Contract# 0013
Matagorda Bay Mitigation Trust

Texas A&M University at Galveston
1001 Texas Clipper Road
Galveston, TX 77554

THIRD INTERIM PERFORMANCE REPORT

FEBRUARY 28TH, 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

Authors:

Ms. Emily Meese (Ph.D. student)
Mr. Asif Mortuza (Ph.D. student)
Mr. Marcus Wharton (Ph.D. student)
Dr. David Hala, Ph.D.
Dr. Karl Kaiser, Ph.D.
Dr. David Wells, Ph.D.
Dr. Lene H. Petersen, Ph.D.
Dr. Antonietta Quigg, Ph.D.

Texas A&M University at Galveston
1001 Texas Clipper Road
Galveston, TX 77554

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

Personnel

Principal Investigator(s):

Drs. David Hala, Karl Kaiser, David Wells, Lene H. Petersen, Antonietta Quigg

Consulting MBMT Project Coordinator:

Mr. Steven J. Raabe

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.

Texas A&M University at Galveston
1001 Texas Clipper Road
Galveston, TX 77554

1. INTRODUCTION

1.1 Background

The pollution of the Matagorda Bay system by microplastics particles released from the Formosa Plastics Corporation (as recorded from 2016-2018) has caused concern for the widespread exposure of resident biota (shellfish and fish) (Conkle, 2018; Wilson, 2018). Microplastics (i.e., particles <5 mm in diameter) can also act as important carriers of pollutants in the marine environment. The ingestion of such tainted plastic particles by aquatic organisms can lead to the increased exposure and body-burdens (or bioaccumulation) of persistent organic pollutants (Hirai et al., 2011; Hüffer and Hofmann, 2016), and contribute to the toxicity of the ingested particles (Vázquez and Rahman, 2021).

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 third quarterly interim report (December 1st, 2021 – February 28th, 2022) we provide a list of key updates as accomplished to date.

2. Key Updates

As of the period encompassing the third interim report (December 1st, 2021 – February 28th, 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 biota (oysters, fish) and water samples from Matagorda Bay has continued. As of current evaluation, the numbers of organisms sampled from Matagorda Bay are listed in **Table 1**.

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).

Common Name	Scientific Name	Numbers Sampled
Gulf menhaden	*Brevoortia patronus*	44
Red drum	*Sciaenops ocellatus*	7
Black drum	*Pogonias cromis*	9
Hardhead catfish	*Ariopsis felis*	77
Flathead grey mullet	*Mugil cephalus*	78
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*	7
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 =	**297**

- The GCMS-pyrolysis method being developed to detect microplastics in the surface waters and biota of Matagorda Bay was recently updated to include phthalate plasticizers, which are commonly used as additives in plastics, and therefore can co-occur with microplastics in the environment and exposed biota. Furthermore, phthalates and have well characterized toxicities (NRC, 2008). The phthalates included were: bis(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl) phthalate (MEHP), benzyl butyl phthalate (BBP), dicyclohexyl phthalate (DCHP), and dibutyl phthalate (DBP). These phthalates were chosen for inclusion in the GCMS-pyrolysis method as they are shortlisted in the Environmental Protection Agencies (EPAs) high-priority chemical contaminants of interest list (EPA, 2020).
- Furthermore, the measurement of these phthalates (if detected) in biota samples from Matagorda Bay will provide an avenue for wildlife and human risk assessment. For example, phthalate body-burdens in biota can be compared against known toxicological effect concentrations, and human exposure can be calculated given knowledge of an

approximate daily intake of sea food. Therefore, the approximated human body-burdens of phthalates can then be compared against reference toxicity doses as determines from rodent toxicity studies, allowing an estimation of the likely hazard of adverse human health effects given an estimated extent of sea food consumption from Matagorda Bay.

- The updated GCMS-pyrolysis analytical method can monitor all common plastics (**Fig. 1**) and phthalate plasticizers (**Fig. 2**).

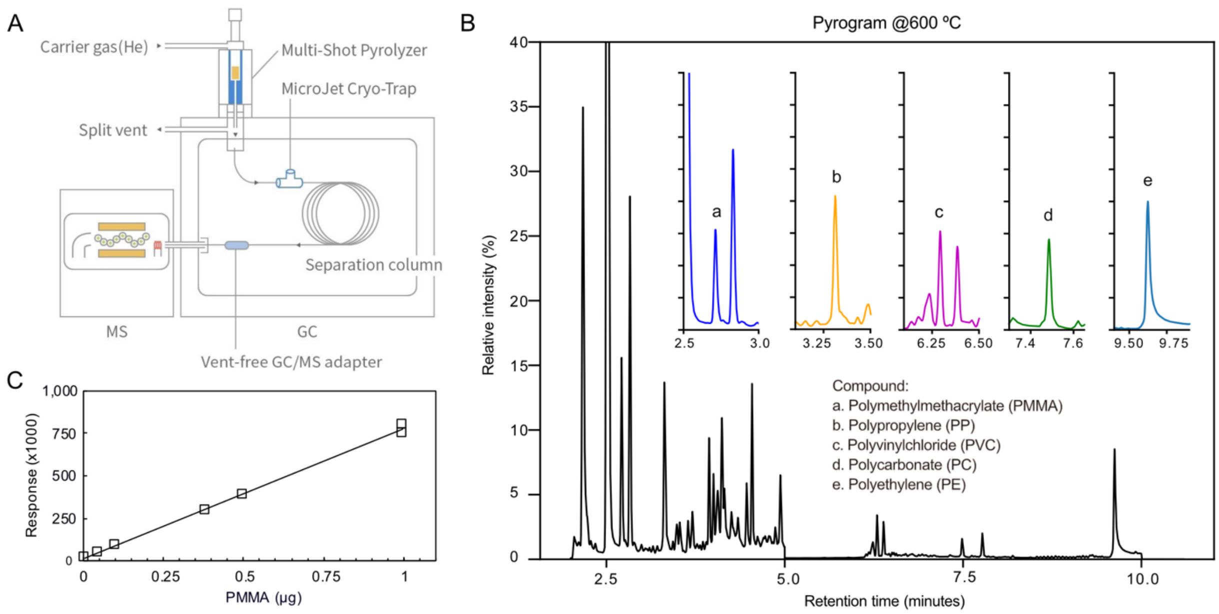

Fig. 1: **(A)** Configuration of the pyrolysis GC-MS/MS system. **(B)** Pyrogram of an oyster sample from Galveston Bay. The oyster was treated with 10% KOH and filtered onto 450 nm mixed cellulose membrane filters. Filters were dissolved in acetone, and an aliquot representing 1% of the oyster digest was used for analysis. **(C)** Calibration curve for PMMA for quantification. Separate calibration curves for each plastic type are constructed using indicator ions of characteristic pyrolysis products.

Phthalate plasticisers are detected and quantified based on their characteristic pyrolyzate product phthalic anhydride (**Fig. 2**).

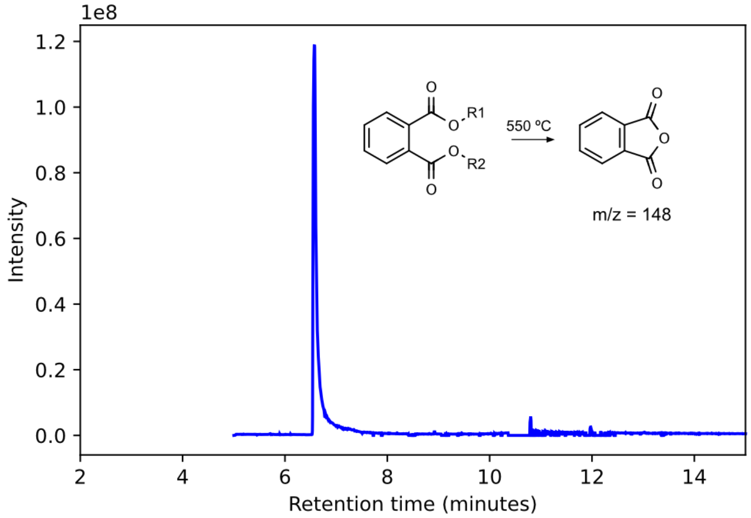

Fig. 2. Pyrogram of phthalate plasticizers. Pyrolysis converts common phthalate esters into phthalic anhydride (m/z = 148) ,that can be identified and quantified parallel to common plastics.

- To date, 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. 2**). 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. 2**). 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.

Texas A&M University at Galveston
1001 Texas Clipper Road
Galveston, TX 77554

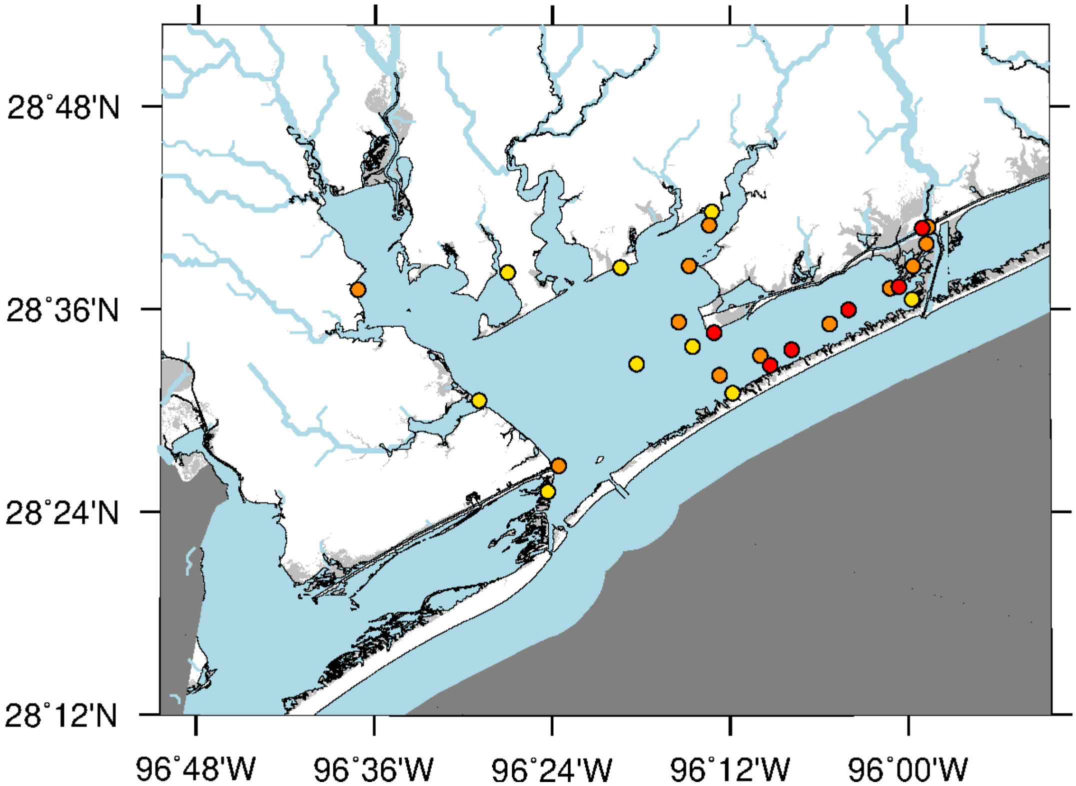

Fig. 3. Map of Matagorda Bay showing the various sites from which water samples have been collected over the past six months of the project duration (Year 1).

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

- 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**).

Texas A&M University at Galveston
1001 Texas Clipper Road
Galveston, TX 77554

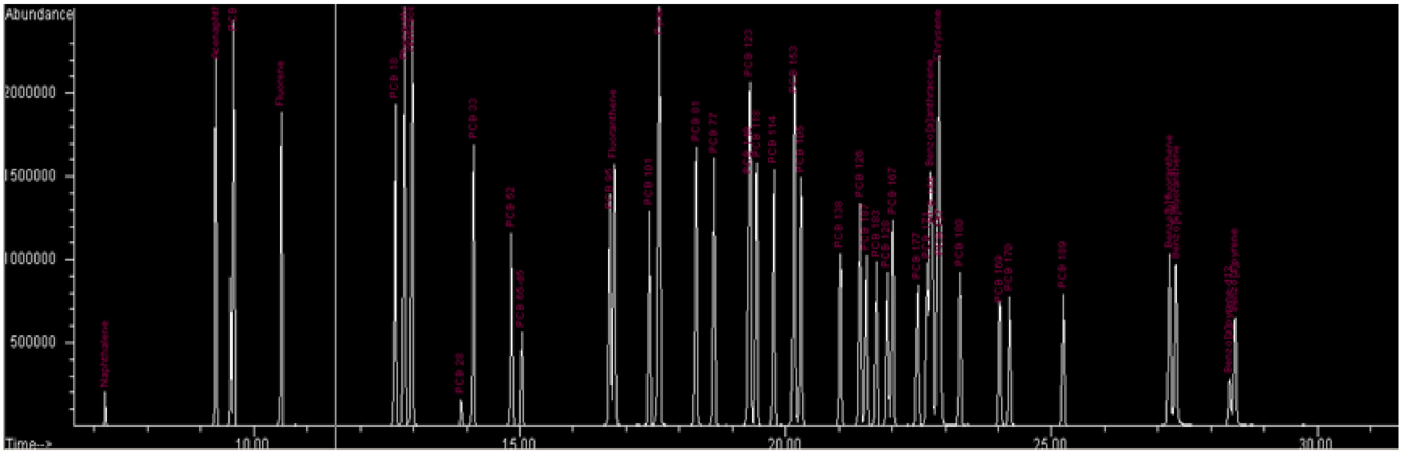

Fig. 4. A chromatograph of PAHs and PCBs as measured using GCMS. A total of 15 PAHs and 29 PCB congeners (all EPA priority pollutants) are being quantified. The 15 PAHs include: naphthalene (NAP), acenaphthene (ACE), fluorene (FLU), anthracene (ANT), phenanthrene (PHE), fluoranthene (FLT), chrysene (CHR), 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 have been completed. Blank samples (i.e., not containing biological samples) were spiked with select PAHs (Benzo[a]pyrene, pyrene) and PCBs (PCB 18, PCB 101). The ASE extraction method and subsequent sample processing method showed a recovery of 72% for Benzo[a]pyrene, 68% for Pyrene, 32% for PCB 18 and 93% for PCB 101. When the PAHs and PCBs were spiked into liver tissue homogenates from fish and subjected to ASE extraction and lipid removal, the recovery was: 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 fish tissue samples (muscle and liver) has commenced. The preliminary analyses of hardhead catfish (*Ariopsis felis*) samples collected on May, June, July, August, and September 2021 (n=2 per time point, n=10 total), indicates a predominance of PAHs vs. PCBs in muscle and liver tissue of catfish (**Fig. 5**). Overall, sum PAH and PCB levels in liver were higher than those in the muscle of fish (2x and 7x respectively) (**Fig. 5**). The higher pollutant body-burdens in the liver tissue are not surprising given the lipophilic nature of the pollutants and the nearly 2.5x higher lipid content of the liver (50.5 ± 5.9 mg lipid) vs. muscle (20.2 ± 5.3 mg lipid).

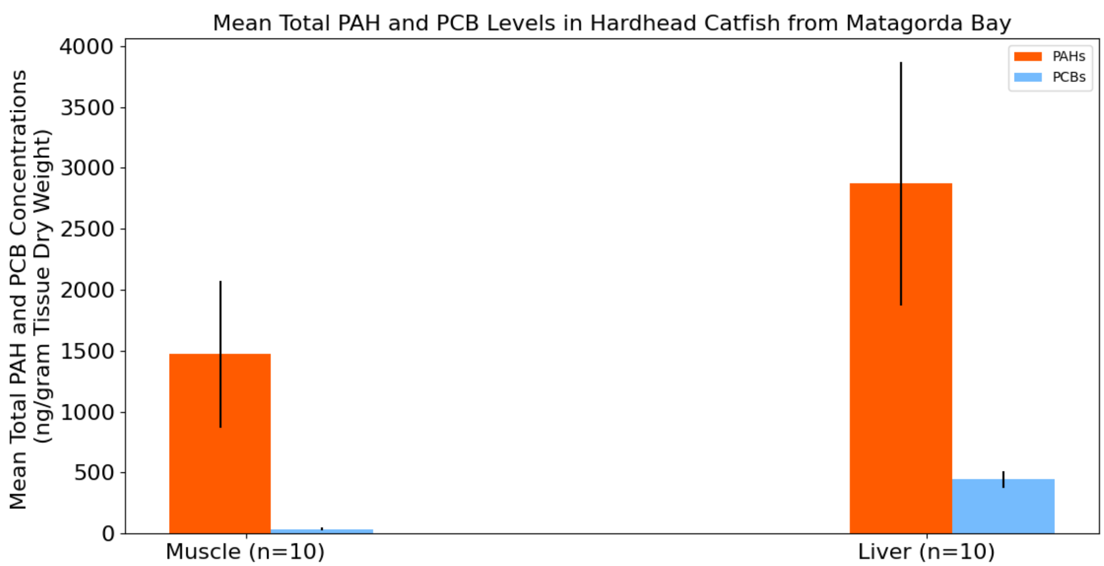

Fig. 5. Mean total PAH and PCB levels in the muscle and liver tissue of hardhead catfish (*Ariopsis felis*) sampled from Matagorda Bay between May and September 2021.

- A closer examination of the individual PAH congeners indicates a predominance of low molecular weight petrogenic PAHs (i.e., mostly oil-derived vs. combustion-derived) (**Table 2**) (**Fig. 6(a)**). The predominance of the low molecular weight PAH, naphthalene (NAP), at approximately 60-70% of total PAHs in liver and muscle tissues (**Fig. 6(a)**). Such high bioaccumulation of NAP is likely due to its greater bioavailability, which is a consequence of its higher water solubility (Djomo et al., 1996). The overall predominance

Texas A&M University at Galveston
1001 Texas Clipper Road
Galveston, TX 77554

of low molecular PAHs, such as naphthalene (NAP) and fluorene (FLU) in the hardhead catfish indicates exposure to petrogenic PAHs in Matagorda Bay (Wolska et al., 2012).

Table 2. Concentrations of individual PAHs and PCB congeners measured in the muscle and liver tissue of hardhead catfish from Matagorda Bay. Levels are shown as average ng/gram tissue dry weight ± standard error.

Compounds: PAHs	**Muscle (n = 10)**	**Liver (n = 10)**
Naphthalene (NAP)	1346.6±586.7	2190.6±967.2
Acenaphthene (ACE)	12.3±2.5	95.0±16.5
Fluorene (FLU)	9.0±1.4	307.0±108.2
Phenanthrene (PHE)	23.1±5.2	23.6±9.3
Anthracene (ANT)	14.4±3.2	43.6±4.0
Fluoranthene (FLT)	28.0±5.1	52.9±12.5
Pyrene (PYR)	31.1±5.2	41.7±10.1
Chrysene (CHR)	0.9±0.6	34.4±14.0
Benzo[a]anthracene (BaA)	2.8±1.9	29.7±12.9
Benzo[b]fluoranthene (BbF)	-	1.6±0.8
Benzo[k]fluoranthene (BkF)	0.3±0.3	2.2±0.9
Benzo[a]pyrene (BaP)	-	6.8±4.5
Indeno[1,2,3-cd]pyrene (IcdP)	-	21.9±7.8
Dibenz[a,h]anthracene (DahA)	2.6±0.8	15.6±6.1
Benzo[ghi]perylene (BghiP)	-	4.1±1.3
∑PAHs	**1,471.0±605.2**	**2,870.6±1000.3**
Compounds: PCBs	Muscle (n = 10)	Liver (n = 10)
Non-ortho (dioxin like)		
PCB 77	1.0±1.0	26.4±12.3
PCB 81	1.3±0.7	7.6±4.5
PCB 126	12.8±8.9	173.5±63.1
PCB 169	1.0±0.3	1.5±1.0
Mono-ortho (dioxin like)		

Texas A&M University at Galveston
1001 Texas Clipper Road
Galveston, TX 77554

PCB 105	3.5±1.9	22.9±5.2
PCB 114	1.2±0.8	8.9±4.5
PCB 118	0.7±0.7	8.0±3.8
PCB 123	0.8±0.8	7.2±3.2
PCB 156	-	0.5±0.5
PCB 167	-	8.2±5.7
PCB 189	-	0.4±0.4
Non-dioxin like		
PCB 1	-	40.0±15.9
PCB 18	2.3±1.4	38.8±11.7
PCB 28	11.4±3.1	51.4±22.8
PCB 33	0.5±0.5	7.3±2.8
PCB 52	-	-
PCB 95	-	17.0±16.4
PCB 101	-	4.1±2.0
PCB 149	-	-
PCB 153	-	-
PCB 138	-	-
PCB 187	-	3.3±2.0
PCB 183	-	2.3±1.7
PCB 128	1.1±0.7	12.1±10.2
PCB 177	-	-
PCB 171	-	-
PCB 157	-	1.0±0.7
PCB 180	-	1.8±1.3
PCB 170	-	-
∑PCBs	**37.6±9.8**	**444.2±70.5**

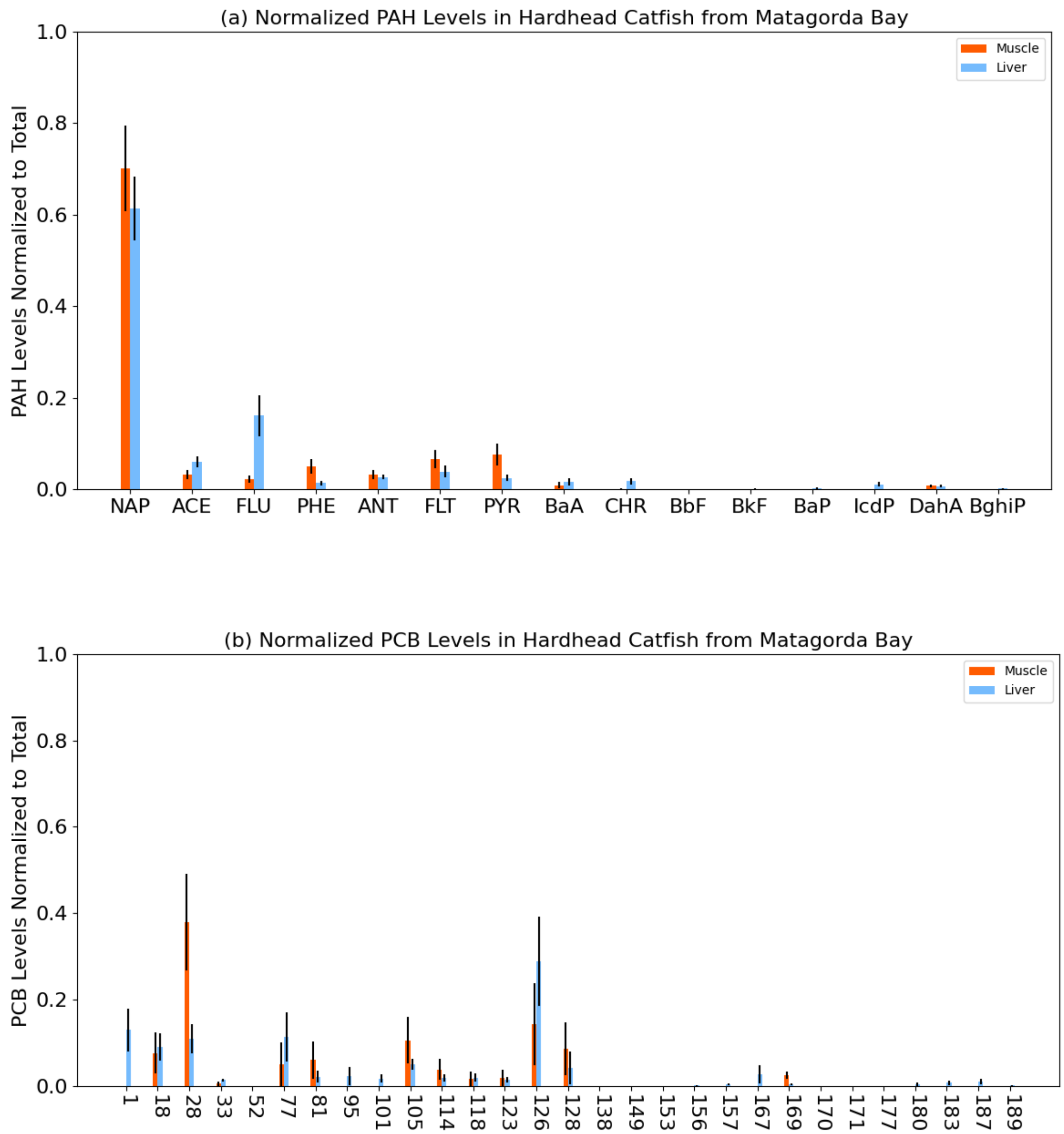

Fig. 6. The profiles of individual **(a)** PAHs, and **(b)** PCB congeners in the muscle and livers of hardhead catfish from Matagorda Bay (mean ± standard error). All mean levels are normalized to ΣPAH and ΣPCB concentrations as ng/gram tissue dry weight.

- The analysis of PCB congeners indicated PCBs 28 and 126 to dominate in the muscle and liver samples of hardhead catfish (**Table 2**) (**Fig. 6(b)**). PCB-28 comprised 11-38% of the total congeners in liver and muscle tissue respectively, whereas PCB-126 comprised 14-29% in the muscle and liver respectively (**Fig. 6(b)**).

- 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. 7(a)**), resulting in an increase in lower chlorinated *ortho*-substituted PCB congeners (Abramowicz, 1995; Tiedje et al., 1993) (**Fig. 7(a)**). PCB-28 appears to be a lower chlorinated PCB (three chlorines) and with a chlorine atom in the *ortho* position (**Fig. 7(b)**). Therefore, it may be likely that PCB-28 represents a biodegraded (by anaerobic bacteria) congener 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, and thus are capable of inducing a similar mode of toxicity (Safe et al., 1985) (**Fig. 7(c)**).
- 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 2**), and likely exposure of humans to the dioxin-like PCB from sea food consumption. A comprehensive risk assessment of will be performed upon the collection of a comprehensive dataset of PCB levels in the various fish species sampled from Matagorda Bay.

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 June 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 Texas A&M University's Institutional Animal Care and Use Committee (IACUC).

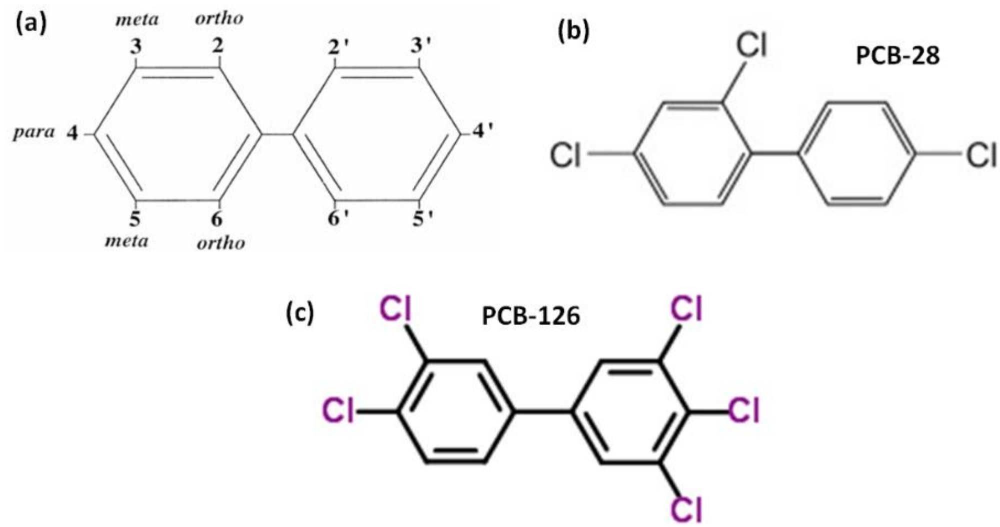

Fig. 7: The structural formula of PCB showing the numbering and locations of chlorine atoms (**a**); and chemical structures of PCB-28 (**b**) and PCB-126 (**c**). (Image of PCB structural formula is from: Anyasi and Atagana (2011)).

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.
- At present, an educational module that involves hands-on learning by students, and includes the assessment of various pollution sources into a Gulf of Mexico estuary (and the complexity associated with their mitigation), has been approved by the Director of Outreach for Texas A&M University at Galveston's Sea Camp Program, Ms. Daisy Dailey.

3. FURTHER WORK

Planned work for completion over the duration of the fourth interim report are as follows:

1) Continue to collect biota and water samples from Matagorda Bay.
2) Commence microplastics analysis in water and biota samples.
3) Continue PAH and PCB analysis in surface water samples and biota samples collected from Matagorda Bay.

Texas A&M University at Galveston
1001 Texas Clipper Road
Galveston, TX 77554

Reviewed by:

Date: 2/28/2022

Dr. David Hala, TAMUG, P.I.

Approved by:

Date: 3/1/2022

Mr. Steven J. Raabe, Trustee

5. REFERENCES

Abramowicz, D. A., 1995. Aerobic and anaerobic PCB biodegradation in the environment. Environ Health Perspect 5, 97-9.

Anyasi, R., Atagana, H., 2011. Biological remediation of polychlorinated biphenyls (PCB) in the soil and sediments by microorganisms and plan. African journal of plant science ISSN 1996-0824 ©2011 Academic Journals, 373-389.

Conkle, J. L., 2018. San Antonio Bay Estuarine Waterkeeper and S. Diane WIlson vs. Formosa Plastics Corp., Texas RioGrande Legal Aid, Inc., pp. 1-112.

Djomo, J. E., Garrigues, P., Narbonne, J. F., 1996. Uptake and depuration of polycyclic aromatic hydrocarbons from sediment by the zebrafish (Brachydanio Rerio). Environmental Toxicology and Chemistry 15, 1177-1181, doi:https://doi.org/10.1002/etc.5620150724.

EPA, 2020. Environmental Protection Agency: Notice memo to open Regulations.gov dockets for 20 high-priority substances. https://downloads.regulations.gov/EPA-HQ-OPPT-2018-0427-0049/content.pdf, 1-4.

Hirai, H., Takada, H., Ogata, Y., Yamashita, R., Mizukawa, K., Saha, M., Kwan, C., Moore, C., Gray, H., Laursen, D., Zettler, E. R., Farrington, J. W., Reddy, C. M., Peacock, E. E., Ward, M. W., 2011. Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. Marine Pollution Bulletin 62, 1683-1692, doi:https://doi.org/10.1016/j.marpolbul.2011.06.004.

Hüffer, T., Hofmann, T., 2016. Sorption of non-polar organic compounds by micro-sized plastic particles in aqueous solution. Environ Pollut 214, 194-201, doi:10.1016/j.envpol.2016.04.018.

NRC, 2008. National Research Council: Phthalates and Cumulative Risk Assessment: The Tasks Ahead. The National Academies Press https://doi.org/10.17226/12528, 1-209.

Safe, S., Bandiera, S., Sawyer, T., Robertson, L., Safe, L., Parkinson, A., Thomas, P. E., Ryan, D. E., Reik, L. M., Levin, W., et al., 1985. PCBs: structure-function relationships and mechanism of action. Environ Health Perspect 60, 47-56.

Tiedje, J. M., Quensen, J. F., Chee-Sanford, J., Schimel, J. P., Boyd, S. A., 1993. Microbial reductive dechlorination of PCBs. Biodegradation 4, 231-240, doi:10.1007/bf00695971.

Vázquez, O. A., Rahman, M. S., 2021. An ecotoxicological approach to microplastics on terrestrial and aquatic organisms: A systematic review in assessment, monitoring and biological impact. Environ Toxicol Pharmacol 84, 103615.

Wilson, D., 2018. Water Keeper Alliance: Report Shows Plastic Pollution a Threat to Texas Gulf Coast, on-line: https://waterkeeper.org/news/report-shows-plastic-pollution-a-threat-to-texas-gulf-coast/ (accessed on 8/16/2021).

Wolska, L., Mechlińska, A., Rogowska, J., Namieśnik, J., 2012. Sources and Fate of PAHs and PCBs in the Marine Environment. Critical Reviews in Environmental Science and Technology 42, 1172-1189, doi:10.1080/10643389.2011.556546.