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

SIXTH INTERIM PERFORMANCE REPORT

NOVEMBER 30TH, 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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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.

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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 sixth interim report (September 1st, 2022 – November 30th, 2022) we provide a list of key accomplishments as per the second quarter of Year 2 of the project.

2. Key Updates

As of the period encompassing the sixth interim report (September 1st, 2022 – November 30th, 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 fifth interim report details the levels of various microplastics measured in the surface waters of Matagorda Bay. Plans are in-place for additional dockside sampling at various locations around Matagorda Bay in Spring 2023. Samples from previous collections with a pump system (> 5 µm) and a tow net (> 200 µm) across the bay and along shore have been processed and analysis will be completed by the end of December. Procedural blanks were also performed to determine any contamination issues during collection, processing, and analysis of samples.

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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 and biota collected in Matagorda Bay were detailed in the previously submitted fifth interim report.

- The PAH and PCB pollutant levels measured in the muscle of fish, and gill/mantle tissue of oysters from Matagorda Bay was compared with biota from Galveston Bay (**Fig. 1** and **2**). The results of this comparative analysis were presented at the Gulf Estuarine Research Society (GERS) Biennial Meeting in Ocean Springs, Mississippi, which was held from October 27th – 29th, 2022 (**Appendix I**).

- The analysis of mean total PAH levels in hardhead catfish from Matagorda Bay indicated overall higher levels vs. other biota (including those from Galveston Bay) (**Fig. 1**). The elevated PAH levels in hardhead catfish from Matagorda Bay appears to be mainly due to elevated naphthalene concentrations, constituting up to 70% of total PAH congeners in this fish (as previously reported in our fifth interim report). Naphthalene appeared to be uniquely elevated in hardhead catfish only, potentially indicating a specific source of exposure.

- The statistical analysis of PAH levels showed no significant difference in biota between the two estuaries (i.e., within same species) (**Fig. 1**). Only significant differences (p<0.05) were observed for Galveston oysters vs. Matagorda spotted seatrout, Galveston oysters vs. Matagorda red drum. And for both comparisons, Galveston oysters ~3x higher mean PAH levels (**Fig. 1**).

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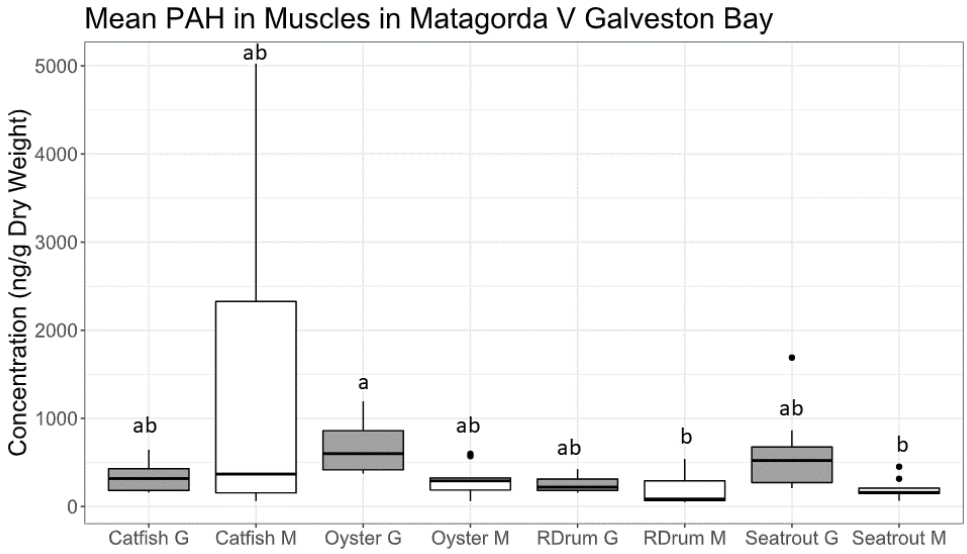

Fig. 1. Box and whisker plots showing mean total PAH levels in the muscle tissues of biota (or gill/mantle for oysters) from Matagorda and Galveston Bays (ng/gram tissue dry weight). G = Galveston Bay, M = Matagorda Bay.

- For the analysis of PCBs in muscle tissue of biota from Matagorda and Galveston Bay, no significant differences in PCB concentrations between the estuaries (i.e., within same species) was observed (**Fig. 2**). Statistically significantly differences (p<0.05) were only observed for Galveston Bay oysters (~8x higher) vs. Matagorda hardhead catfish, Galveston Bay oysters (~4x higher) vs. Matagorda spotted seatrout, and Galveston Bay oysters (~11x higher) vs. Galveston Bay red drum.

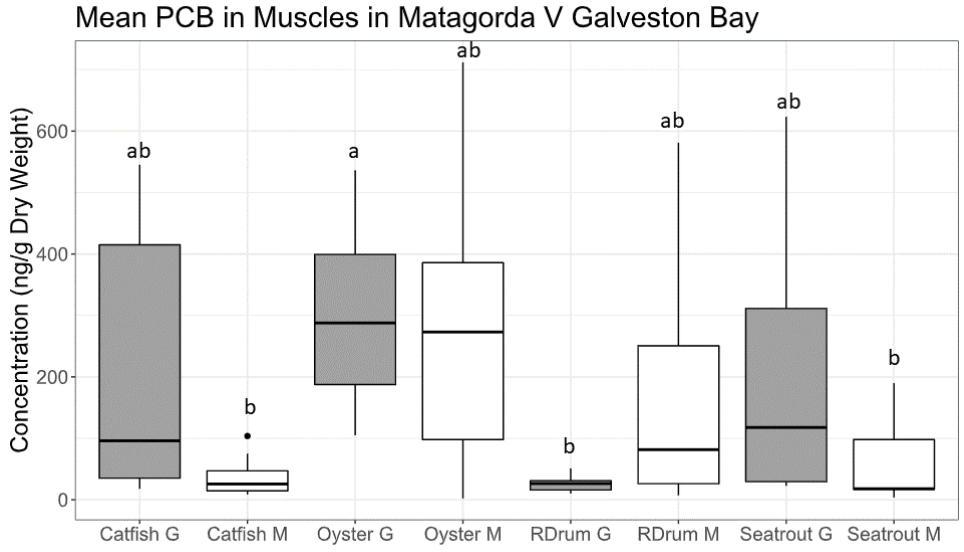

Fig. 2. Box and whisker plots showing mean total PCB levels in the muscle tissues of biota (or gill/mantle for oysters) from Matagorda and Galveston Bays (ng/gram tissue dry weight). G = Galveston Bay, M = Matagorda Bay.

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- Given that certain low-to-high molecular weight PAH ratios can be used to indicate the prominent sources pollution. We characterized the major sources of PAHs into Matagorda and Galveston Bay by using the ratios detailed in **Table 1**.

Table 1. The predominant source of pollution based on PAH ratios. Ant= Anthracene, Phe= Phenanthrene, Flu= Fluoranthene, Pyr= Pyrene.

Ant/(Ant+Phe)	Flu/(Flu+Pyr)	Predominant source
<0.1 (Li et al., 2016)	<0.4 (Li et al., 2016)	Petroleum
-	0.4-0.5 (Li et al., 2016)	Gasoline engine
-	>0.5 (Ravindra et al., 2008)	Diesel engine
0.24 (Kong et al., 2010)	0.57 (Li et al., 2016)	Coal combustion

- PAH source analysis showed that the biota in Matagorda Bay were predominantly exposed to petroleum-derived PAHs (55%), and with Petroleum-combustion (29%) and combustion-only sources (16%) contributing to the remainder of PAHs sources (**Fig. 3**). In contrast to Matagorda Bay, the biota from Galveston Bay were exclusively exposed to mainly petroleum-derived PAHs (100%) (**Fig. 3**).

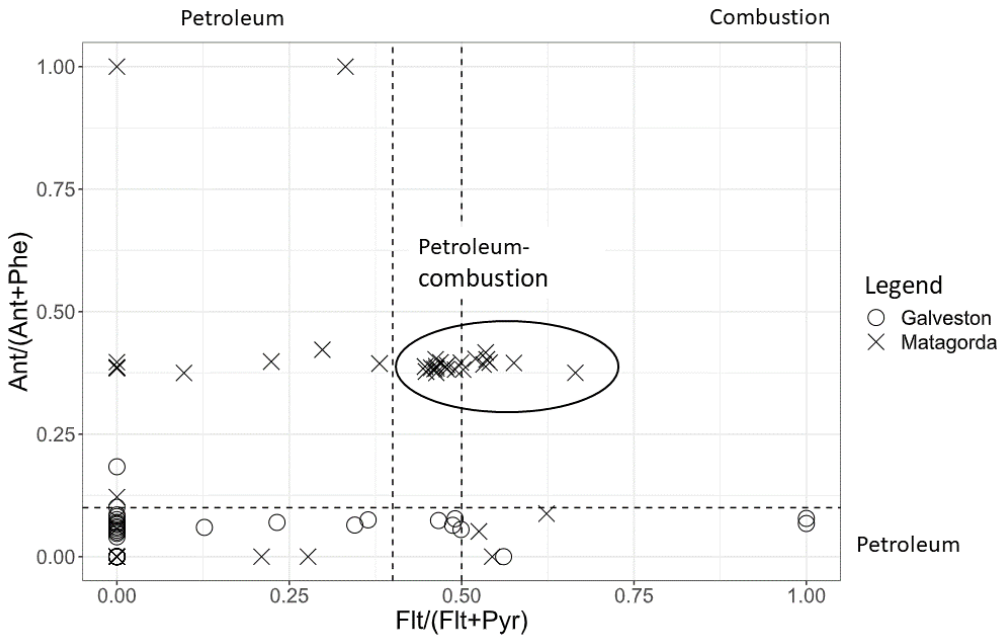

Fig. 3. The comparison of PAH sources based upon the ratios of specific PAH congeners in the body-burdens of biota from Matagorda and Galveston Bays. Please see the discussion for an interpretation of the major sources of PAHs identified from this analysis.

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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 2023 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).

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

- A description of public educational outreach engagement has been previously described in the fifth interim report. Educational engagement through the TAMUG Sea Camp (during the summer months) will continue throughout the remainder duration of the project and will be reported in the eighth interim report.

3. FURTHER WORK

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

1) Prepare a manuscript for publication by Summer 2023 on the PAH and PCB data generated as part of the research performed in this project (comparing pollutant levels in Matagorda vs. Galveston Bays).
2) Commence microplastics analysis in water and biota samples. The preparation of a manuscript describing the microplastics analysis methods will be prepared in Summer 2023.
3) Plan the initiation of toxicological studies on the effects of microplastics and PAH/PCB mixtures on embryo-larval life stages of fish.

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Reviewed by:

11/29/2022

_____ Date: _____

Dr. David Hala, TAMUG, P.I.

Approved by:

_____ Date: 11/29/2022

Mr. Steven J. Raabe, Trustee

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Appendix I

Platform Presentation Slides as Presented by Mr. Asif Mortuza (PhD Student Funded by this MBMT Award)

This Presentation was given at the Gulf Estuarine Research Society (GERS) Biennial Meeting in Ocean Springs, Mississippi (October 27th – 29th, 2022)

TEXAS A&M UNIVERSITY
GALVESTON CAMPUS

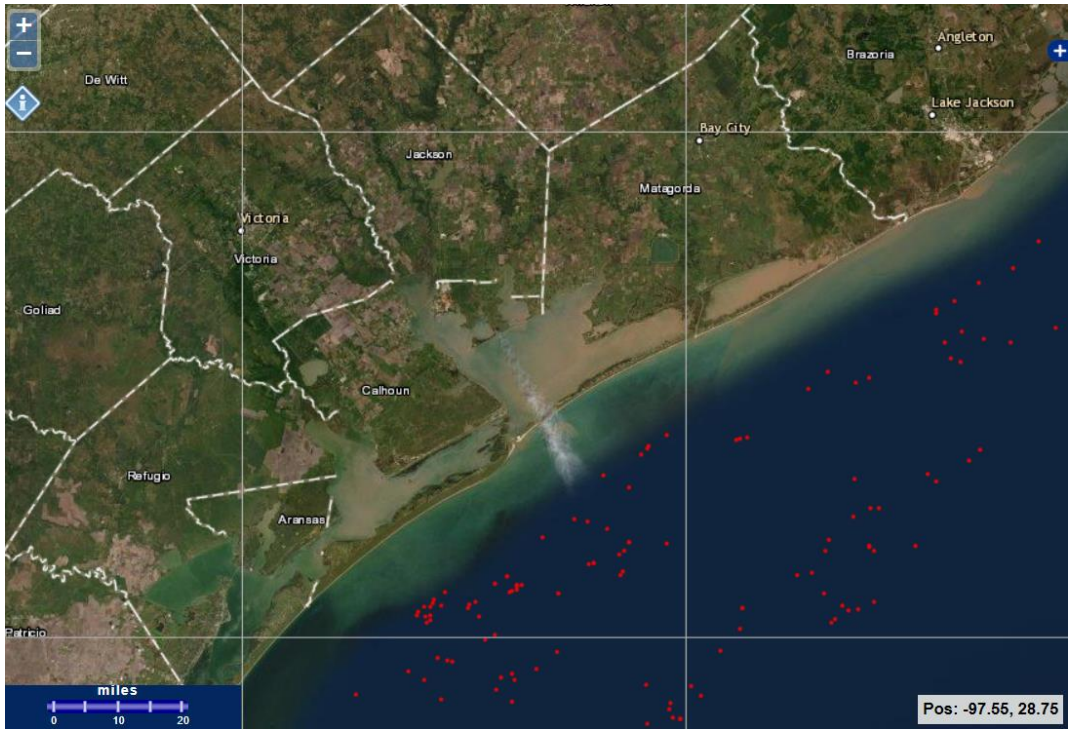
Comparison of PAH and PCB Body-burdens in Biota From Two Sub-tropical Estuaries

ASIF MORTUZA, EMILY MEESE, MARCUS WHARTON, KARL KAISER, LENE H. PETERSEN, DAVID WELLS, ANTONIETTA QUIGG, DAVID HALA

Introduction

Introduction

- **Gulf of Mexico** contributes to **30% of oil** and **10% of U.S. natural gas** productions.
- Leads to accumulation of
  - Oil derived polycyclic aromatic hydrocarbons (**PAHs**)
  - ‘Legacy’ industrial pollution polychlorinated biphenyls (**PCBs**)
- Galveston Bay
  - **30-50% U.S. capacity of oil** and chemical industries
- Matagorda Bay
  - **Fisheries industry**, relatively less industrialized



Biota and Pollutants

BIOTA

- **Hardhead Catfish**
  - *Ariopsis felis*
- **Spotted Seatrout**
  - *Cynoscion nebulosus*
- **Red Drum**
  - *Sciaenops* ocellatus
- **Eastern Oyster**
  - *Crassostrea virginica*
  - Benthic, filter feeder

POLLUTANTS

- All EPA priority pollutants

https://marinefishesofgeorgia.org/hardhead-catfish/

https://deq.nc.gov/about/divisions/marine-fisheries/public-information-fact-sheets/shellfish/oysters

https://fishspecies.dnrec.delaware.gov/fishspecies.aspx?habitat=2&species=150

Objective

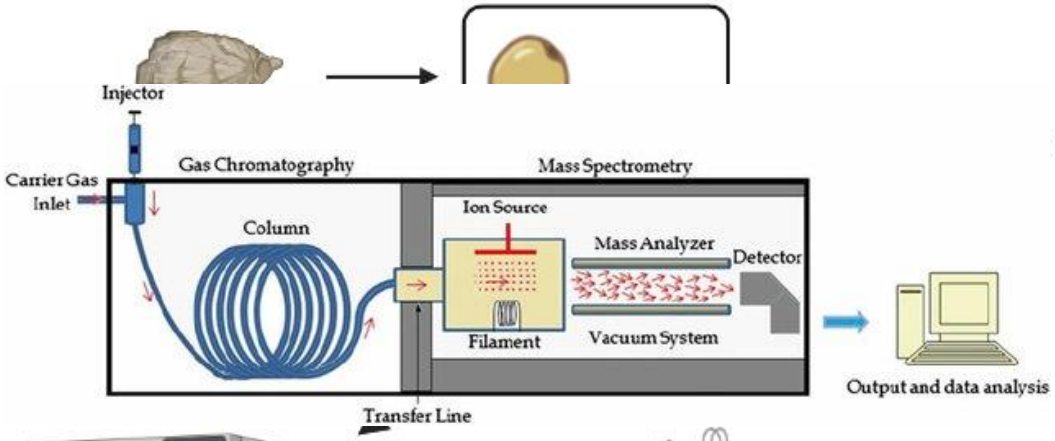
To study the difference in **PAHs** and **PCBs body burdens** in biota

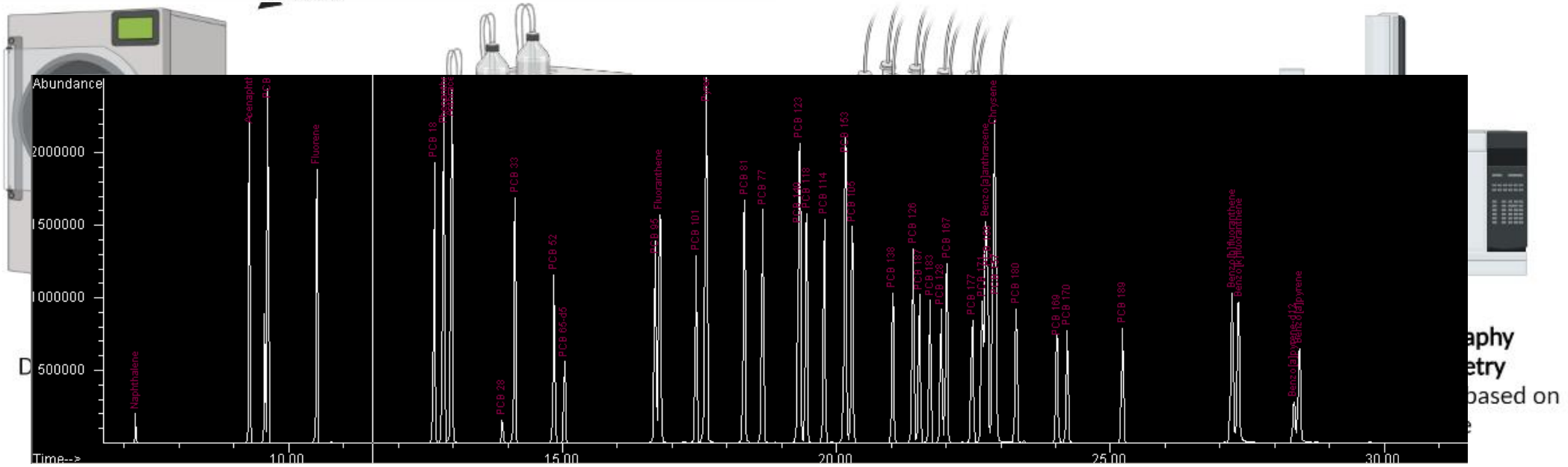
- **Matagorda Bay** vs. **Galveston Bay**

Will **Galveston Bay** biota PAH PCB body burdens be higher than **Matagorda Bay**?

Methods

Tissue Extraction and PAH/ PCB Quantification Method





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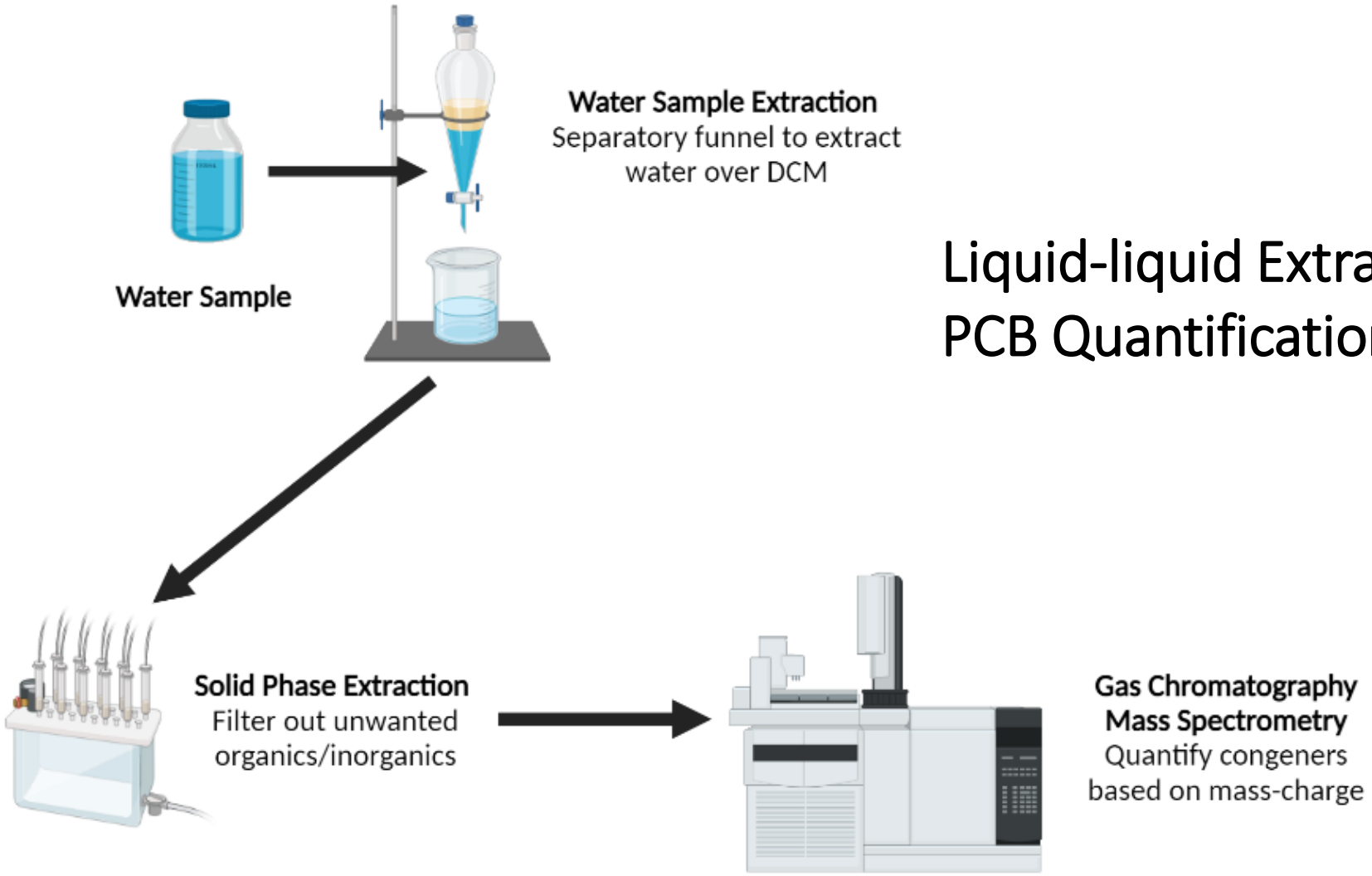
https://www.researchgate.net/publication/273955959_Gas_Chromatography-Mass_Spectrometry_of_Biofluids_and_Extracts/figures?lo=1&utm_source=google&utm_medium=organic

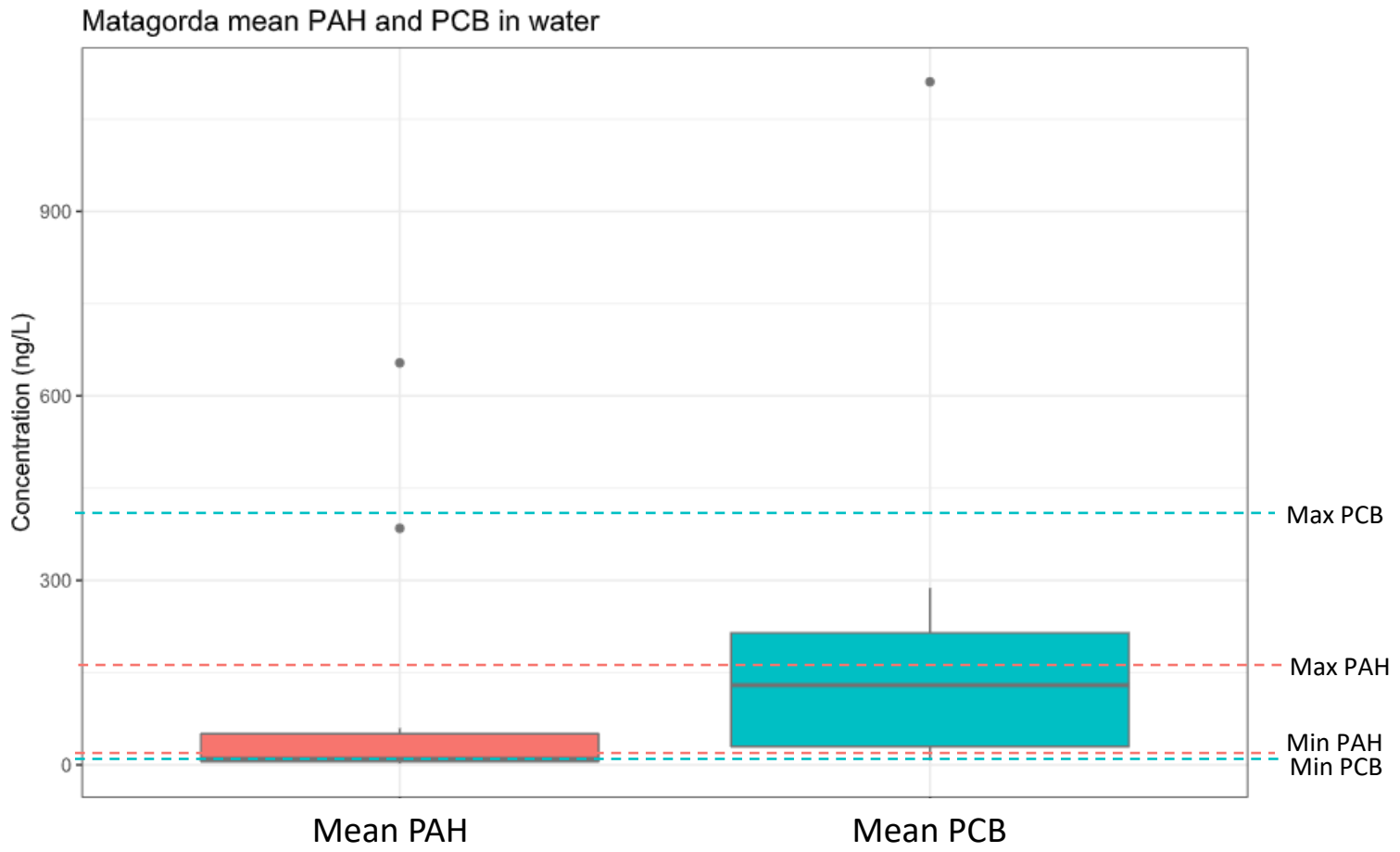
Liquid-liquid Extraction and PAH/ PCB Quantification Method



Results

Matagorda Water

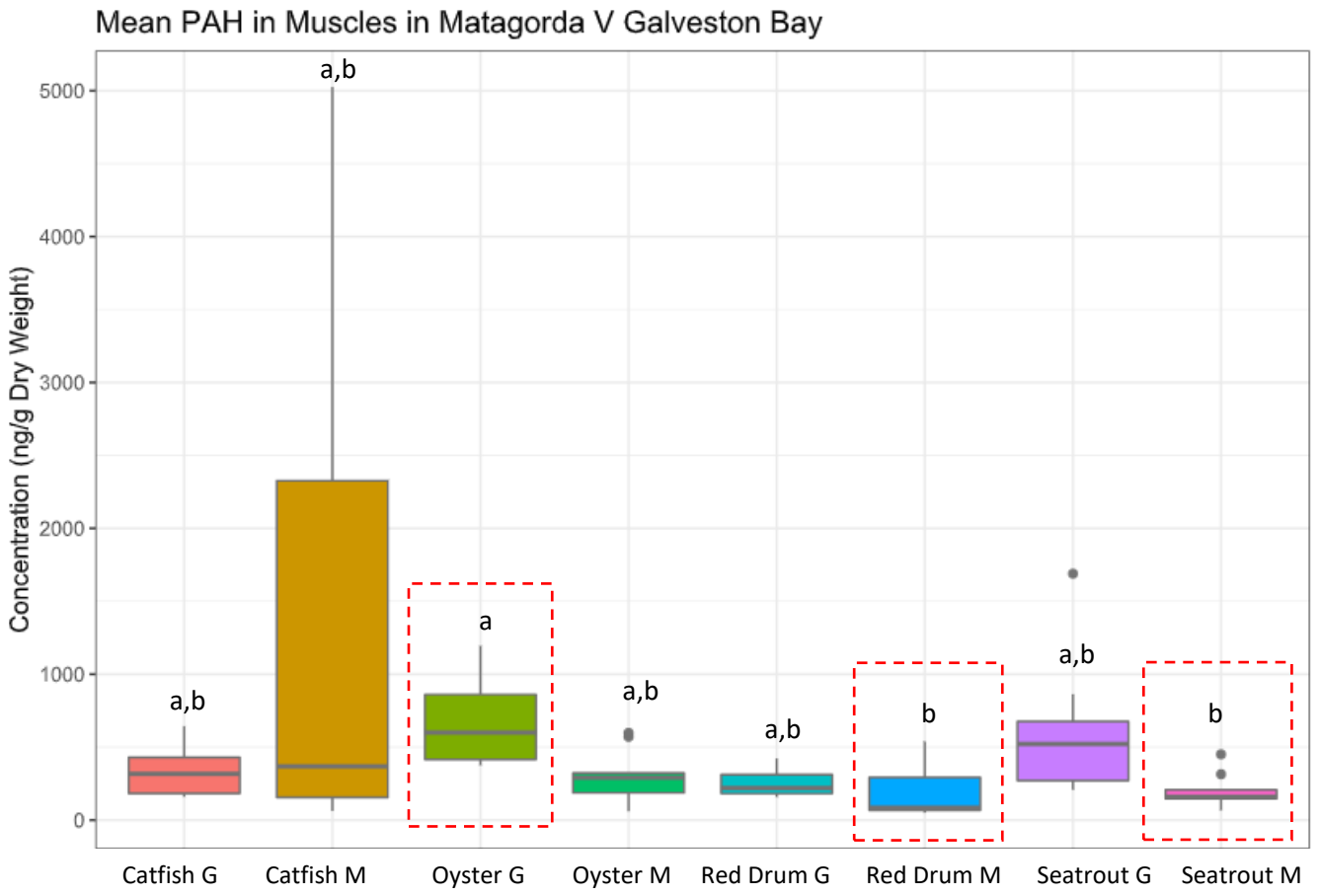
- **Mean PAH** in Matagorda Bay water is **within range** of Galveston Bay.
- **Mean PCB** in Matagorda Bay is **within range** of Galveston Bay.



(Bacosa et al. 2020)

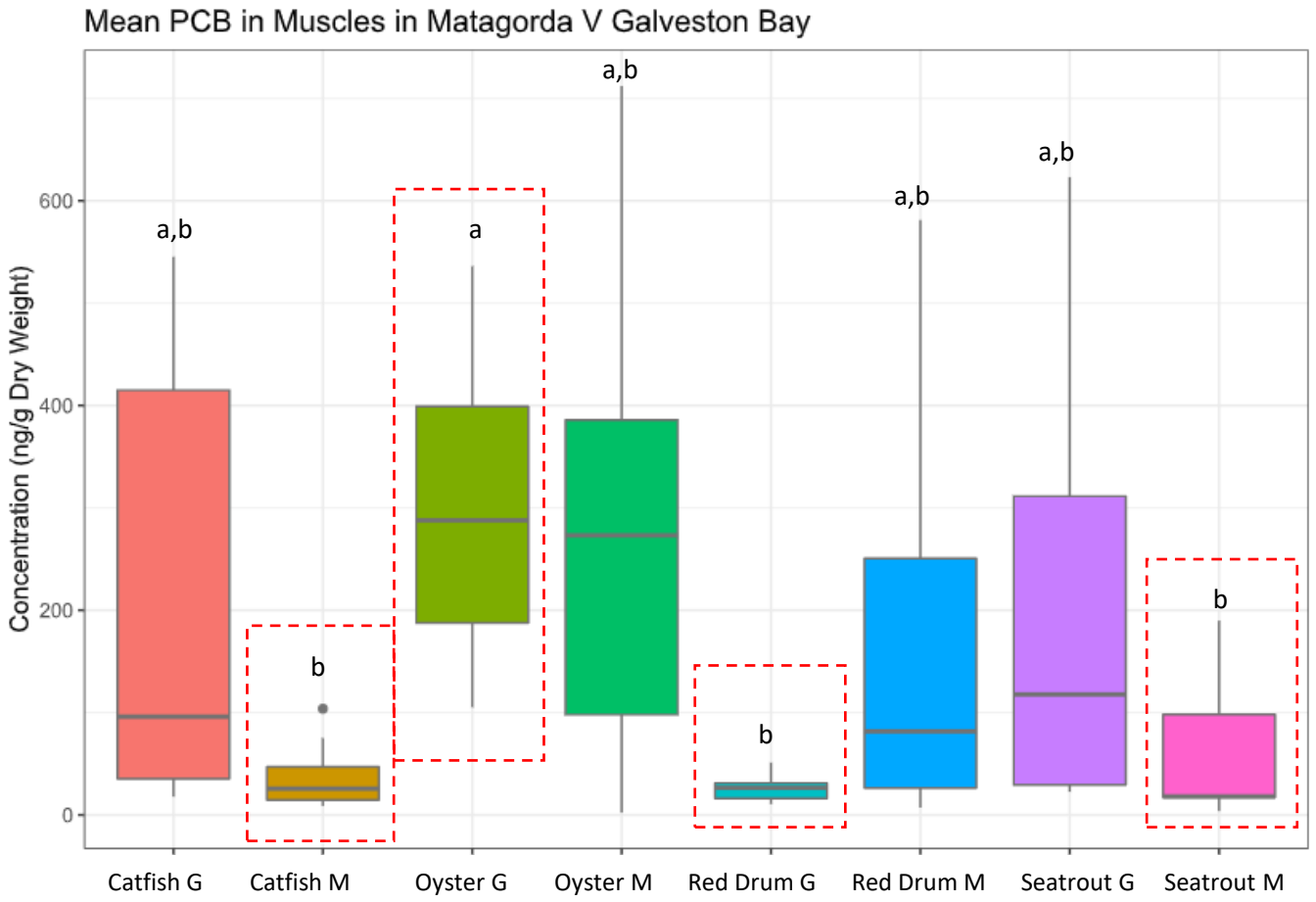
Biota Mean PAH

- **No significant difference** in biota PAH accumulation between the estuaries (**within same species**).
- Significantly different (P<0.05):
  - Galveston Oyster vs. Matagorda Spotted Seatrout
  - Galveston Oyster vs. Matagorda Red Drum
  - Both cases, Galveston Oyster **~3x mean PAH**



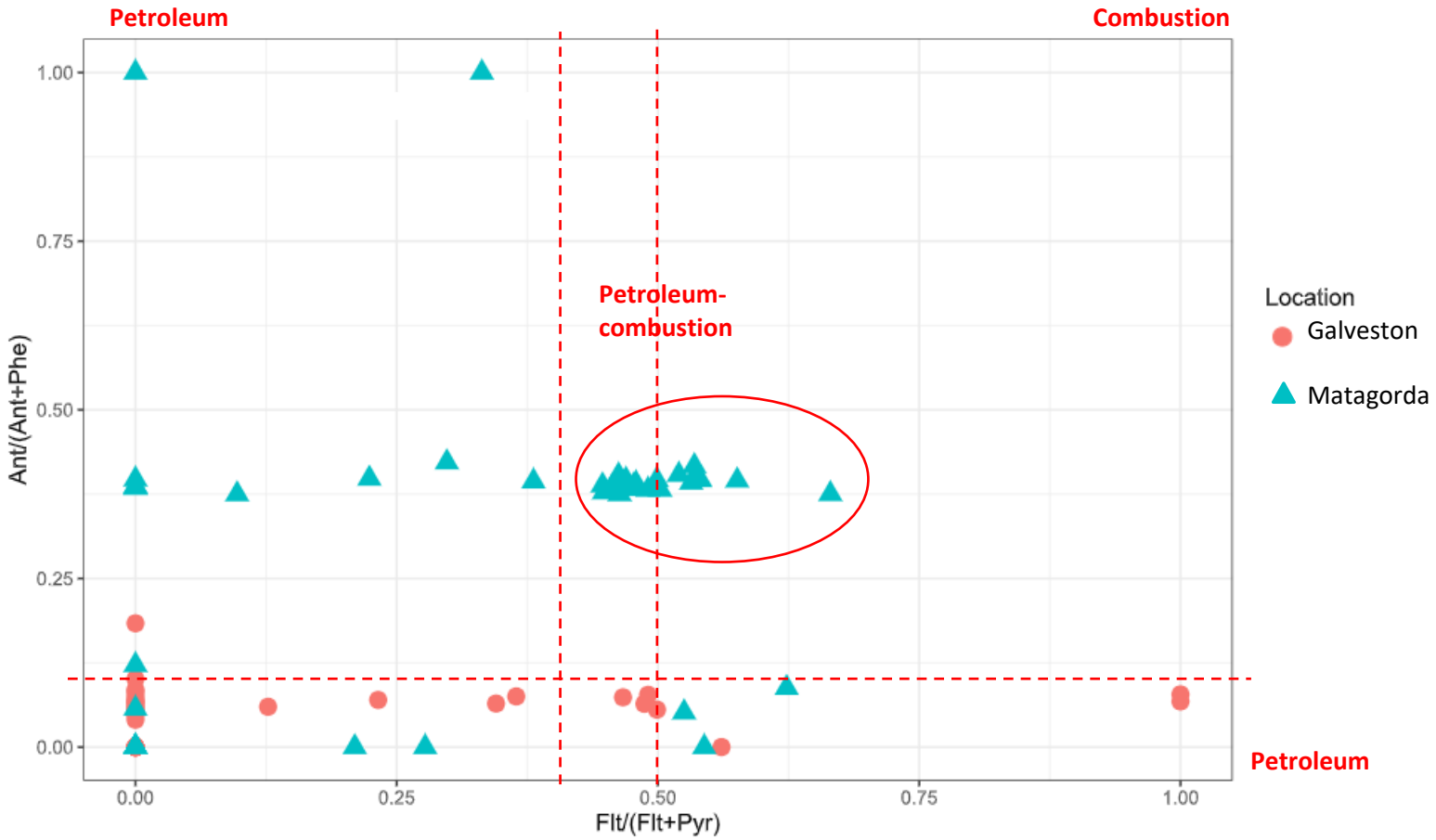
Biota Mean PCB

- **No significant difference** in biota PCB accumulation between the estuaries (**within same species**).
- Significantly different (P<0.05):
  - Galveston Oyster **(~8x)** vs. Matagorda Hardhead Catfish
  - Galveston Oyster **(~4x)** vs. Matagorda Spotted Seatrout
  - Galveston Oyster **(~11x)** vs. Galveston Red Drum



Source Ratio

- **Matagorda Bay** biota PAH accumulation source predominantly **Petroleum (55%).**
  - **29% Petroleum-combustion**
  - **16% Combustion**
- **Galveston Bay** biota PAH accumulation source **Petroleum (100%)**



FDA Cancer Risk

- **LOC = (RL x BW x AT x CF)/(CSF x CR x ED)**

	Finfish	Oyster
RL (Risk level)	0.00001	0.00001
BW (Avg consumer body weight)	80 kg	80 kg
AT (Avg time)	78 yrs	78 yrs
CF (Conversion factor)	1000 µg/mg	1000 µg/mg
CSF (Cancer slope or potency factor of BaP)	7.3 mg/kg/day	7.3 mg/kg/day
CR (Consumption rate)	49 g/day	12 g/day
ED (Assumed exposure duration)	5 yrs	5 yrs
LOC (Level of concern) =	0.035 µg/g/BaPE	0.142 µg/g/BaPE

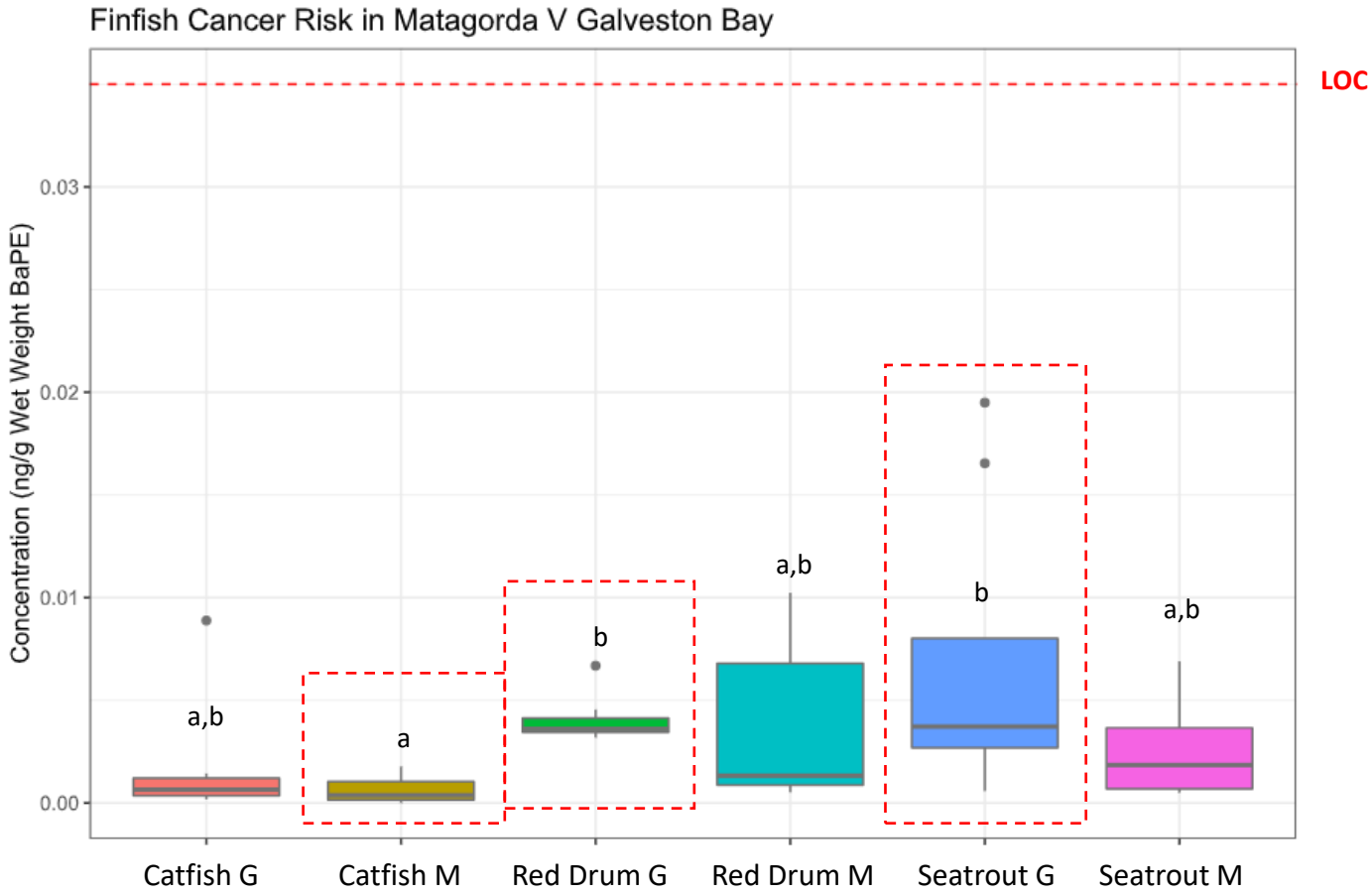
- **BaPE concentration= BaP concentration+ (sum of other PAH concentrations x TEF)**
  - Other PAHs: Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Indeno[123-cd]pyrene, Dibenzo[a,h]anthracene

https://www.fda.gov/food/food-safety-during-emergencies/protocol-interpretation-and-use-sensory-testing-and-analytical-chemistry-results-re-opening-oil

FDA Cancer Risk

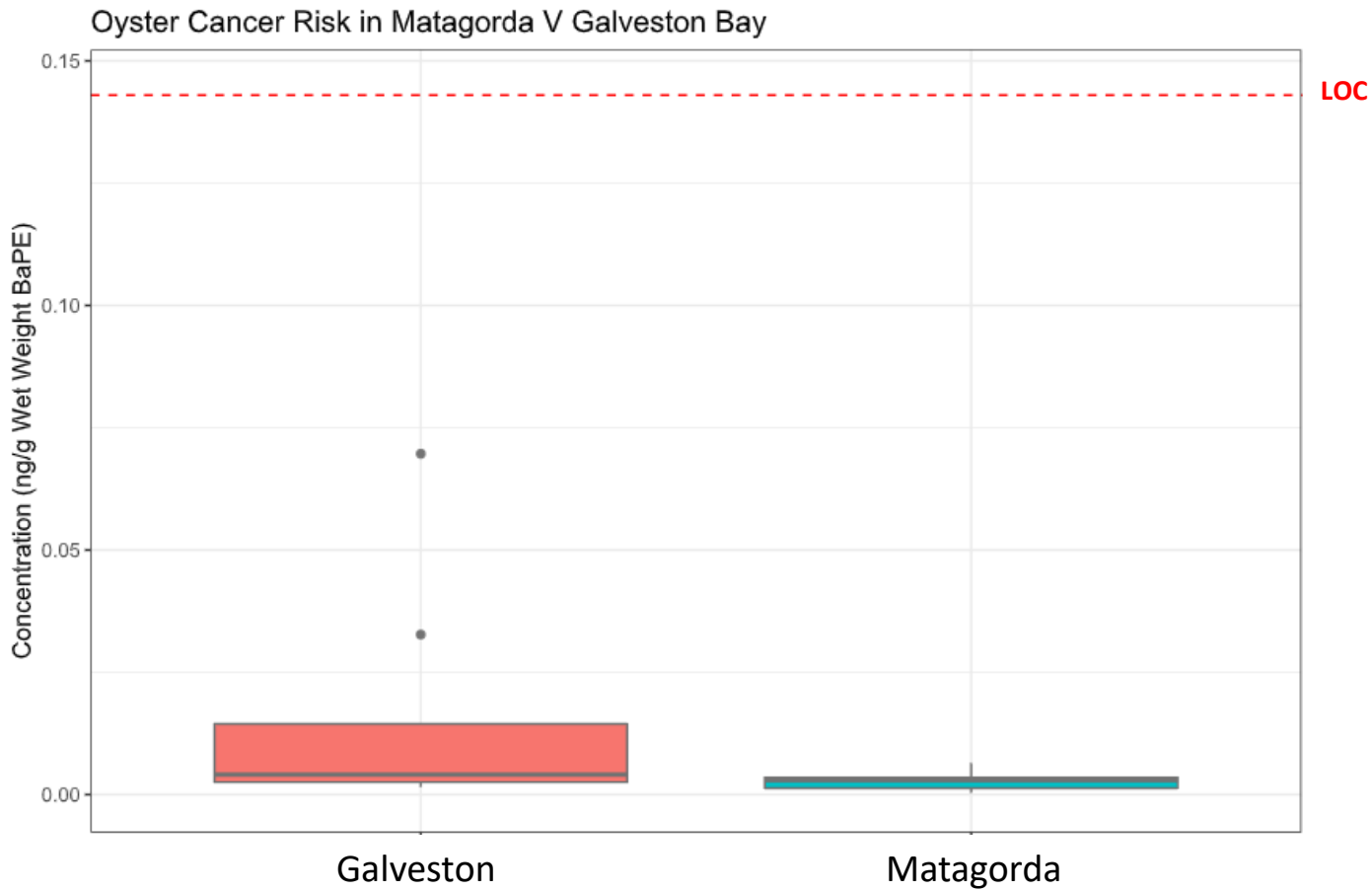
- Fin fish BaPE **~5- 56x less than** the LOC

- **Matagorda Hardhead Catfish** risk (P<0.05)
  - **~24x less than** Galveston Oyster
  - **~11x less than** Galveston Spotted Seatrout
  - **~6x less than** Galveston Red Drum



FDA Cancer Risk

- Oyster BaPE **~10- 53x less than** the LOC



Conclusion

- **No significant difference** in biota PAH and PCB accumulation between the estuaries (**within same species**).
  - PAH: **Galveston Oyster** > Matagorda Spotted Seatrout, Red Drum muscle
  - PCB: **Galveston Oyster**> Matagorda Spotted Seatrout, Hardhead Catfish and Galveston Red Drum muscle
- Predominant source of PAH in **Matagorda** and **Galveston Bay**- **Petrogenic**
  - **Matagorda Bay: 45%** from petroleum-combustion+ **combustion**
- All biota BaPE concentrations **lower than FDA level of concern for cancer risk**

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  - Dr. David Hala
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  - Dr. Lene Petersen
  - Dr. David Wells
  - Dr. Antonietta Quigg
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  - Marcus Wharton (PhD)
  - Emily Meese (PhD)
  - Brady Samuelson (Undergraduate)
  - Hayley Valdez (Undergraduate)
  - Raquel Guerrero (Undergraduate)
  - Avery Franklin (Undergraduate)
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Thank you!

Questions?

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