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

**TWELFTH INTERIM PERFORMANCE REPORT**

**MAY 31ST, 2024**

# 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. candidate)

Mr. Asif Mortuza (Ph.D. candidate)

Mr. Marcus Wharton (master's 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 Investigators:**

Drs. David Hala, Karl Kaiser, Robert (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

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 twelfth interim report (March 1st, 2024 – May 31st, 2024) we provide a list of key accomplishments as per the fourth quarter of Year 3 of the project.

# 2. Key Updates

As of the period encompassing the twelfth interim report (March 1st, 2024 – May 31st, 2024), 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.***

- We have completed all analysis of microplastics in the body-burdens (muscle and liver) of biota from Matagorda Bay.
- We are currently preparing manuscripts for intended submission in Spring/Summer 2024.

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

- We have completed all analyses of PAHs and PCBs in the body-burdens (muscle and liver) of biota from Matagorda Bay.
- Our current focus is on preparing a high-impact manuscript for intended submission in Spring/Summer 2024.

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

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

- We have completed all single and mixtures toxicity studies with select PAHs, PCBs, and microplastics (0.1 µm polystyrene particles).
- The toxicological study design involved exposing two days post fertilized (dpf) zebrafish (*Danio rerio*) embryos to select pollutants or their mixtures for up to 4 days (96 hours) or up to 6 dpf. Fish were exposed under a semi-static renewal exposure design which involved replacing 50% of the exposure aquaria for each treatment group for each day of the trial. Up to 25 embryo/larval fish were exposed to each test chemical. At test termination, n=10 fish/treatment were placed in a multi-well micro-respirometer plate to measure oxygen consumption (or metabolic rate) over a duration of several hours (≤6 hours). Another subset of n=5 fish were observed under a microscope to quantify key morphological features, such as fish size, shape, presence of anomalous features, etc.). The final sub-set of n=10 fish were placed on a solution containing a chromogenic substrate that changes color to reflect the metabolic biotransformation capacity of the fish.
- The single compound toxicity trials included exposure to select PAHs and PCBs, which were chosen based upon their abundance and detection frequency in the biota sampled from Matagorda Bay. For example, the selection criteria for PAHs and PCBs for single or mixtures toxicity studies involved selecting only those chemicals that represented ≥20% of the sum total body-burdens for ≥20% of the fish species tested.
- The shortlisted PAHs (and their concentrations) to be tested as single compounds included (summarized in **Table 1**): phenanthrene (0.2 µM), pyrene (0.1 µM), Benzo(a)anthracene (0.6 µM), and indeno[1,2,3-cd]pyrene (0.1 µM). And the shortlisted PCBs (and their concentrations) to be tested as single compounds included: PCBs 18 (0.1 µM), 81 (0.02 µM), 105 (0.04 µM), and 0.02 (µM). We chose polystyrene (PS) latex beads (0.1 µm) as a representative microplastic at a final concentration of 10 µg/L.

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

**Table 1.** A list of the chemical compounds and their respective concentrations tested as single compounds or in mixtures.

| |
|---|
| (a) Solvent control (freshwater 0.1% DMSO) |
| |
| (b) PAHs |
| Phenanthrene (0.2 µM) |
| Pyrene (0.1 µM) |
| Benzo[a]anthracene (BaA, 0.6 µM) |
| Indeno[123-cd]pyrene (IcdP, 0.1 µM) |
| |
| (c) PCBs |
| PCB 18 (0.1 µM) |
| PCB 81 (0.02 µM) |
| PCB 105 (0.04 µM) |
| |
| (d) Plastic - PS (polystyrene, 10 µg/L) |

- We also performed a series of mixtures toxicity trials in which each single compound (i.e., PAH or PCB) was combined with the PS microplastic (10 µg/L). In addition to these tests, the PAHs or PCBs were also combined as separate PAHs only or PCBs only mixtures, PAHs only mixture + PS, PCBs only mixture + PS, or PAHs + PCBs + PS mixture (summarized in **Table 2**).

**Table 2.** A summary of the chemical compounds used to compose mixtures toxicity studies (PS = Polystyrene, 10µg/L).

| |
|---|
| (a) PAH mixtures |
| Phenanthrene (0.2 µM) + PS |
| Pyrene (0.1 µM) + PS |
| Benzo[a]anthracene (BaA, 0.6 µM) + PS |
| Indeno[123-cd]pyrene (IcdP, 0.1 µM) + PS |
| |
| (b) PCB mixtures |
| PCB 18 (0.1 µM) + PS |
| PCB 81 (0.02 µM) + PS |
| PCB 105 (0.04 µM) + PS |
| |
| (c) Complete mixtures |
| All PAHs |
| All PCBs |
| All PAHs + PCBs |
| All PAHs + PS |
| All PCBs + PS |
| All PAHs + PCBs + PS |

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

- In **Fig. 1** we show data on the effects of single or mixtures chemical exposures on the mass-specific oxygen consumption rate of $MO_2$ (µg $O_2$/mg fish/hour) in embryo-larval zebrafish. The data are shown as a percent change relative to the respective solvent control (0.1% $^{v}/_{v}$ dimethyl sulfoxide (DMSO)) used for the exposure trials. Please consult **Tables 1** and **2** for information on dosing concentrations and chemical composition for the mixture's trials. In **Fig. 1**, red dashed boxes are drawn around the treatment responses that show a marked induction of metabolic rate relative to the solvent control (statistical analysis is pending).

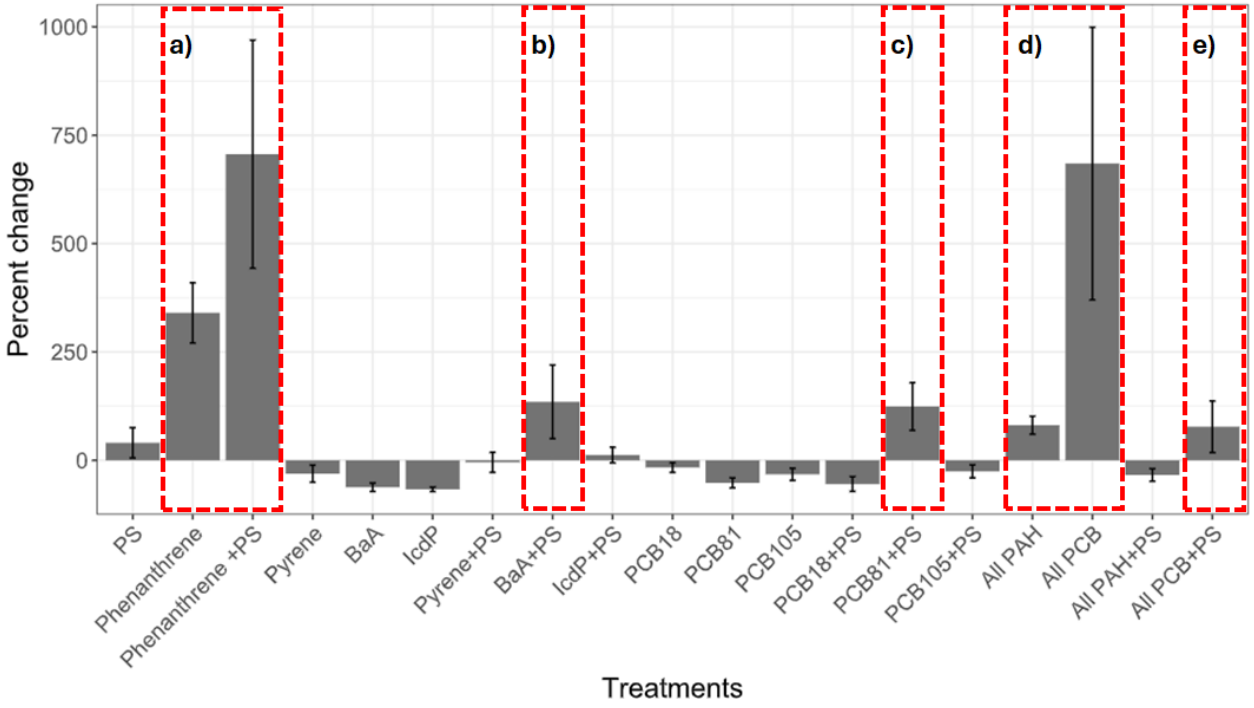


**Fig. 1.** The effects of exposure of embryo/larval zebrafish metabolic rate (or oxygen consumption rate) to single or mixtures test compounds. All data shown relative to their respective solvent control groups (0.1 $^{v}/_{v}$ DMSO). Five datasets are demarcated in the Figure, with each indicating an induction of metabolic rate in the fish exposed to: a) phenanthrene or phenanthrene + microplastics (PS); b) Benzo[a]anthracene (BaA) + PS; c) PCB 81 + PS; d) All PAHs only and all PCBs only mixture; and e) all PCBs + PS mixture. Please see **Tables 1** and **2** for a list of the chemical concentrations used for the single compound and mixtures studies. The red dashed boxes highlight treatment groups that show marked increases in metabolic rate relative to their

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

respective solvent controls. PS = polystyrene, BaA = Benzo[a]anthracene, IcdP = Indeno[1,2,3-cd]pyrene. Statistically significant effects are to be determined.

- While statistically significant effects remain to be tested for, our data highlights some key trends. For example, for the PAHs phenanthrene (**Fig. 1 (a)**) and BaA (**Fig. 1 (b)**), an elevated metabolic rate is evident when co-exposed with the microplastic polystyrene. For the PCBs, only the mixture comprising PCB 81 + PS showed an elevated metabolic rate (**Fig. 1 (c)**). Mixtures comprising all PAHs or all PCBs only also showed induction of metabolic rate, with an ~5x higher induction of metabolic rate for the all PCB mixture relative to the all PAH mixture (**Fig. 1 (d)**). Whereas, co-exposure of the fish to the all PCBs mixture + PS showed a marginal induction of metabolic rate (**Fig. 1 (e)**).
- Therefore, our results indicate a synergistic effect of microplastics with only select PAHs (phenanthrene, BaA) and a PCB (PCB-81).
- We are also characterizing the effects of exposure on the cardiac morphology of larval fish and expect to report on our findings for the final report.

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

- Educational outreach engagement is completed and was pursued in collaboration with the TAMUG Sea Camp program in Summer 2022. Outcomes from the outreach activity were reported in the *sixth* interim report.

**3. FURTHER WORK**

Planned work for completion for the Final Project report are as follows:

1) Prepare and submit manuscripts for publication over Summer 2024 on PAHs, PCBs, and microplastics levels in biota from Matagorda Bay.

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

2) Prepare a manuscript describing the microplastics analysis methods and application to measuring levels in biota from Matagorda Bay (Summer 2024).
3) Complete all statistical and data analyses of toxicological datasets on the effects and prepare for a manuscript submission (to be completed by Fall 2024).

## 4. REFERENCES

None reported for this interim report.

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

Reviewed by:

5/29/2024

_____ Date: _____

Dr. David Hala, TAMUG, P.I.

Approved by:

_____ Date: 5/30/2024

Mr. Steven J. Raabe, Trustee