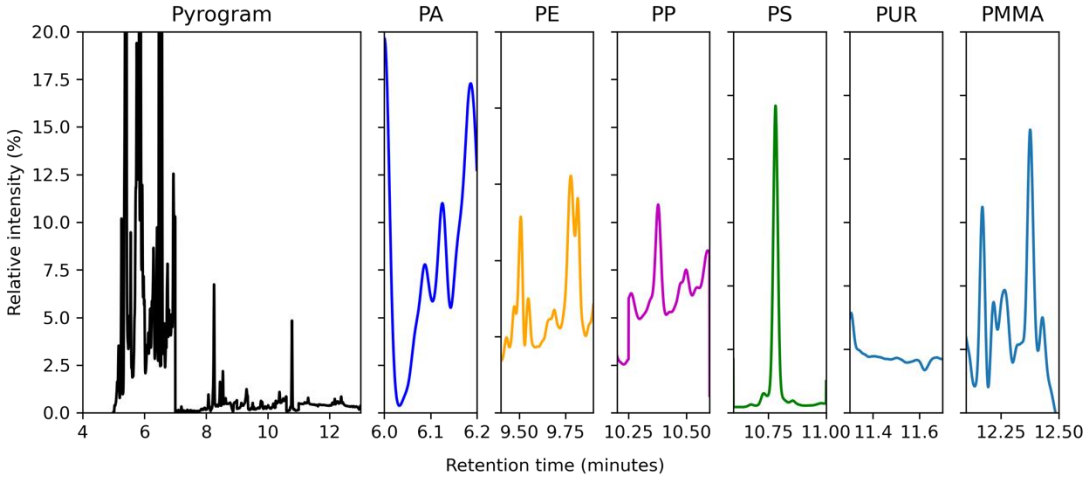

Fig. 1 Pyrogram of a surface water sample. Sample was collected with a 10' stainless steel filter with a 5 µm pore size and ~100 L were filtered. Pyrolysis is performed at 600ºC, and pyrolysis products are separated and detected by GC-MS/MS. PS, polystyrene; PA, polyamide; PVC, polyvinyl chloride; PC, polycarbonate; PE, polyethylene; PP, polypropylene; PMMA, polymethyl methacrylate; PUR; polyurethane.

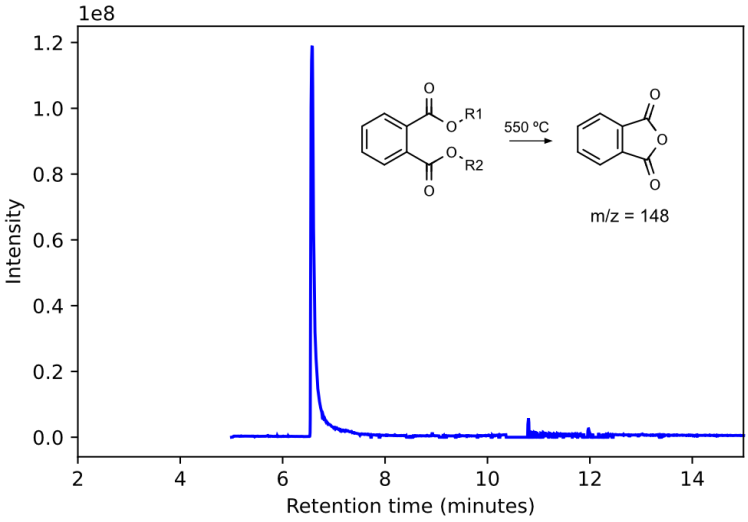

Fig. 2. Pyrogram of phthalate plasticizers. Pyrolysis converts common phthalate esters into phthalic anhydride (m/z = 148), that can be identified and quantified parallel to common plastics.

- In addition, microplastics have also been measured in the surface waters of Matagorda Bay and are presented in **Table 2**.

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Table 2. Concentrations of microplastics in surface water from Matagorda Bay. Sample was collected with 10' inch stainless steel filters (5 µm pore size). PS, polystyrene; PA,, polyamide; PVC, polyvinyl chloride; PC, polycarbonate; PET, polyethylene terephthalate; PE, polyethylene; PP, polypropylene; PMMA, polymethyl methacrylate; PUR; polyurethane.

Plastic type	Concentration (ng/L)
PS	213
PA	258
PVC	0
PC	28
PET	0
PE	1080
PP	1082
PMMA	672
PUR	648

- In Year 1, three water sampling trips on Matagorda Bay have been completed. The first trip was on August 17th, 2021, during this trip we filtered and collected surface water samples from 7 locations in the Matagorda Bay waters (please see **Fig. 3**). The second trip was on September 12th, 2021, we collected samples from 6 locations from the beaches of Port O'connor, Magnolia Beach, Port Lavaca, Weedhaven, Palacios and Wadsworth around the bay (not shown on the map in **Fig. 3**). On the second trip we used a pump to filter the surface water and about 13-26 gallons was filtered each time and then collected in mason jars. A third trip of water sampling in the Matagorda Bay waters was conducted on December 16th, 2021. During this trip, samples were collected with a filtration system and a 200 µm tow net. Filtered samples were collected with a 5 µm stainless steel filter cartridge and 10-24 gallons were filtered.

- Additional water sampling trips are planned in Year 2.

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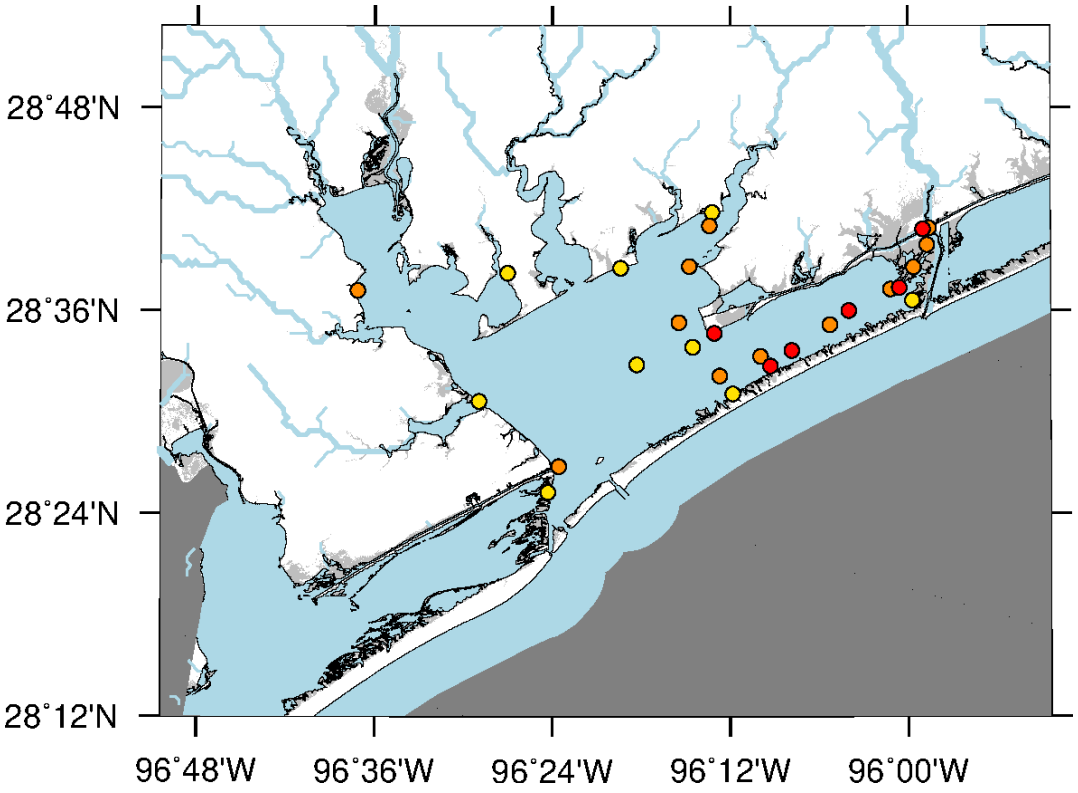

Fig. 3. Map of Matagorda Bay showing the various sites from which water samples have been collected over the Year 1 project duration. The May – July 2021 water sampling locations are shown as yellow circles; August 2021 sites are shown as red circles; and the December 2021 sites are shown as orange circles.

Objective 2: Measure levels of persistent pollutants in surface waters, adsorbed to microplastics, and bioaccumulated in the biota of Matagorda Bay.

- As per the end of Year 1, PAH and PCB body-burdens in biota samples from Matagorda Bay have been nearly completed (with exception of analysis in oysters, which is currently in-progress).
- An accelerated solvent extraction (ASE) and gas chromatography mass spectrometry (GCMS) method is being used for the analysis of select persistent organic pollutants, namely polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), in biota from Matagorda Bay (**Fig. 4**).

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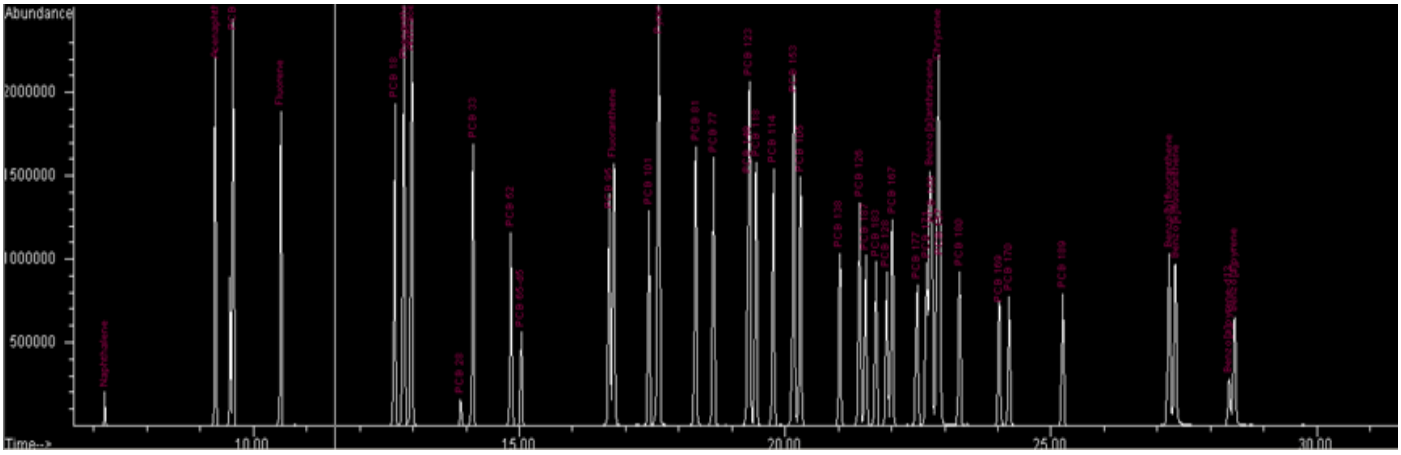

Fig. 4. A chromatograph of PAHs and PCBs as measured using GCMS. A total of 15 PAHs and 29 PCB congeners (all EPA priority pollutants) are being quantified. The 15 PAHs include: naphthalene (NAP), acenaphthene (ACE), fluorene (FLU), anthracene (ANT), phenanthrene (PHE), fluoranthene (FLT), chrysene (CHR), pyrene (PYR), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DahA), benzo[g,h,i]perylene (BghiP), and indeno[1,2,3-cd]pyrene (IcdP). The 29 PCB congeners include PCBs 1, 18, 28, 33, 52, 95, 101, 81, 77, 149, 123, 118, 114, 153, 105, 138, 126, 187, 183, 128, 167, 177, 171, 156, 157, 180, 169, 170, and 189. Of the 29 PCB congeners, 12 are dioxin-like: PCBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189. All PCBs are identified according to the IUPAC numbering system.

- Quality assurance studies for PAH and PCB analysis involving the standard addition of PAHs and PCBs into liver tissue homogenates from fish and subjected to ASE extraction and lipid removal, yield a recovery of 77% for Benzo[a]pyrene, 38% for Pyrene, 90% for PCB 18 and 63% for PCB 101. These recoveries adequately demonstrate the effectiveness of the pollutant extraction and analysis methods.

- The analysis of PAHs and PCBs in the tissue samples (muscle and liver) of select fish species expected to represent various trophic levels is completed. The species selected for analysis include hardhead catfish (*Ariopsis felis*), spotted seatrout (*Cynoscion nebulosus*),

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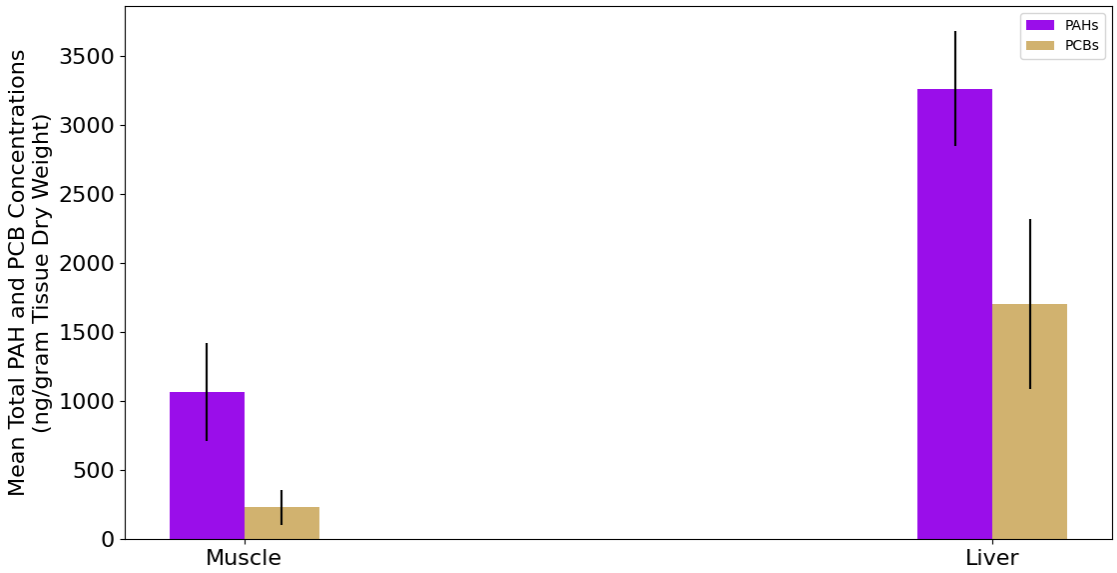
flathead grey mullet (*Mugil cephalus*), and red drum (*Sciaenops ocellatus*). The mean total PAH levels in liver were higher than those in the muscle of fish (~50 – 95%) (**Fig. 5**). Whereas PCB levels were 40 – 97% higher in the liver vs. muscle of fish (**Fig. 5**). Overall, PAH levels were 5x higher than PCB levels in muscle, and 2x higher than PCB levels in the livers of fish. The higher pollutant body-burdens in the liver tissue are not surprising given the lipophilic nature of the pollutants and the ~2x – 10x higher lipid content of the



liver vs. muscle.

Fig. 5. Mean total PAH and PCB levels in the muscle and liver tissue of the fish sampled from Matagorda Bay. Data is shown as mean ± standard errors.

- A closer examination of the individual PAH congeners indicates a predominance of the low molecular weight petrogenic PAHs (i.e., mostly oil-derived vs. combustion-derived), naphthalene (NAP), acenaphthene (ACE), fluorene (FLU), in the muscle and liver tissues of fish (**Table 3**) (**Fig. 6(a)** and **(b)**). However, the high molecular pyrogenic PAH, and indeno[1,2,3-cd]pyrene (IcdP), was also evident only in the muscle tissue of spotted seatrout, flathead grey mullet, and red drum (**Fig. 6(a)**).

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- The high bioaccumulation of the low molecular weight PAHs is likely due to their greater bioavailability, which is a consequence of their higher water solubility (Djomo et al., 1996). However, it is unclear whether the high bioaccumulation of IcdP is indicative of trophic transfer. The overall predominance of low molecular PAHs, such as NAP, ACE, and FLU in fish tissues indicates exposure to mostly petrogenic PAHs in Matagorda Bay (Wolska et al., 2012).

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Table 3. Concentrations of individual PAHs and PCBs measured in the muscle and liver tissue of fish from Matagorda Bay. Levels are shown as average ng/gram tissue dry weight ± standard error. (- indicates values below limits of detection and therefore not reported).

	Hardhead Catfish		Spotted Seatrout		Flathead Grey Mullet		Red Drum	
Compounds: PAHs	**Muscle (n=10)**	**Liver (n=10)**	**Muscle (n=9)**	**Liver (n=3)**	**Muscle (n=13)**	**Liver (n=13)**	**Muscle (n=7)**	**Liver (n=8)**
Naphthalene (NAP)	1346.6 ±586.7	2190.6 ±967.2	2.3 ±2.3	823.8 ±140.0	334.9 ±163.8	516.0 ±163.7	-	1522.4 ±335.8
Acenaphthene (ACE)	12.3 ±2.5	95.0 ±16.5	15.8 ±3.0	431.4 ±135.5	21.7 ±4.1	76.3 ±11.1	6.5 ±0.7	581.2 ±168.5
Fluorene (FLU)	9.0 ±1.4	307.0 ±108.2	8.3 ±4.0	2287.9 ±1498.6	18.8 ±6.1	1528.1 ±588.8	1.8 ±1.3	1876.7 ±760.9
Phenanthrene (PHE)	23.1 ±5.2	23.6 ±9.3	44.9 ±20.7	47.3 ±24.0	64.1 ±19.7	47.3 ±11.5	11.5 ±1.1	45.7 ±11.8
Anthracene (ANT)	14.4 ±3.2	43.6 ±4.0	8.1 ±3.1	3.5 ±1.8	42.3 ±12.9	38.4 ±8.4	3.8 ±1.4	22.7 ±9.1
Fluoranthene (FLT)	28.0 ±5.1	52.9 ±12.5	9.6 ±5.8	0.8 ±0.8	20.9 ±2.8	-	2.2 ±2.2	2.6 ±1.2
Pyrene (PYR)	31.1 ±5.2	41.7 ±10.1	22.7 ±3.6	19.6 ±5.7	26.2 ±5.8	23.4 ±6.2	6.6 ±1.7	15.8 ±3.8
Chrysene (CHR)	0.9 ±0.6	34.4 ±14.0	13.1 ±9.4	2.4 ±2.4	9.3 ±3.6	22.5 ±12.6	19.1 ±15.2	26.8 ±14.8
Benzo[a]anthracene (BaA)	2.8	29.7	31.0	4.0	36.6	14.8	114.7	97.9

	±1.9	±12.9	±16.3	±4.0	±14.5	±8.9	±59.5	±70.2
Benzo[b]fluoranthene (BbF)	-	1.6 ±0.8	7.3 ±1.8	-	11.2 ±2.6	0.1 ±0.1	3.4 ±1.3	-
Benzo[k]fluoranthene (BkF)	0.3 ±0.3	2.2 ±0.9	8.9 ±1.7	-	8.4 ±2.9	-	1.8 ±1.2	-
Benzo[a]pyrene (BaP)	-	6.8 ±4.5	2.8 ±2.1	4.1 ±3.6	0.6 ±0.4	12.7 ±9.0	1.4 ±0.9	2.1 ±1.3
Indeno[1,2,3-cd]pyrene (IcdP)	-	21.9 ±7.8	20.2 ±2.3	11.6 ±7.5	209.1 ±30.2	40.4 ±19.6	22.7 ±1.9	7.0 ±1.9
Dibenz[a,h]anthracene (DahA)	2.6 ±0.8	15.6 ±6.1	2.6 ±1.5	-	2.6 ±1.3	10.0 ±3.9	2.5 ±0.9	1.5 ±0.8
Benzo[ghi]perylene (BghiP)	-	4.1 ±1.3	1.1 ±0.7	-	0.6 ±0.4	8.0 ±1.6	0.8 ±0.8	13.7 ±4.3
∑PAHs	**1,471.0 ±605.2**	**2,870.6 ±1,000.3**	**1,788.5 ±40.0**	**3,636.4 ±1,734.7**	**807.2 ±236.1**	**2,338.0 ±629.9**	**198.9 ±73.6**	**4,216.1 ±1,222.0**
Compounds: PCBs								
Non-ortho (dioxin like)								
PCB 77	1.0 ±1.0	26.4 ±12.3	-	16.2 ±11.8	1.9 ±0.9	32.5 ±17.5	4.1 ±3.2	37.3 ±20.1
PCB 81	1.3 ±0.7	7.6 ±4.5	-	3.0 ±1.5	2.1 ±1.4	51.4 ±26.2	7.3 ±4.1	35.5 ±23.1
PCB 126	12.8 ±8.9	173.5 ±63.1	0.6 ±0.4	-	11.0 ±8.8	0.9 ±0.9	2.8 ±2.2	2.7 ±1.8

PCB 169	1.0 ±0.3	1.5 ±1.0	2.2 ±1.1	-	-	7.7 ±2.7	0.7 ±0.7	-
Mono-ortho (dioxin like)								
PCB 105	3.5 ±1.9	22.9 ±5.2	7.4 ±4.3	17.5 ±17.5	4.8 ±2.4	361.9 ±75.0	6.0 ±2.0	71.1 ±24.2
PCB 114	1.2 ±0.8	8.9 ±4.5	2.0 ±1.0	176.7 ±84.5	4.4 ±2.6	21.7 ±6.7	3.8 ±2.1	167.9 ±51.0
PCB 118	0.7 ±0.7	8.0 ±3.8	1.7 ±0.8	19.5 ±6.2	-	21.3 ±4.8	5.7 ±4.0	53.8 ±26.3
PCB 123	0.8 ±0.8	7.2 ±3.2	1.6 ±0.8	26.3 ±22.0	17.2 ±9.9	25.3 ±7.1	2.4 ±1.8	42.6 ±25.1
PCB 156	-	0.5 ±0.5	1.1 ±1.1	1.6 ±1.6	0.4 ±0.4	17.5 ±12.9	11.5 ±7.5	8.5 ±3.6
PCB 167	-	8.2 ±5.7	4.6 ±4.6	102.2 ±22.5	-	142.3 ±78.6	-	51.3 ±18.4
PCB 189	-	0.4 ±0.4	-	-	-	0.4 ±0.4	-	6.4 ±4.8
Non-dioxin like								
PCB 1	-	40.0 ±15.9	-	44.6 ±5.6	44.5 ±14.1	42.8 ±9.8	-	34.6 ±10.9
PCB 18	2.3 ±1.4	38.8 ±11.7	31.1 ±14.6	65.1 ±33.0	11.7 ±7.0	2062.3 ±751.4	86.4 ±54.8	1106.6 ±585.2
PCB 28	11.4 ±3.1	51.4 ±22.8	3.2 ±1.6	295.9 ±246.4	1.1 ±0.8	249.3 ±102.9	16.3 ±14.1	333.2 ±137.0

PCB 33	0.5 ±0.5	7.3 ±2.8	0.8 ±0.8	5.6 ±0.7	6.1 ±2.7	19.1 ±6.4	6.6 ±3.8	28.1 ±6.4
PCB 52	-	-	0.2 ±0.2	-	-	0.1 ±0.1	1.3 ±0.6	0.3 ±0.3
PCB 95	-	17.0 ±16.4	-	-	-	1.5 ±0.8	1.3 ±0.9	18.7 ±11.9
PCB 101	-	4.1 ±2.0	-	17.0 ±9.9	3.0 ±1.5	27.5 ±8.6	2.0 ±2.0	55.4 ±16.7
PCB 149	-	-	-	-	-	6.4 ±3.8	4.4 ±3.1	6.7 ±2.9
PCB 153	-	-	0.8 ±0.8	0.3 ±0.3	1.1 ±0.6	1.5 ±0.7	0.6 ±0.6	0.5 ±0.5
PCB 138	-	-	0.5 ±0.5	9.8 ±4.0	-	26.1 ±16.2	-	1.3 ±0.8
PCB 187	-	3.3 ±2.0	0.5 ±0.5	-	-	-	-	-
PCB 183	-	2.3 ±1.7	-	-	-	-	-	0.5 ±0.5
PCB 128	1.1 ±0.7	12.1 ±10.2	7.9 ±7.9	196.0 ±39.4	0.8 ±0.5	65.2 ±54.9	0.6 ±0.6	90.7 ±35.7
PCB 177	-	-	0.5 ±0.5	-	-	-	3.4 ±2.2	0.6 ±0.6
PCB 171	-	-	-	-	-	-	3.7 ±1.9	1.6 ±1.6

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PCB 157	-	1.0 ±0.7	-	3.4 ±1.7	1.2 ±0.8	18.1 ±12.0	2.5 ±1.6	4.8 ±4.2
PCB 180	-	1.8 ±1.3	-	2.2 ±2.2	-	0.6 ±0.6	-	1.2 ±0.8
PCB 170	-	-	0.6 ±0.6	2.4 ±2.4	-	1.9 ±1.2	1.3 ±1.3	2.3 ±1.1
∑PCBs	**37.6 ±9.8**	**444.2 ±70.5**	**605.6 ±25.2**	**1,005.2 ±378.8**	**111.2 ±37.7**	**3,205.1 ±819.3**	**174.8 ±84.2**	**2,164.5 ±657.4**

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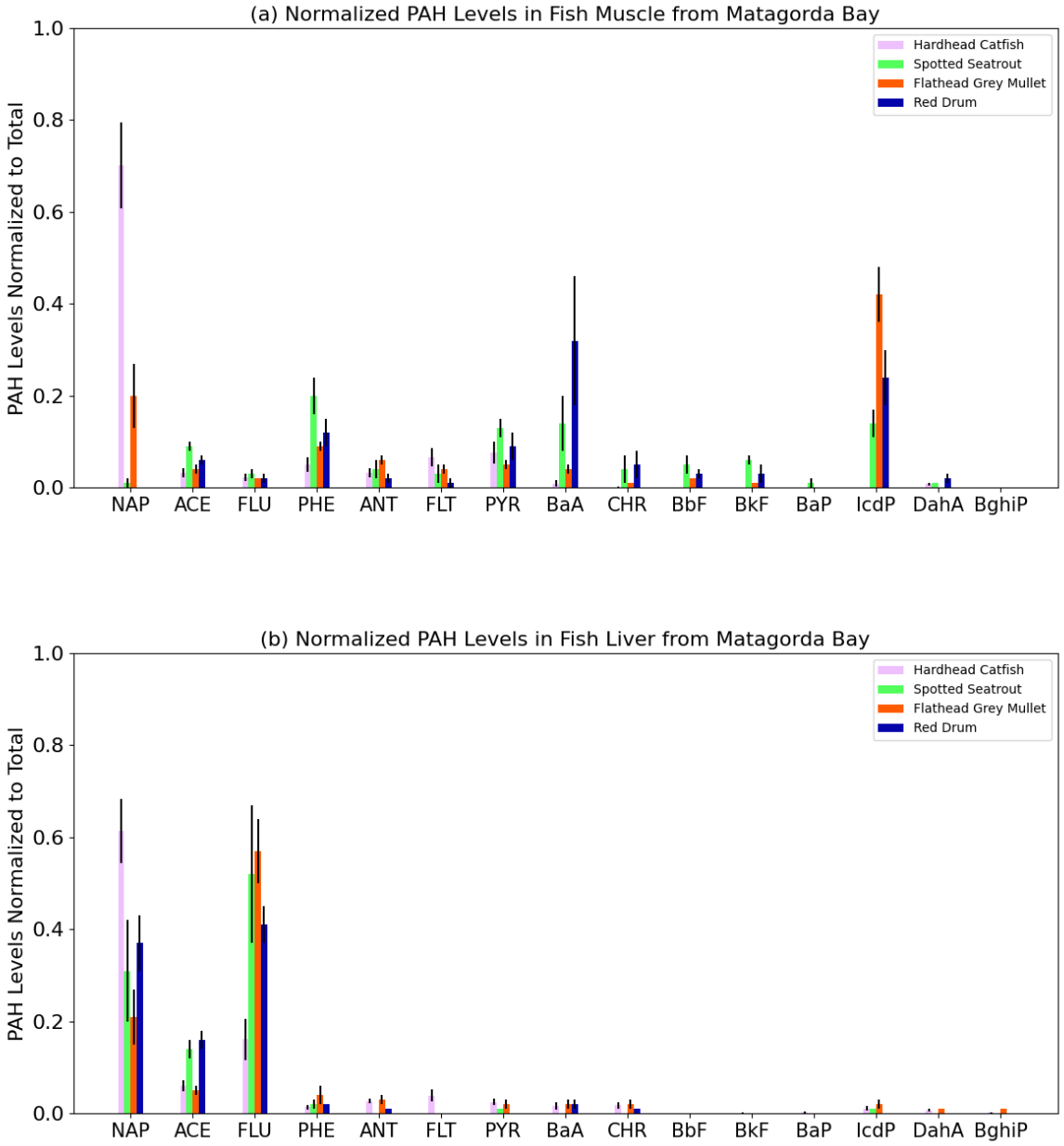

Fig. 6. The profiles of individual PAHs in **(a)** muscle, and **(b)** livers of fish from Matagorda Bay (shown as mean ± standard error). All mean levels are normalized to ΣPAH concentrations as ng/gram tissue dry weight.

- The analysis of PCB congeners indicated PCBs-18 and 28 to dominate in the muscle and liver samples of fish (**Table 3**) (**Fig. 7(a)** and **(b)**). As an exception, PCB-1 was

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predominant only in the muscle tissue flathead grey mullet (**Fig. 7(a)**), and PCB-126 was predominant only in the muscle and livers of hardhead catfish (**Fig. 7(a)** and **(b)**).

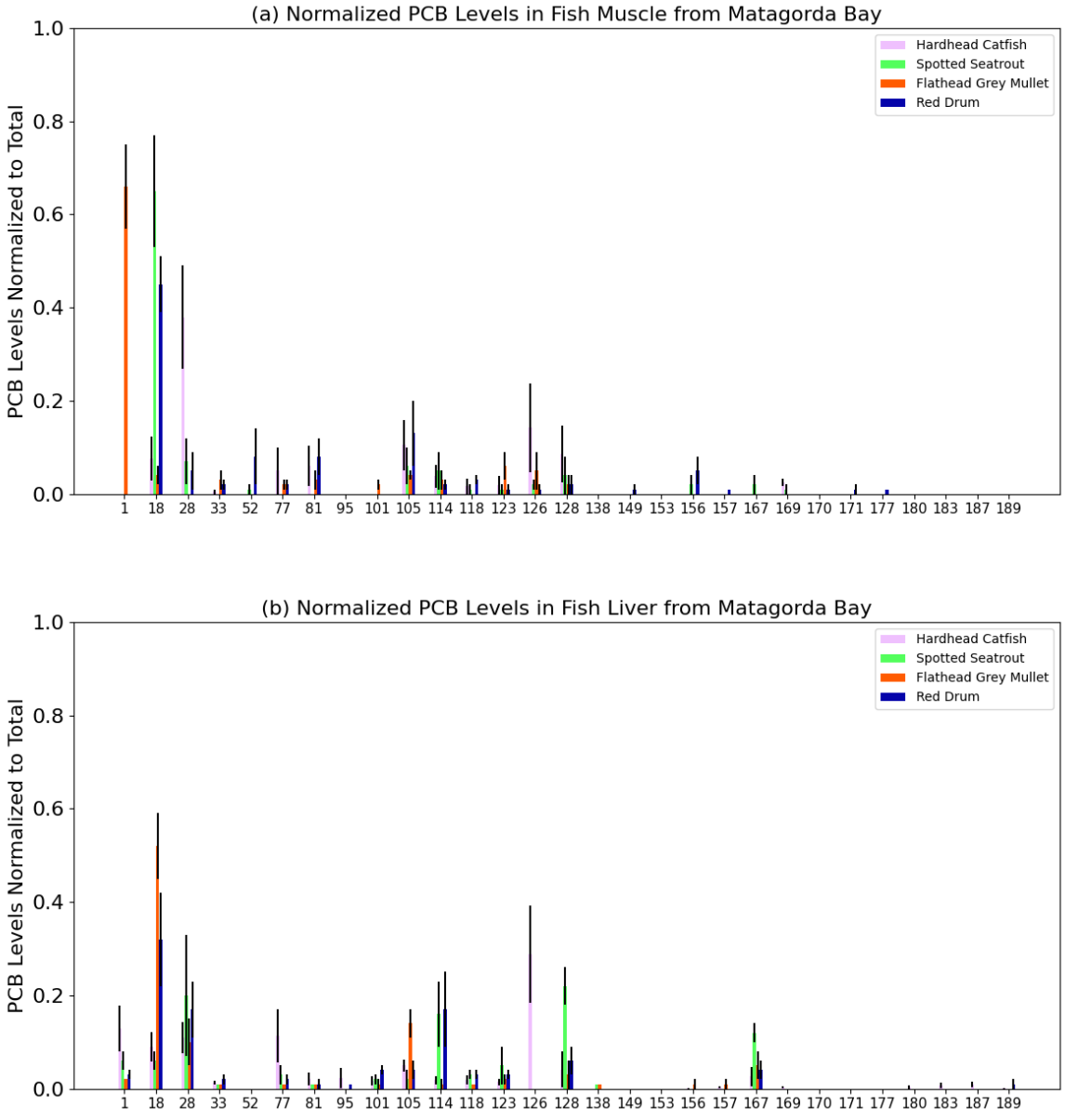

Fig. 7. The profiles of individual PCB congeners in **(a)** muscle, and **(b)** livers of fish from Matagorda Bay (shown as mean ± standard error). All mean levels are normalized to ΣPCB concentrations as ng/gram tissue dry weight.

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- In the environment, the microbial biodegradation of PCBs via anaerobic dechlorination proceeds from the preferential removal of chlorine atoms (in highly chlorinated PCB congeners) from the *meta* and *para* positions (**Fig. 8(a)**), resulting in an increase in lower chlorinated *ortho*-substituted PCB congeners (Abramowicz, 1995; Tiedje et al., 1993) (**Fig. 8(a)**). PCBs-18 and 28 appears to be a lower chlorinated PCB (three chlorines) and with a chlorine atom in the *ortho* position (**Fig. 8(b)** and **(c)**). Therefore, it may be likely that these two PCBs represent biodegraded (by anaerobic bacteria) congeners in Matagorda Bay.

- Some of the most toxic PCB congeners are those with chlorine atoms at both *para*, and at two or more *meta* positions. These include 3,4,4',5-tetra- (PCB-81), 3,3',4,4'-tetra- (PCB-77), 3,3',4,4',5-penta- (PCB-126) and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB-169). The chlorine atom substitutions on these four PCBs results in a coplanar structure (i.e. all atoms lie in the same geometric plane), which is similar to 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzodioxin), and thus are capable of inducing a similar mode of toxicity (Safe et al., 1985) (**Fig. 8(d)**).

- Therefore, the relatively high presence of PCB-126 in the muscle (12.8 $\pm$ 8.9 ng/gram dry weight) and liver tissue (173.5 $\pm$ 63.1 ng/gram dry weight) of hardhead catfish may be of concern for toxicity to the fish itself (**Table 3**), and likely exposure of humans to the dioxin-like PCB from sea food consumption. A comprehensive risk assessment of will be performed in Year 2 to assess such risk.

Objective 3: Study the toxicity of microplastics and adsorbed pollutants using embryo-larval life stages of sheepshead minnow.

- This objective will be engaged with starting in September 2022 and onwards.
- An Animal Use Protocol (AUP) to perform *in vivo* experimentation with early life-stages of embryo-larval sheepshead minnows (*Cyprinodon variegatus*) has already been approved by the A&M Institutional Animal Care and Use Committee (IACUC).

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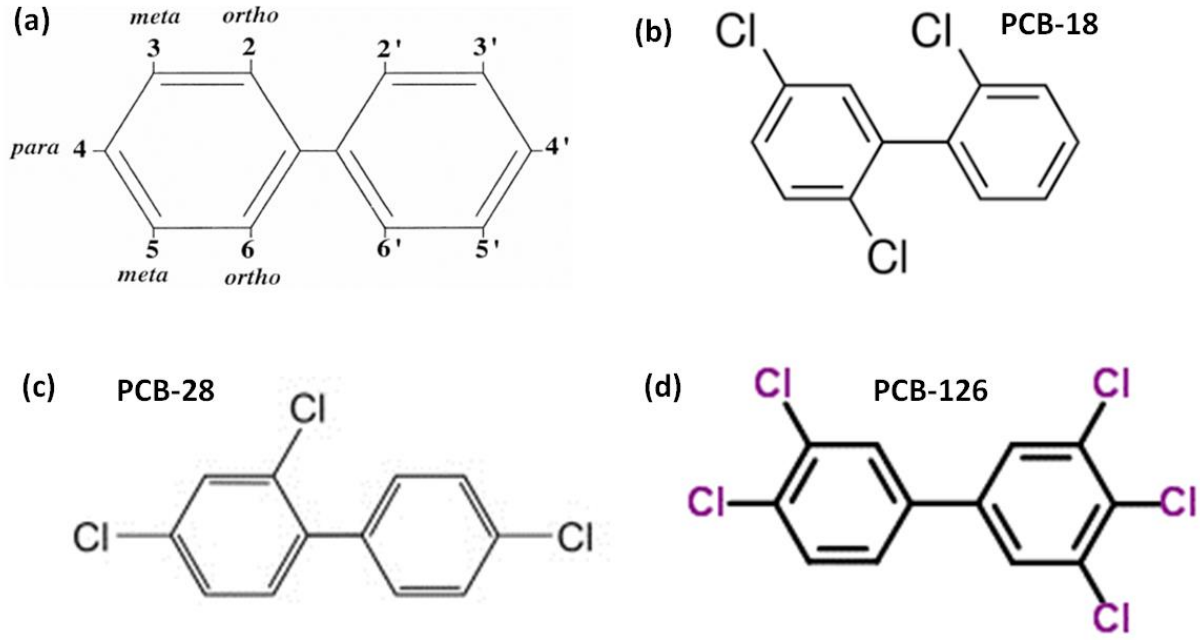

Fig. 8: The structural formula of PCB showing the numbering and locations of chlorine atoms (**a**); and chemical structures of PCB-18 **(b)**, PCB-28 (**c**), and PCB-126 (**d**). (Image of PCB structural formula is from: Anyasi and Atagana (2011)).

Objective 4: Public educational outreach to local high school students on the science of ecosystem health monitoring.

- This objective will be engaged with in summer (June/July) 2022. We have 3 events planned as part of TAMU Galveston's SeaCamp Ocean Conversation Camp course on June 14th, 28th, and July 26th. Students between the age of 14-18 will be exposed to classroom and lab-based activities describing the problems with plastic pollution and performing collection of field samples and analysis of microplastics by infrared spectroscopy.

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3. FURTHER WORK

Planned work for completion over the duration of the fifth interim report (Year 2) are as follows:

1) Complete PAH/PCB analysis for oysters from Matagorda Bay. The preparation of a manuscript describing this data will be prepared in Fall 2022.
2) Complete PAH/PCB analysis of water samples from Matagorda Bay.
3) Commence microplastics analysis in water and biota samples. The preparation of a manuscript describing the microplastics analysis methods will be prepared in Fall 2022.

Reviewed by:

5/30/2022

_____ Date: _____

Dr. David Hala, TAMUG, P.I.

Approved by:

_____ Date: 5/30/2022

Mr. Steven J. Raabe, Trustee

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