Texas A&M University at Galveston
1001 Texas Clipper Road
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FIFTH INTERIM PERFORMANCE REPORT

AUGUST 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

Principal Investigators:

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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 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 fifth interim report (June 1st, 2022 – August 31st, 2022) we provide a list of key accomplishments as per the first quarter of Year 2 of the project.

2. Key Updates

As of the period encompassing the fifth interim report (June 1st, 2022 – August 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 surface water samples and biotas (oysters, fish) has been completed and microplastics analysis of these samples is in-progress.
- At present, various sample clean-up methods are being optimized to ensure the accurate quantification of microplastics particles in the biota samples. Specifically, chemical and enzymatic digestion protocols are validated to test recovery of microplastics.

- As previously reported in the Fourth Interim report (encompassing the project duration from March 1st – May 31st, 2022), microplastics have been measured in the surface waters of Matagorda Bay and are presented in **Table 1**. The sample was collected at Halfmoon Reef, Matagorda Bay.

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Table 1. Concentrations of microplastics in surface water from Halfmoon Reef, 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
	Σ 3981 ± 147

- The microplastics levels presented in **Table 1** are approximately an order of magnitude higher than those of persistent organic pollutants, such as PAHs and PCBs, also measured in the surface waters of Matagorda Bay (**Table 2**).

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

- The levels of PAHs and PCBs measured in surface waters collected in Matagorda Bay are detailed in **Table 2**.
- The analysis of PAHs and PCBs in the gill/mantle tissue of eastern oysters and muscle/liver of fish collected from Matagorda Bay has been completed (**Table 3**).

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Table 2. Concentrations of individual PAHs and PCBs measured in the surface waters of Matagorda Bay. Levels are reported as mean ng/L ± standard error and were quantified in surface water samples collected near shorelines (i.e., beachside, dockside sites) or in the bay itself. Beachside and dockside water samples were collected from Port O'Connor, Magnolia Beach, Port Lavaca, Weedhaven, Palacios, and Wadsworth. (- indicates values below limits of detection and therefore not reported) (please see **Table 3** for the full names of PAHs matched to acronyms)

(a) PAHs	Concentrations (ng/L) in Matagorda Bay beachside and dockside water samples	Concentrations (ng/L) in Matagorda Bay surface water samples
NAP	33.5 ± 33.5	-
ACE	63.6 ± 63.6	74.9 ± 74.9
FLU	0.9 ± 0.6	-
PHE	13.8 ± 5.7	0.7 ± 0.4
ANT	-	2.8 ± 0.3
FLT	3.6 ± 3.6	0.5 ± 0.5
PYR	2.5 ± 2.0	0.1 ± 0.1
BaA	-	-
CHR	3.2 ± 1.3	-
BbF	7.5 ± 5.2	2.6 ± 1.1
BkF	-	-
BaP	2.3 ± 0.9	0.7 ± 0.5
IcdP	-	-
DahA	0.6 ± 0.6	-
BghiP	-	-
	Σ 131.4 ± 116.9	**Σ 82.3 ± 77.9**

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(b) PCBs	Concentrations (ng/L) in Matagorda Bay beachside and dockside water samples*	Concentrations (ng/L) in Matagorda Bay surface water samples#
PCB_1	1.0 ± 1.0	-
PCB_18	104.4 ± 89.8	-
PCB_28	29.4 ± 13.3	7.2 ± 5.5
PCB_33	14.3 ± 6.0	-
PCB_52	0.7 ± 0.7	3.6 ± 2.0
PCB_77	3.2 ± 2.1	4.4 ± 4.4
PCB_81	0.3 ± 0.3	1.2 ± 0.9
PCB_95	-	-
PCB_101	-	2.8 ± 2.8
PCB_105	51.8 ± 40.9	-
PCB_114	18.7 ± 11.2	6.7 ± 3.7
PCB_118	3.7 ± 2.3	3.0 ± 3.0
PCB_123	5.6 ± 3.7	1.4 ± 1.4
PCB_126	1.3 ± 0.9	-
PCB_128	2.3 ± 1.7	6.4 ± 5.0
PCB_138	2.4 ± 2.4	-
PCB_149	1.6 ± 1.2	-
PCB_153	3.9 ± 3.1	0.4 ± 0.4
PCB_156	-	-
PCB_157	0.3 ± 0.3	-
PCB_167	28.1 ± 8.5	-
PCB_169	27.8 ± 10.4	-
PCB_170	1.6 ± 0.8	0.6 ± 0.6
PCB_171	7.7 ± 3.3	2.9 ± 2.7
PCB_177	16.8 ± 2.2	9.2 ± 4.3
PCB_180	2.5 ± 1.6	1.5 ± 1.5
PCB_183	1.1 ± 1.1	-

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PCB_187	0.1 ± 0.1	1.3 ± 1.3
PCB_189	0.4 ± 0.4	-
	Σ 330.9 ± 209.1	**Σ 52.7 ± 39.8**

- An assessment of the sum total concentrations of congeners indicates overall higher levels of PAHs and PCBs at sites close to shorelines versus sampling sites within the bay (**Table 2**). A comparison of the highest levels of sum total PAH and PCB levels in Matagorda Bay versus levels previously measured in Galveston Bay by our laboratory, shows PAH levels in Galveston Bay (2131.9 ± 1731.6 ng/L) to be 16x higher than those reported in Matagorda Bay (131.4 ± 116.9 ng/L). And PCB levels to be 4x higher in Galveston Bay (1319.5 ± 485.7 ng/L) versus Matagorda Bay (330.9 ± 209.1 ng/L). Such a disparity in concentrations of PAHs and PCBs between the two northwestern Gulf of Mexico bay systems is likely a reflection of the high petrochemical and chemical industrialization of Galveston Bay.

- 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 gill/mantle of oysters; and muscle and liver tissues of fish (**Table 2** and **3**) (**Fig. 1(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. 1(a)**).

- 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 biota 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 gill/mantle of oysters; 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).

	Eastern Oysters	Hardhead Catfish		Spotted Seatrout		Flathead Grey Mullet		Red Drum	
Compounds: PAHs	**Gill/Mantle (n=10)**	**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)	65.3 ±44.1	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)	17.8 ±5.7	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)	19.5 ±11.2	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)	28.5 ±8.3	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)	1.3 ±0.5	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)	4.5 ±2.5	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)	8.5 ±2.6	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

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Benzo[a]anthracene (BaA)	114.5 ±40.5	2.8 ±1.9	29.7 ±12.9	31.0 ±16.3	4.0 ±4.0	36.6 ±14.5	14.8 ±8.9	114.7 ±59.5	97.9 ±70.2
Chrysene (CHR)	15.7 ±3.2	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[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)	1.1 ±1.0	0.3 ±0.3	2.2 ±0.9	8.9 ±1.7	-	8.4 ±2.9	-	1.8 ±1.2	-
Benzo[a]pyrene (BaP)	4.6 ±2.4	-	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)	3.0 ±1.3	-	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)	6.2 ±2.2	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)	5.3 ±1.2	-	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	**295.8 ±126.6**	**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	62.1	1.0	26.4	-	16.2	1.9	32.5	4.1	37.3

	±33.9	±1.0	±12.3		±11.8	±0.9	±17.5	±3.2	±20.1
PCB 81	17.1 ±9.8	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	1.0 ±1.0	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 **Mono-ortho (dioxin like)**	-	1.0 ±0.3	1.5 ±1.0	2.2 ±1.1	-	-	7.7 ±2.7	0.7 ±0.7	-
PCB 105	3.2 ±1.4	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	26.8 ±12.8	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	4.7 ±1.9	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	3.6 ±1.2	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	5.9 ±2.0	-	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	2.7 ±1.5	-	8.2 ±5.7	4.6 ±4.6	102.2 ±22.5	-	142.3 ±78.6	-	51.3 ±18.4
PCB 189	0.3	-	0.4	-	-	-	0.4	-	6.4

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	±0.3		±0.4				±0.4		±4.8
Non-dioxin like									
PCB 1	4.7 ±1.8	-	40.0 ±15.9	-	44.6 ±5.6	44.5 ±14.1	42.8 ±9.8	-	34.6 ±10.9
PCB 18	109.2 ±29.5	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	4.1 ±2.3	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	36.6 ±12.2	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	0.3 ±0.3	-	-	0.2 ±0.2	-	-	0.1 ±0.1	1.3 ±0.6	0.3 ±0.3
PCB 95	1.2 ±0.8	-	17.0 ±16.4	-	-	-	1.5 ±0.8	1.3 ±0.9	18.7 ±11.9
PCB 101	3.0 ±1.2	-	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	0.9 ±0.5	-	-	-	-	-	6.4 ±3.8	4.4 ±3.1	6.7 ±2.9
PCB 153	0.3 ±0.2	-	-	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	0.9 ±0.5	-	-	0.5 ±0.5	9.8 ±4.0	-	26.1 ±16.2	-	1.3 ±0.8

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PCB 187	0.5 ±0.5	-	3.3 ±2.0	0.5 ±0.5	-	-	-	-	-
PCB 183	0.4 ±0.4	-	2.3 ±1.7	-	-	-	-	-	0.5 ±0.5
PCB 128	1.9 ±1.3	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	0.8 ±0.8	-	-	-	-	-	-	3.7 ±1.9	1.6 ±1.6
PCB 157	0.9 ±0.5	-	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	1.0 ±0.5	-	1.8 ±1.3	-	2.2 ±2.2	-	0.6 ±0.6	-	1.2 ±0.8
PCB 170	0.3 ±0.3	-	-	0.6 ±0.6	2.4 ±2.4	-	1.9 ±1.2	1.3 ±1.3	2.3 ±1.1
∑PCBs	**294.4 ± 119.4**	**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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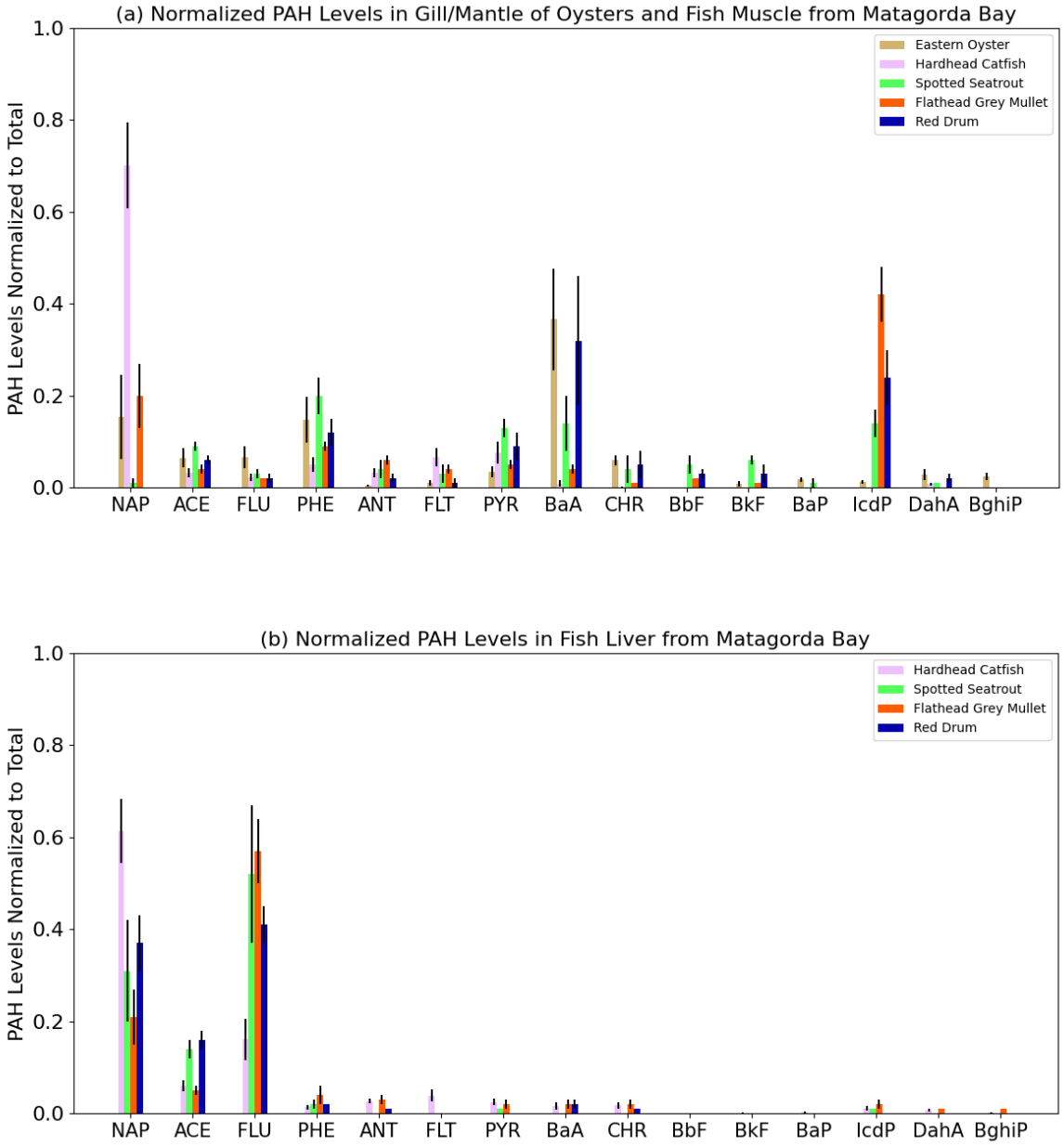

Fig. 1. The profiles of individual PAHs in **(a)** gill/mantle of oysters and muscle of fish, and **(b)** livers of fish from Matagorda Bay (shown as mean ± standard error). All mean levels are normalized to ΣPAH concentrations as ng/gram tissue dry weight.

- The analysis of PCB congeners indicated PCBs-18 and 28 to dominate in all (**Table 3**) (**Fig. 2 (a)** and **(b)**). As an exception, PCB-1 was predominant only in the muscle tissue

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flathead grey mullet (**Fig. 2 (a)**), and PCB-126 was predominant only in the muscle and livers of hardhead catfish (**Fig. 2 (a)** and **(b)**).

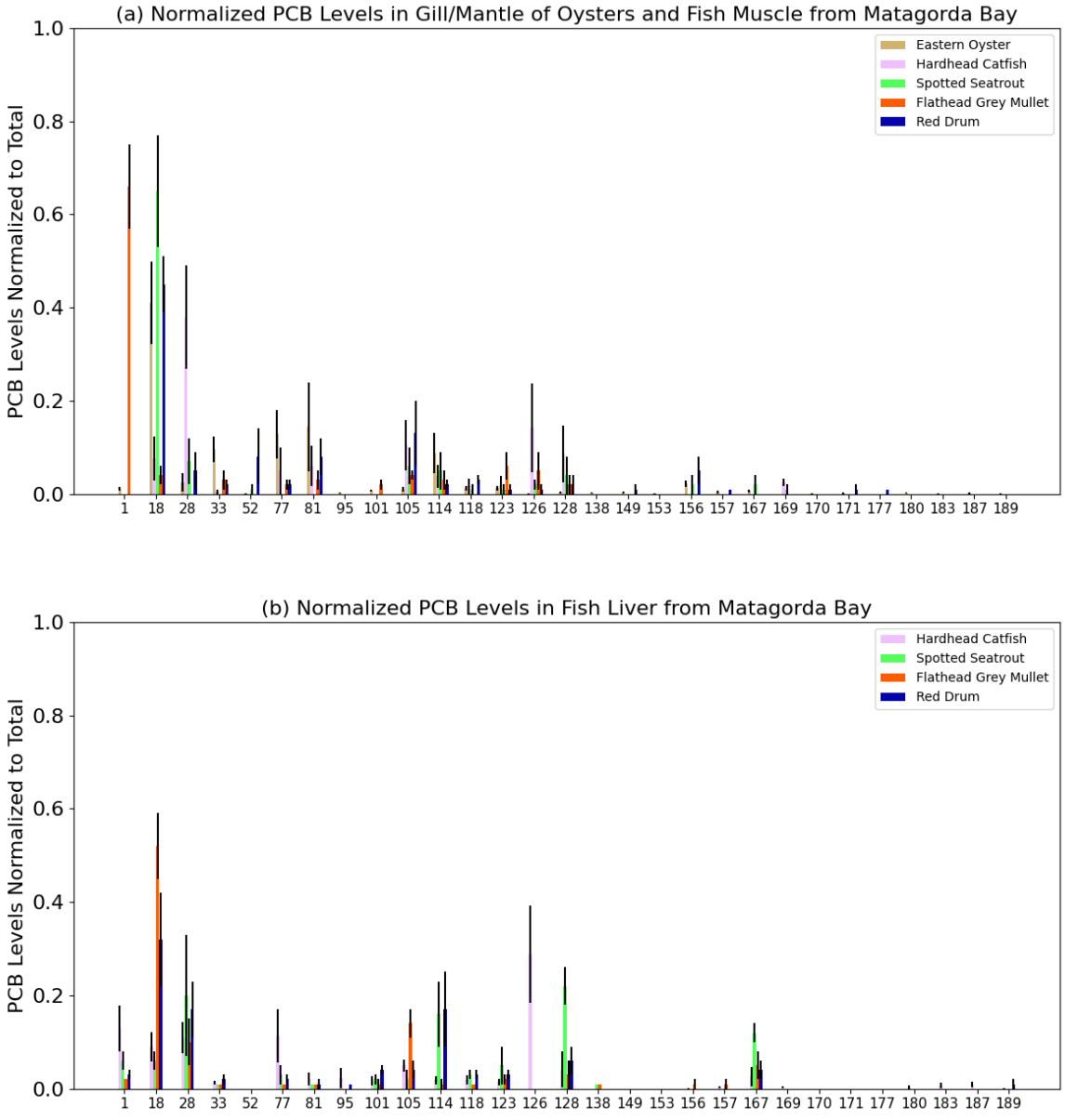

Fig. 2. The profiles of individual PCB congeners in **(a)** gill/mantle of oysters and muscle of fish, and **(b)** livers of fish from Matagorda Bay (shown as mean ± standard error). All mean levels are normalized to ΣPCB concentrations as ng/gram tissue dry weight.

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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. 3 (a)**), resulting in an increase in lower chlorinated *ortho*-substituted PCB congeners (Abramowicz, 1995; Tiedje et al., 1993) (**Fig. 3 (a)**). PCBs-18 and 28 appears to be a lower chlorinated PCB (three chlorines) and with a chlorine atom in the *ortho* position (**Fig. 3 (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. 3 (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 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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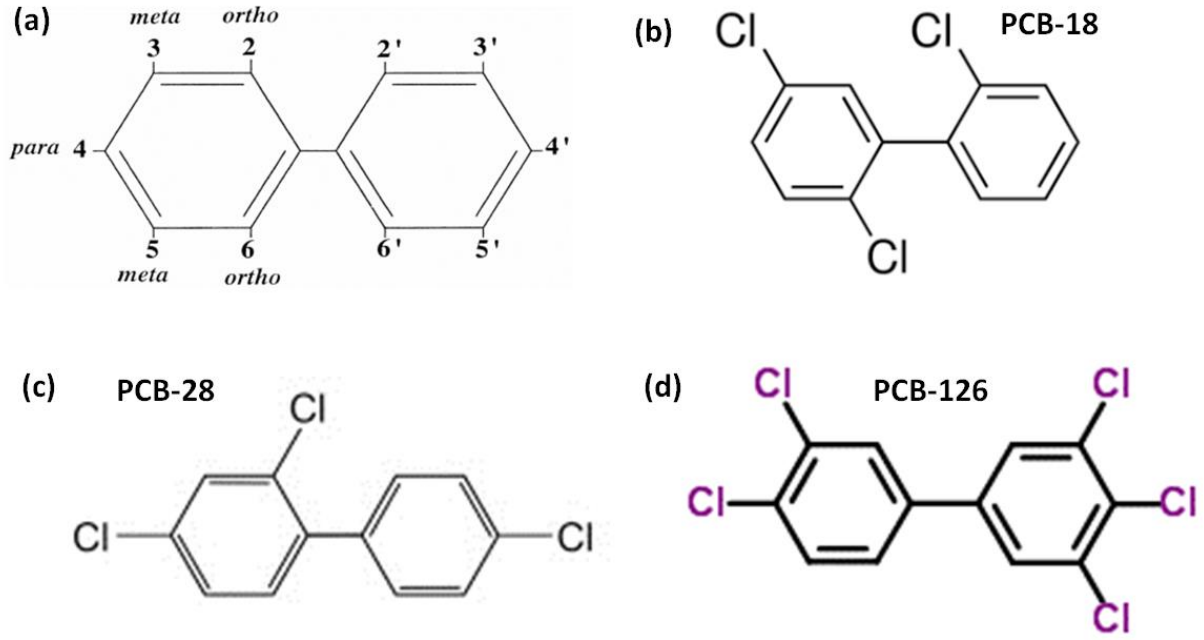

Fig. 3. The structural formula of PCB showing the numbering and locations of chlorine atoms (**a**); and chemical structures of PCB-18 **(b)**, PCB-28 (**c**), and PCB-126 (**d**). (Image of PCB structural formula is from: Anyasi and Atagana (2011)).

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

- Public educational outreach was engaged with on July 27th, 2022. Twenty-four high school senior students attending the summer TAMUG Sea Camp program participated in a hands-on Toxicology laboratory experiment led by Dr. David Hala and Mr. Asif Mortuza (graduate student recruited on the MBMT project) (**Fig. 4**). The lab involved generating a dose-response curve of daphnia (*Daphnia magna*) immobility versus increasing saline concentrations. The purpose of this lab was to demonstrate how to experimentally determine safe versus adverse levels of a chemical, given its exposure effects on an observable biological endpoint (such as mobility in daphnia).

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- Three outreach activities were performed on plastics in coastal and marine environments as part of the TAMUG Sea Camp program. The effort was led by Dr. Karl Kaiser, Marcus Wharton (graduate student recruited on the MBMT project), Emily Summers, and Katie Miller.

  Dates of the activities were:

  Ocean Conservation Camp: June 14th

  Ocean Careers Awareness: June 27th

  Marine Science Research July 27th

  Activities were structured to give students an overview of current issues with plastics and microplastics in coastal and marine environments and demonstrate collection and analysis of microplastics in various samples collected along Galveston beaches. Students learned to use simply analytical techniques to identify plastics such as infrared spectroscopy. At the end of the class, students visited the PI's research lab, and they were given a short introduction on the state-of-the-art analysis of microplastics in biological tissues by pyrolysis-gas chromatography mass spectrometry.

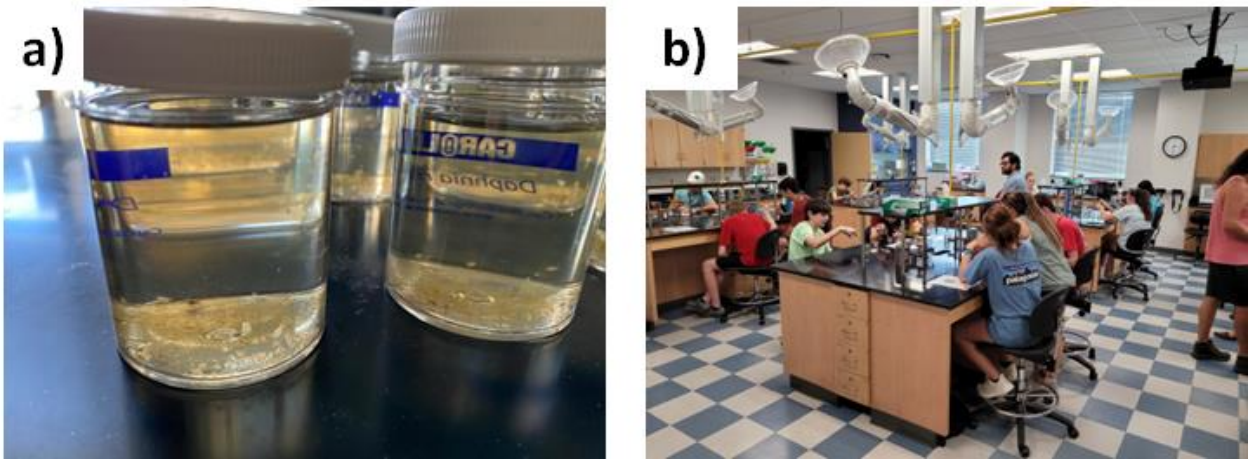

Fig. 4. Images showing the Toxicology lab performed as part of educational outreach on July 27th, 2022. Senior high school students (from various regional schools) participated in a hands-on lab to study how dose-response assessments are used to assess the safety of chemicals. The lab utilized live daphnia **(a)** and involved twenty-four students in a hands-on lab **(b)**.

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3. FURTHER WORK

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

1) Prepare a manuscript for publication in Fall 2022 on the PAH and PCB data generated as part of the research performed in this project.
2) Commence microplastics analysis in water and biota samples. The preparation of a manuscript describing the microplastics analysis methods will be prepared in Fall 2022.
3) Plan the initiation of toxicological studies on the effects of microplastics and PAH/PCB mixtures on embryo-larval life stages of fish.

Reviewed by:

8/30/2022

_____ Date: _____

Dr. David Hala, TAMUG, P.I.

Approved by:

_____ Date: 8/31/2022

Mr. Steven J. Raabe, Trustee

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