



7TH ANNUAL

# TEXAS PLASTIC POLLUTION SYMPOSIUM

THE HOUSTON ZOO  
- APRIL 3, 2025 -

[www.TexasPlasticPollutionSymposium.com](http://www.TexasPlasticPollutionSymposium.com)

# Poster Titles & Presenters

The primary poster session for this symposium is scheduled from 12:45 – 1:45 p.m. on Thursday, April 3, 2025, in the education classrooms within the Brown Education Center at the Houston Zoo. The poster authors will be present during this time to answer questions and discuss their projects.

For those attending the meeting virtually: You will receive an email following the meeting containing PDF copies of the posters that were provided by the authors. Check them out, and feel free to reach out to the authors with any questions or comments you may have.

## **Aerial Invasion: Atmospheric Deposition of Microplastics in Mosquito Lagoon, Florida**

Madison Serrate\*, Tanillesse Gonzalez, Stephanie Fletcher, Paul Sacks, Joshua Fnu, Sara Kim, Lei Zhai, Abby Frey, Julia Kruger, Tara Blanchard, Emily Hays, Linda Walters

## **Analysis of Microplastic Concentrations in Dried Algae Mats and Sediment Collected from Detention Basins in the Edwards Aquifer Recharge Zone**

Paulina Quinonez\*§, Andre Felton, Jeffery Hutchinson

## **Digestion of Polyethylene Terephthalate Fibers by *Zophobas morio* Larvae**

Isabel Li\*§, Dr. Kasia J. Dinkeloo

## **Dynamics of Marine Litter Post-Hurricane Beryl: Assessing the Ultimate Fate of Flotsam**

William Bailey\*§, David Mohrig, Cornel Olariu, Kutalmis Saylam

## **Effects of PET Microfiber Exposure on Mating Behavior, Foraging Behavior, and Problem Solving in *Gambusia affinis***

Adrienne Lihou\*§, Rivers Hartzell\*, and Jing Gruber\*

## **Examining the Plastic Degrading Potential of Marine Fungi Found on the Texas Coast**

Jaden Acevedo\*§, Kristen Garsaud\*, Dr. Kasia Dinkeloo

## **Fluorescent Detection of Nile Red-stained Microplastic Uptake in the Roots of *Arabidopsis thaliana***

Kailyn Nonhof\*§, Jing Gruber, Kasia Dinkeloo

## **Influence of Microplastics on Sediment Transport Dynamics**

Marufa A. Upoma, Min Y. Pack\*

## **Making Space for Migratory Birds: An Urban Conservation Program Highlight**

Kiara Carrasco\*§, Chloe Dannenfelser, Liz Virgl, Nancy Brown

\* Indicates presenter

§ Indicates student presentation

**Microbial Marvels: Investigating Dubia Roach Microbiota in Relation to Polyethylene Biodegradation**

Roland Quinones\*§, Kasia Dinkeloo

**Non-Plastic Solutions for Oyster Reef Restoration: Efficacy and Environmental Impacts of Novel Restoration Materials**

Cara Womacks\*§, Madison Serrate, Otis Woolfolk, Fnu Joshua, Lei Zhai, Paul Sacks, and Linda Walters.

**Plastic-Free Restored Habitats: Reducing Plastic Pollution in Community-Based Restoration of Oyster Reefs**

Dr. Jennifer Beseres Pollack, Dr. Linda Walters, Dr. Lisa Chambers, Jace Tunnell, Dr. Zhanfei Liu, Dr. Terry Palmer, Natasha Breaux, Erin Hill, Mckenna Reinsch\*

**The Nurdleome: Identification and Characterization of Microbes Found On Gulf Coast Nurdles**

Vibha Annaswamy\*§, Kasia Dinkeloo

**Threads of Change: Zooplankton Community Shifts in Response to Fiber Disturbances**

Caitlyn Lankford, Heaven Thompson, Ashton Fisher, Addison Lehew, Dr. Mary Kay Johnston

\* Indicates presenter

§ Indicates student presentation

## Background

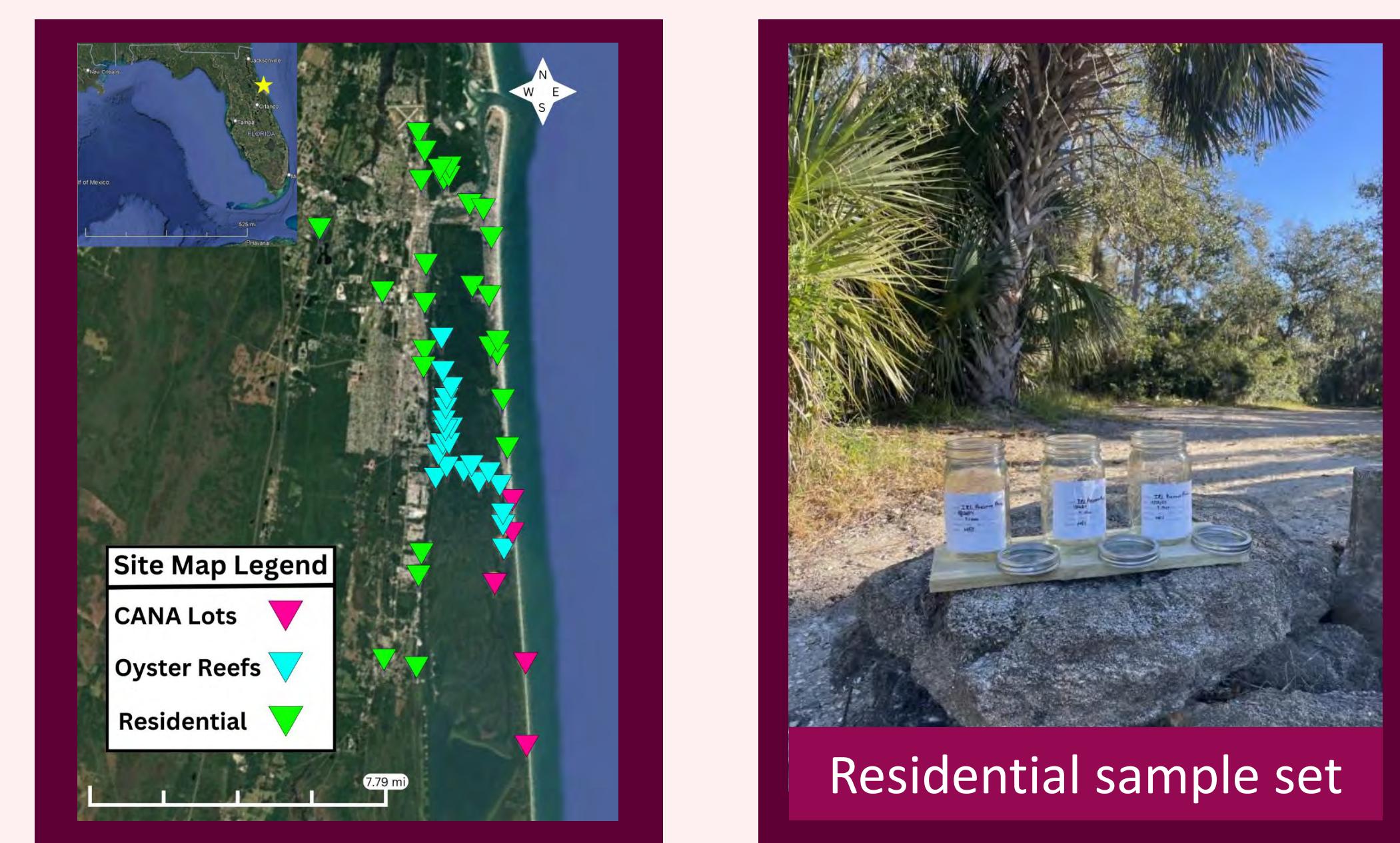
- Microplastics (MP, < 5 mm) have been found in remote regions, suggesting atmospheric deposition (AD) facilitates long-distance transportation<sup>1</sup>
- For example, in the Pyrenees Mountains,  $365 \pm 69 \text{ MP m}^{-2}\text{d}^{-1}$  were found in AD samples<sup>2</sup>
- A 2021 study found that the Indian River Lagoon (IRL) in Florida is a microplastic hotspot, with a mean density ( $\pm \text{SE}$ ) of  $1.47 \pm 0.05 \text{ MP per liter of lagoon water}$ <sup>3</sup>
- The role of AD in MP abundance around the Indian River Lagoon and the surrounding communities is unknown

## Objectives

- Assess whether MP abundance varies between site types (CANA parking lots, intertidal oyster reefs, residential)
- Evaluate potential differences in MP characteristics between site types

## Field Methods

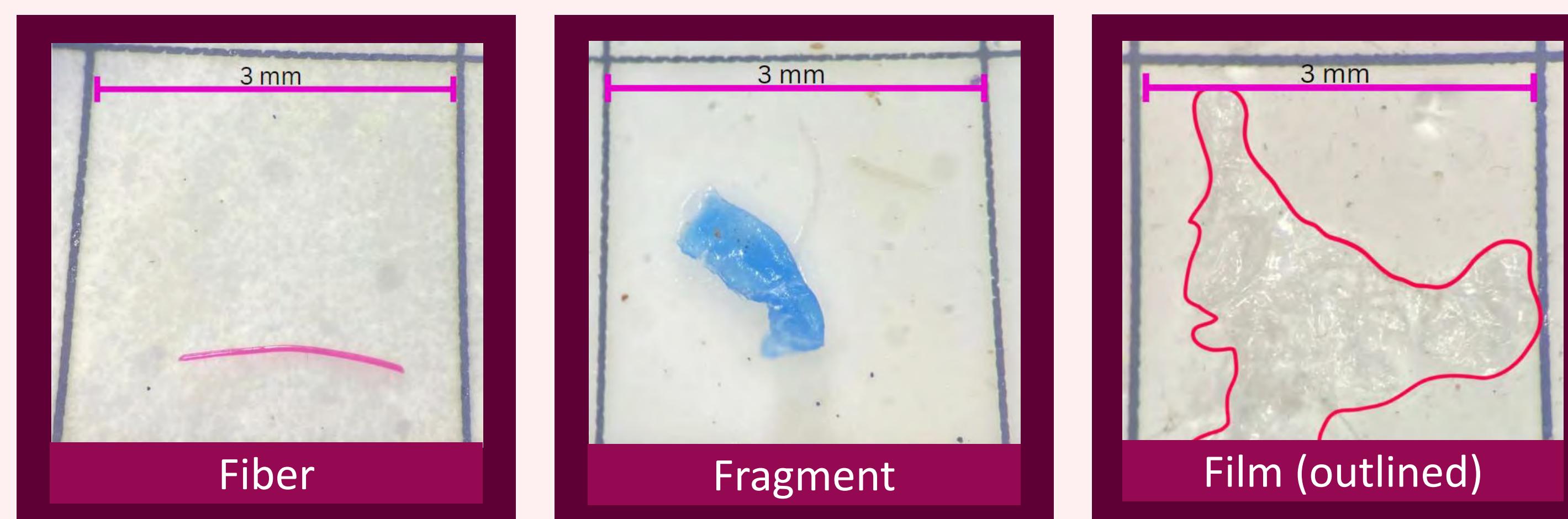
- Collection jars are placed around the IRL at 3 site types: Canaveral National Seashore parking lots (CANA lots), oyster reefs, and residential sites
- Each sample set contains 3 replicate glass jars (1000 mL jar) containing 10 mL of filtered (0.45  $\mu\text{m}$ ) deionized (DI) water to trap particle deposits
- Samples are left at the site for 3 hours to collect particle deposits before being sealed and transported back to the lab for processing



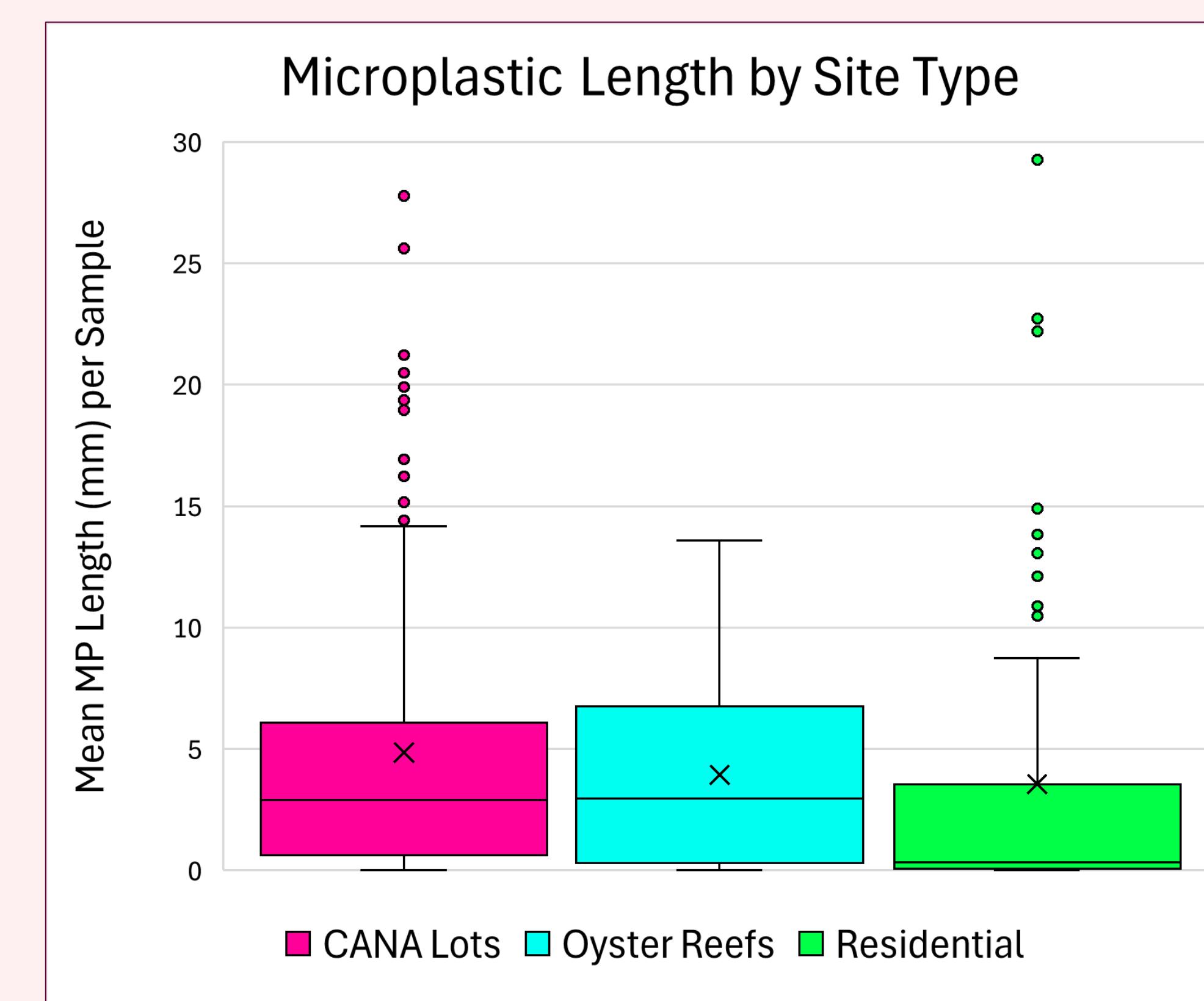
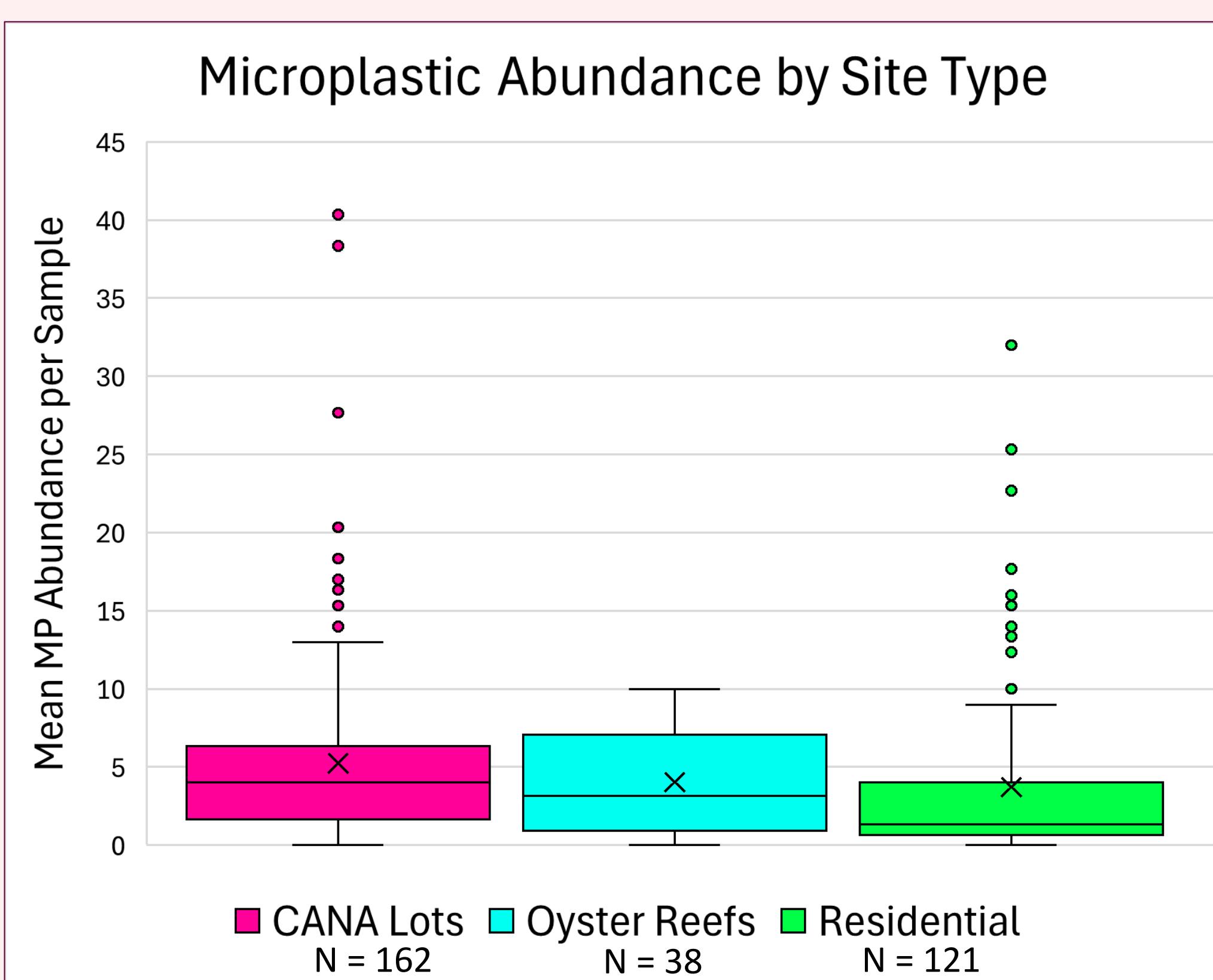
## Methods

### Laboratory Methods

- To remove particles from the jars, each was rinsed 3 times with 200 mL filtered (0.45  $\mu\text{m}$ ) DI water and filtered three times through a vacuum-pump filtration system using 0.45  $\mu\text{m}$  gridded filter paper
- A 40x microscope is used to examine filter paper contents and the size, color, morphology (fiber, fragment, film, foam, bead, pellet), and suspected material type is recorded for each particle
- To account for aerial contamination, 5 blanks with filter papers dampened with filtered (0.45  $\mu\text{m}$ ) DI water are set around each microscope during microscopy processing
- After samples are processed, blanks are examined the same way
- Fourier transform infrared spectroscopy (FTIR) is used for definitive determination of suspected MPs



## Preliminary Results



## Abundance

- Kruskal-Wallis Test: There was a significant difference in mean MP abundance between site types ( $p = 1.70 \times 10^{-5}$ )
- CANA lots and oyster reefs had the highest mean MP abundance with a mean of 5.2 and 4.0 MPs per sample, respectively
- Contamination Rate:** Blanks had an aerial MP contamination rate of  $0.00006688 \text{ MP/minute}$  during processing

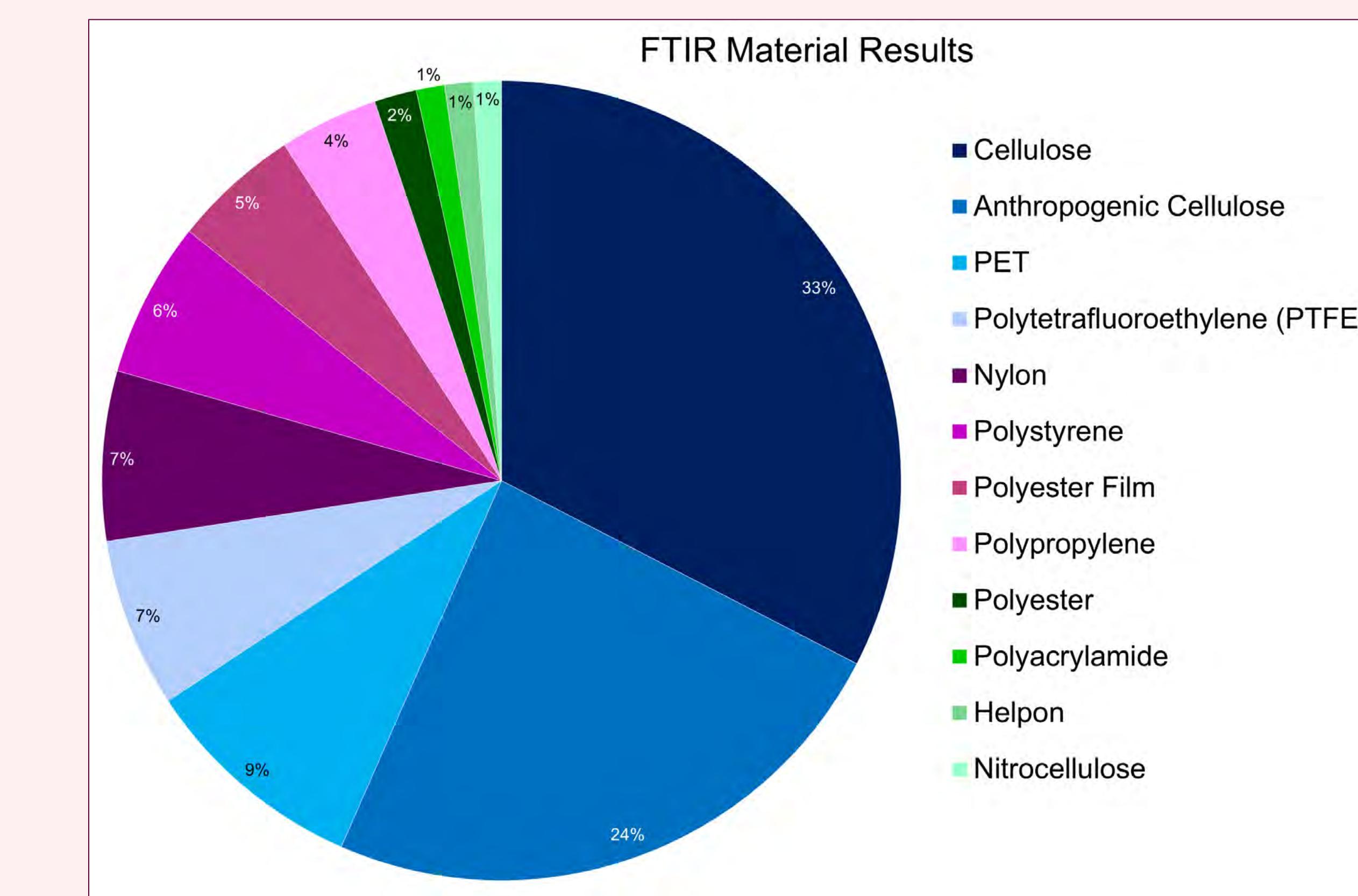
## Characteristics

- Kruskal-Wallis Test: There was a significant difference in mean MP length between site types ( $p = 3.72 \times 10^{-5}$ )
- CANA lots had the longest mean MP length with a mean of 4.86 mm

## Top Three MP Colors and Morphologies

	Clear	Light blue	Black	Fiber	Fragment	Film
CANA Lot	56%	8%	9%	90%	3%	7%
Oyster Reef	65%	8%	8%	93%	3%	3%
Residential	61%	8%	6%	87%	6%	5%

## FTIR Results



- So far, FTIR has been performed on 23% of CANA lot samples, 26% of oyster reef samples, and 13% of residential samples
- 25% of particles tested were materials used in the textile industry, such as nylon, polyester, and PET
- 24% of the materials matched highly similar forms of anthropogenically modified cellulose, such as microcrystalline cellulose, Kayocel 10W80, and powdered cellulose
- Polytetrafluoroethylene (PTFE), commonly known as Teflon™, was matched to 7% of the tested materials
- 6% of particles were polystyrene, which is commonly used to make Styrofoam™ products such as single-use coolers

## Discussion

- CANA lot and oyster reef samples are in areas accessible to park visitors, which may contribute to the higher mean MP abundance compared to residential sites
- Several residential samples had very high mean MP abundance, potentially due to nearby home construction
- The mean length ( $\pm \text{SE}$ ) of MPs across all site types ( $4.26 \pm 8.43 \text{ mm}$ ) was larger than the mean length of MP found in 2021 in lagoon water ( $1.94 \pm 0.13 \text{ mm}$ )<sup>1</sup>, suggesting other MP sources are important in and around the IRL
- The presence of Teflon™ highlights the need to consider MP sources beyond the commonly studied MPs (PET, PP, PE etc.)
- Inhaling MPs serves as a pathway for bioaccumulation, a recent study found MP concentrations of  $3345 \mu\text{g g}^{-1}$  in human brain tissue samples<sup>4</sup>
- This study will help policymakers consider strategies that will aid in reducing microplastic pollution by limiting certain single-use plastics (e.g., Styrofoam™)

## Future Directions

- This project will conclude a full year of collecting samples in May 2025
- As concern over the risks associated with inhaling MPs rises, continued research on the AD of MPs is needed worldwide
- Informed policies and regulations are essential to reduce MP pollution and protect vulnerable environments and public health

## Acknowledgments

Thank you to all the citizen scientists and CEELab members who helped collect and process samples. Thank you to MDC for facilitating residential collections, Canaveral National Seashore for site access, and the IRLNEP Small Grant Project for funding.

## Citations

<sup>1</sup>Zhang, Y., et al. (2020). Atmospheric microplastics: A review on current status and Perspectives. *Earth-Science Reviews*, 203 v.2020.103118 <sup>2</sup>Allen, S., et al. Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nat. Geosci.* 12, 339–344 (2019). <sup>3</sup>Walters, L., et al. (2021). *Microplastics, oysters, and the Indian River Lagoon - UCF stars, STARS*. <sup>4</sup>Nihart, A., et al. (2025). Bioaccumulation of microplastics in decedent human brains. *Nature Medicine*. <https://doi.org/10.1038/s41591-024-03453-1>



# Analysis of Microplastic Concentrations in Dried Algae Mats and Sediment Collected from Detention Basins in the Edwards Aquifer Recharge Zone

## Introduction

- Plastic pollution is growing exponentially
- Stormwater runoff from roads contains multiple pollutants that end up in surface waters or groundwater
- Detention basins are used to capture, temporarily store, and allow infiltration of stormwater runoff
- Algae might act as the first line of defense for capturing microplastics (MPs) in freshwater systems
- Sediment can tell us more about the movement of MP through detention basins



Roadway runoff



Detention pond holding roadway runoff

## Objectives

- Quantify the amount and types of MPs within algae mats and sediment layers of the basin
- Compare MP concentrations between the upper and lower layers
- Examine differences in MP accumulation across various detention basins.

## Study Area

Map of the US showing the location of the study area in Texas.



a

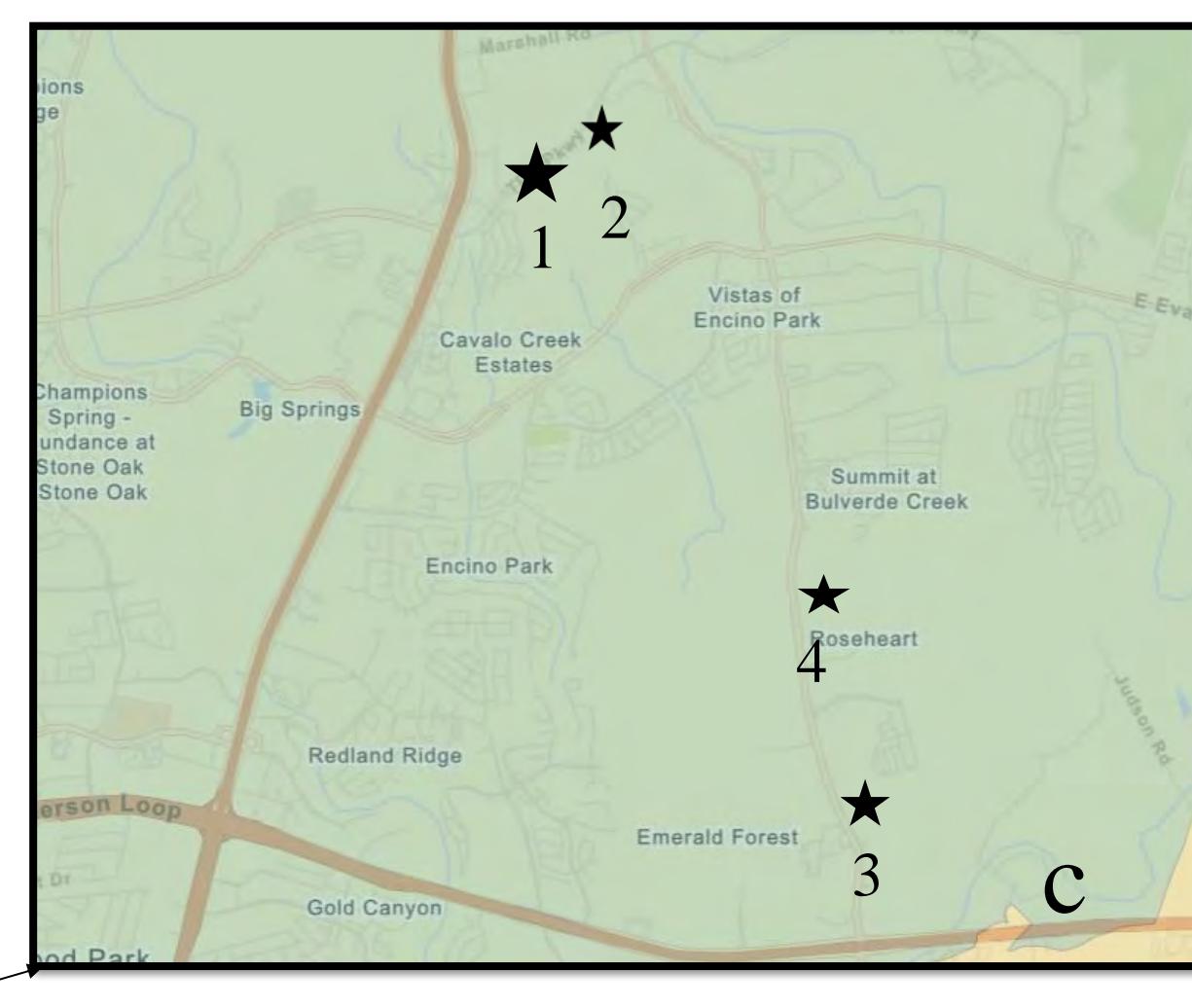
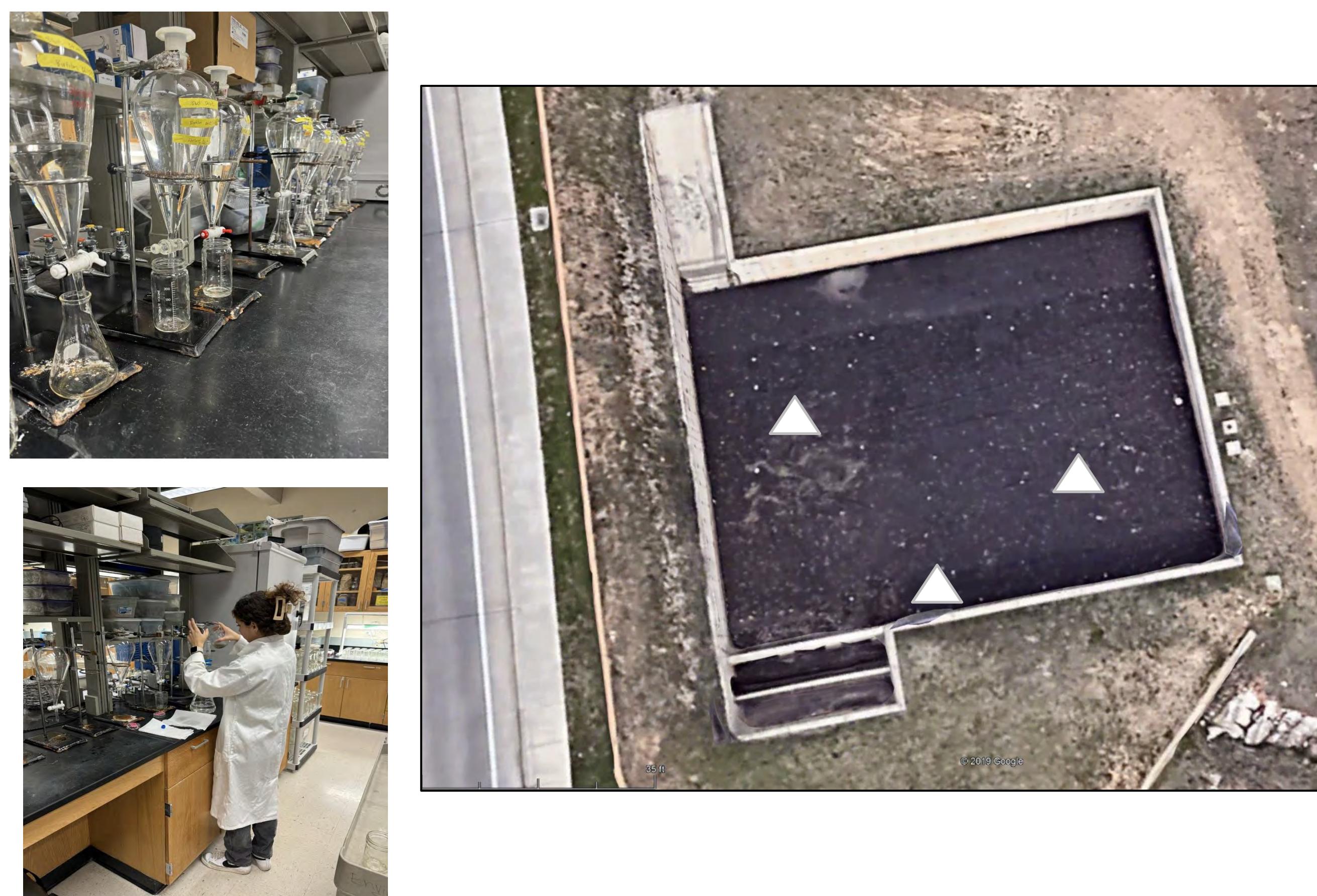


Figure 1. a) Map of the US to show which state the study is being conducted in b) Edwards Aquifer Region (blue is the recharge zone), c) location of detention basins along the recharge zone

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## Methods

- Sediment and algae mats were processed under similar conditions
- Digestion using peroxide, separation by flotation and vacuum filtration were used to isolate MPs
- MPs were visually identified. Unidentifiable MPs were identified with FTIR



## Preliminary Results

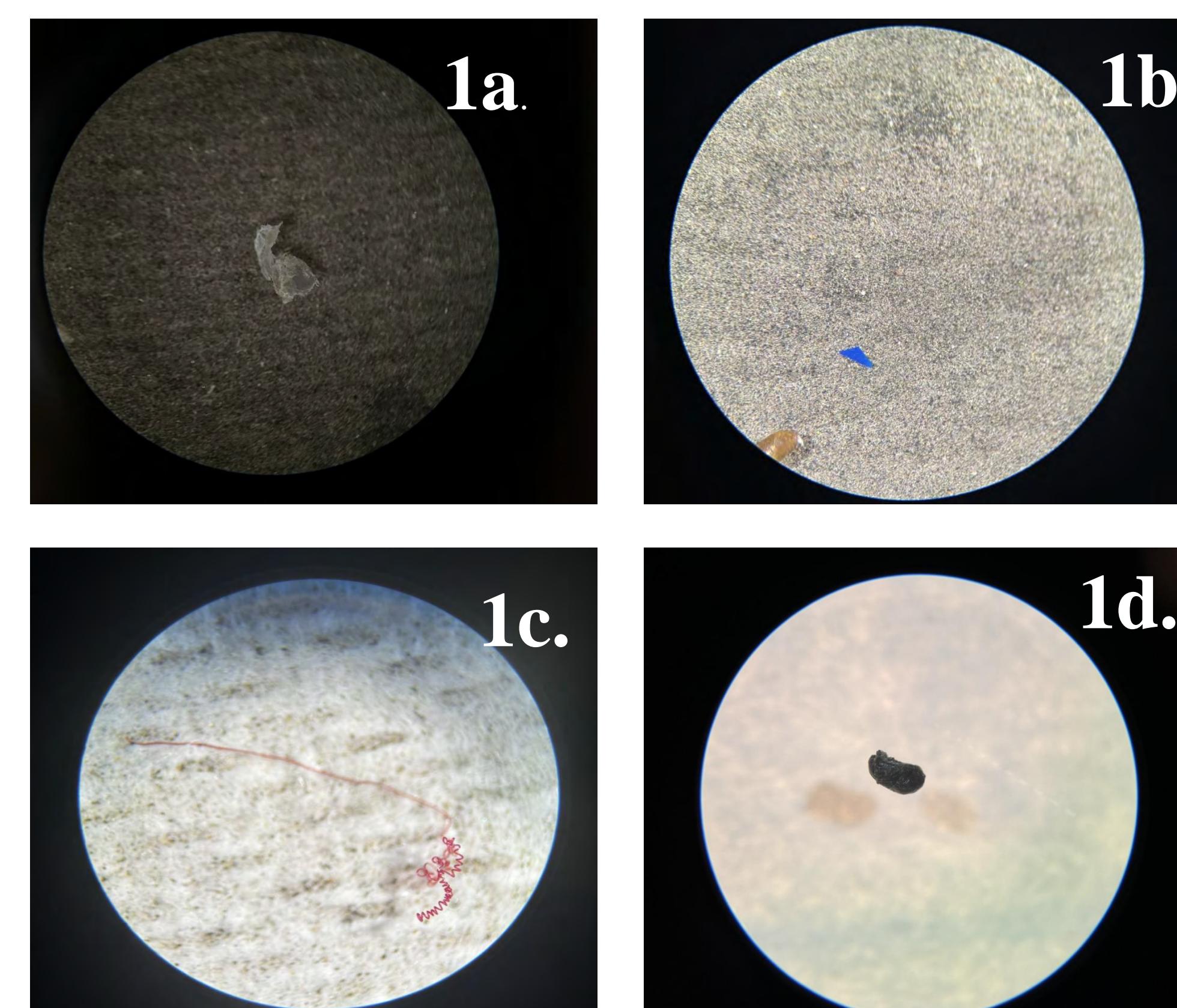


Figure 2. Examples of MP found in detention ponds found in the Edwards Aquifer recharge zone; 1a. Teflon, 1b. PMMA, 1c. Polyamide 1.d. Polyurethane foam

## Preliminary Results (cont.)

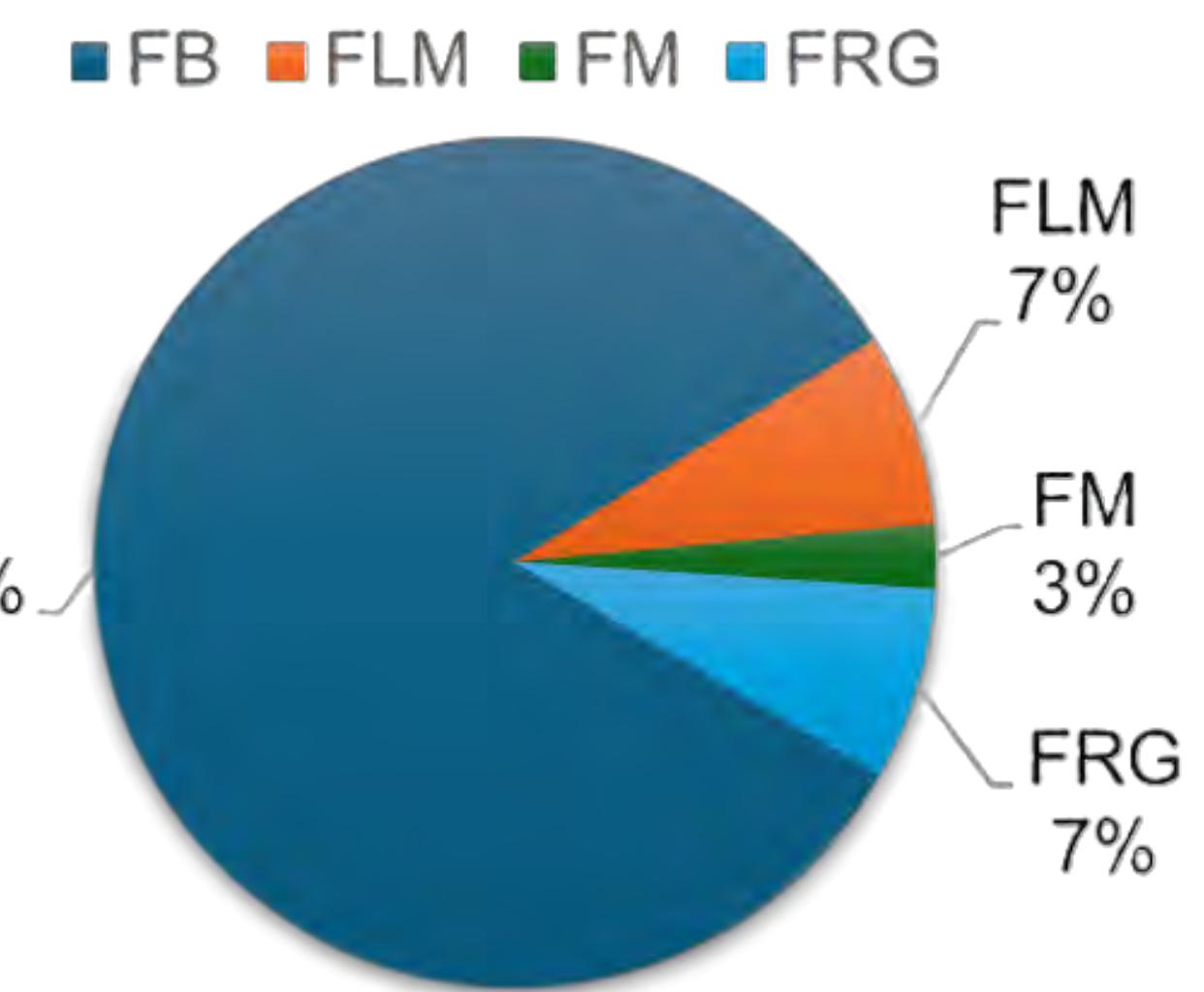


Figure 3. Percent morphotype of polymers: Fiber (FB), Film (FLM), Foam (FM), and Fragment (FRG)

Basin 2 Basin 3 Basin 4

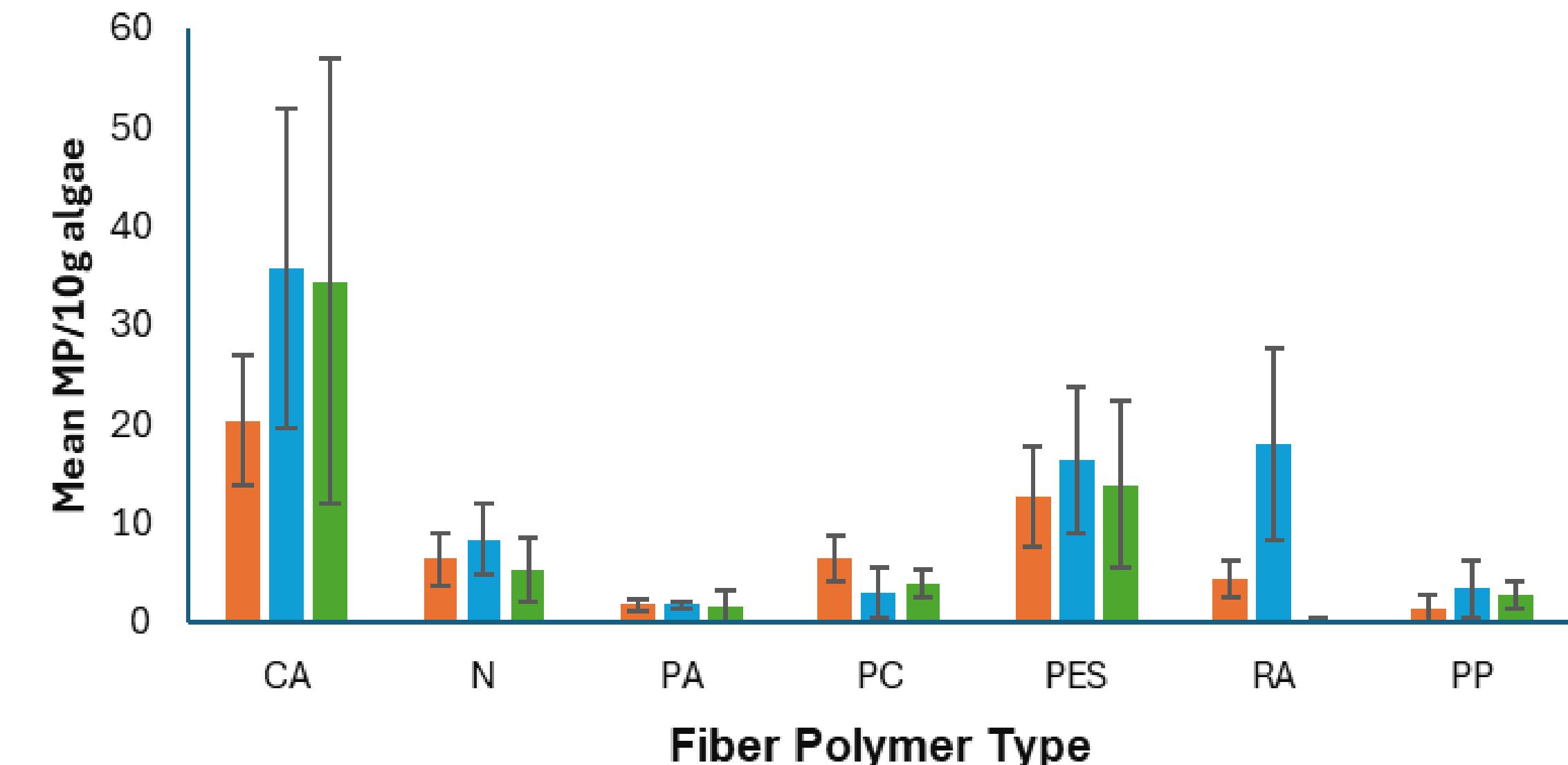


Figure 4. Mean bar graph of mean MP/10g algae with standard error bars for 3 basins. Most common fiber polymer type are abbreviated as follows: Cellulose Acetate (CA), Nylon (N), Polyamide (PA), Polycarbonate (PC), Polyester (PES), Rayon, (RA), and Polypropylene (PP).

## Conclusion

- Fibers are the most abundant throughout the study
- While MP such as Teflon and PMMA are found in low abundance, it should be of concern for potential carcinogens getting into our ground water
- Pharmaceuticals are also present in the basins
- Prelim evidence suggest detention basins are effective at capturing MP
- Data suggest maintenance plan for these basins from the city should be revised

## Acknowledgements

City of San Antonio and associated partners for providing funding



# Degradation of Polyethylene Terephthalate Fibers by *Zophobas morio* Larvae

Isabel Li, Dr. Kasia Dinkeloo, Bioprospecting 2.0

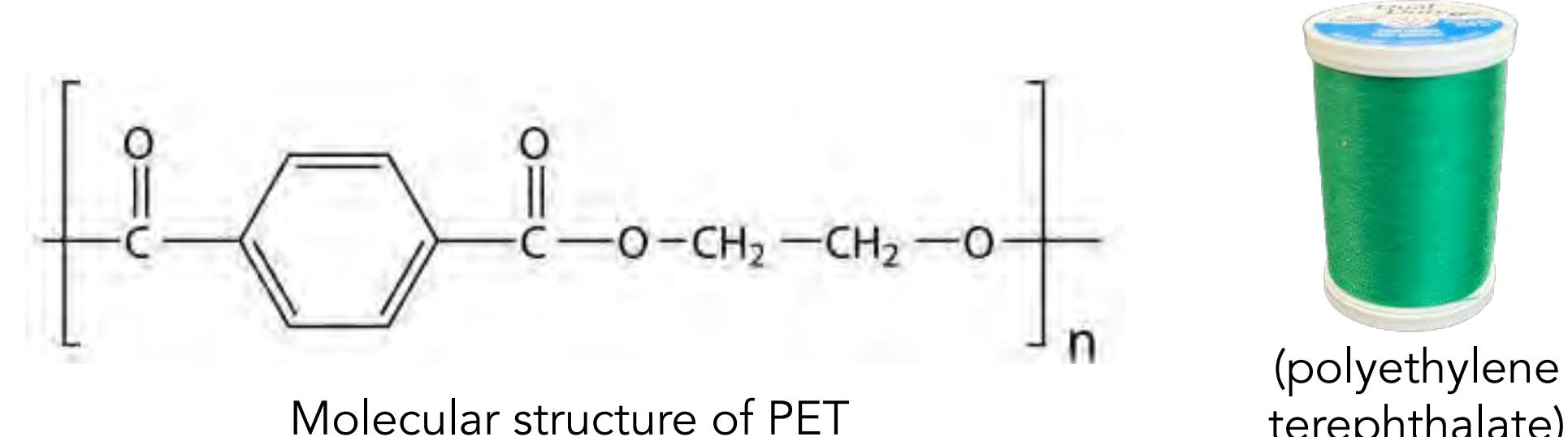
## Introduction

With an estimated 13-25 million metric tons of plastic emitted into terrestrial environments and 9-23 million metric tons of plastic emitted into rivers, lakes, and oceans per year (MacLeod et al., 2021), plastic pollution is a growing threat to the environment and human health. Moreover, this pollution has proven difficult to mitigate: of the 8,300 million tonnes of plastic materials manufactured in the past 70 years, only 6% was recycled (Liu et al., 2024). Therefore, some researchers have explored enzymatic breakdown of plastic as a solution to current pollution and disposal issues. A possible source of plastic-degrading enzymes lies in the gut microbiota of *Zophobas morio* larvae. These superworms are capable of breaking down certain plastics with contributions from microbes in their gut (Yang et al., 2020). Harnessing these plastic-degrading microbes or their associated enzymes could facilitate a bioprocess for large scale plastic recycling. Though investigation into the gut microbiota of superworms is still in its early stages, prior research has already been conducted on superworm ingestion of plastics like polystyrene, polyurethane, and ethylene vinyl acetate, among others (Weng et al., 2024). However, comparatively less research has been done on superworm consumption of PET (polyethylene terephthalate), a polymer commonly found in textiles as polyester. Because textile fibers, more than half of which are made from oil-based PET (Palacios-Mateo et al., 2021), are estimated to be the largest microfiber source in the environment (Liu et al., 2021), finding methods to reduce PET emissions is highly relevant.

## Research Questions

The primary research questions this project seeks to answer are:

- Can larvae of *Zophobas morio* degrade PET fibers?
- If so, what microbes from the gut of *Zophobas morio* larvae are involved?



## Methods and Materials

Superworms were kept in an enclosure at room temperature and were fed with 1.29g of polyethylene terephthalate in the form of 100% polyester thread (n=25) or fed with carrots as a control group. The feeding trial took place over two weeks, during which the weight and frass of the superworms was monitored. After the trial concluded, ten superworms were selected for gut extraction. One of the dissected gut was used for microscopic examination. Three of the gut samples were used for DNA extraction with column-based purification. First, the cells in the gut tissue were lysed using buffers and mechanical disruption methods like vortexing. The lysate mixture was transferred onto a column, where further purification occurred using wash buffers and a centrifuge. DNA was then eluted out of the column with an elution buffer, concluding the purification process. The resulting DNA sample was evaluated with both a NanoDrop Spectrophotometer and a Qubit 4 Fluorometer. PCR was used to amplify the 16S region of DNA in various microbial species from the sample to check the quality. This DNA contained genomic information of microbes in the superworm gut and was then used for metagenomic sequencing with Oxford Nanopore's MinION device. This device uses tethering proteins to run DNA fragments through nanopores, where the voltage change is measured to find base sequences. The sequencing was then analyzed with EPI2ME, a bioinformatics application for Nanopore sequencing. Specifically, the sequence data was aligned against a microbial genomic DNA database using BLAST, and the sequence reads were subsequently quantified. In addition, three of the dissected guts were used for culturing. The guts were mixed with saline solution and centrifuged. The resulting product was cultured in LCFBM (Liquid Carbon-Free Basal Media) with polyester thread acting as a carbon source. After 1.5 months of growth, microbes were isolated, identified, and used for further testing by growing colonies on selective cultures. The microbial DNA was extracted, sequenced, and analyzed according to the procedures outlined above.

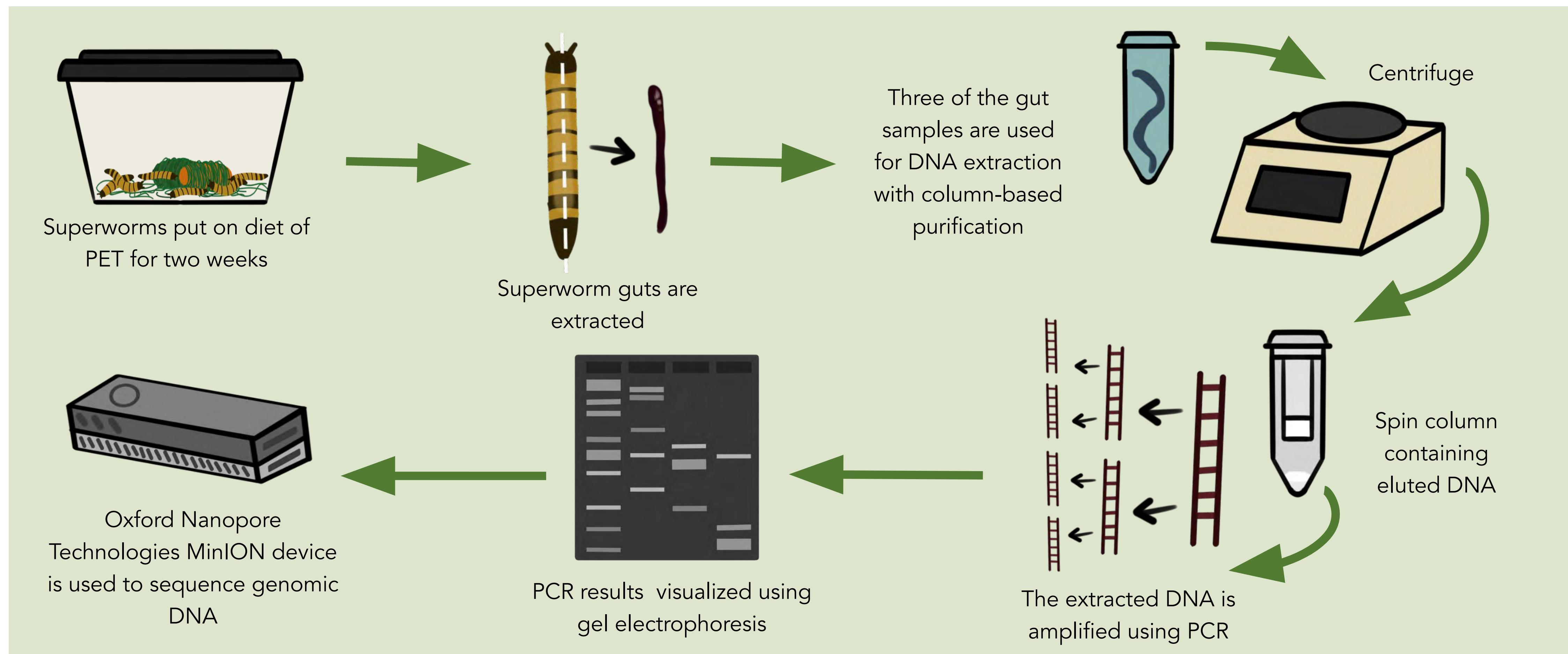
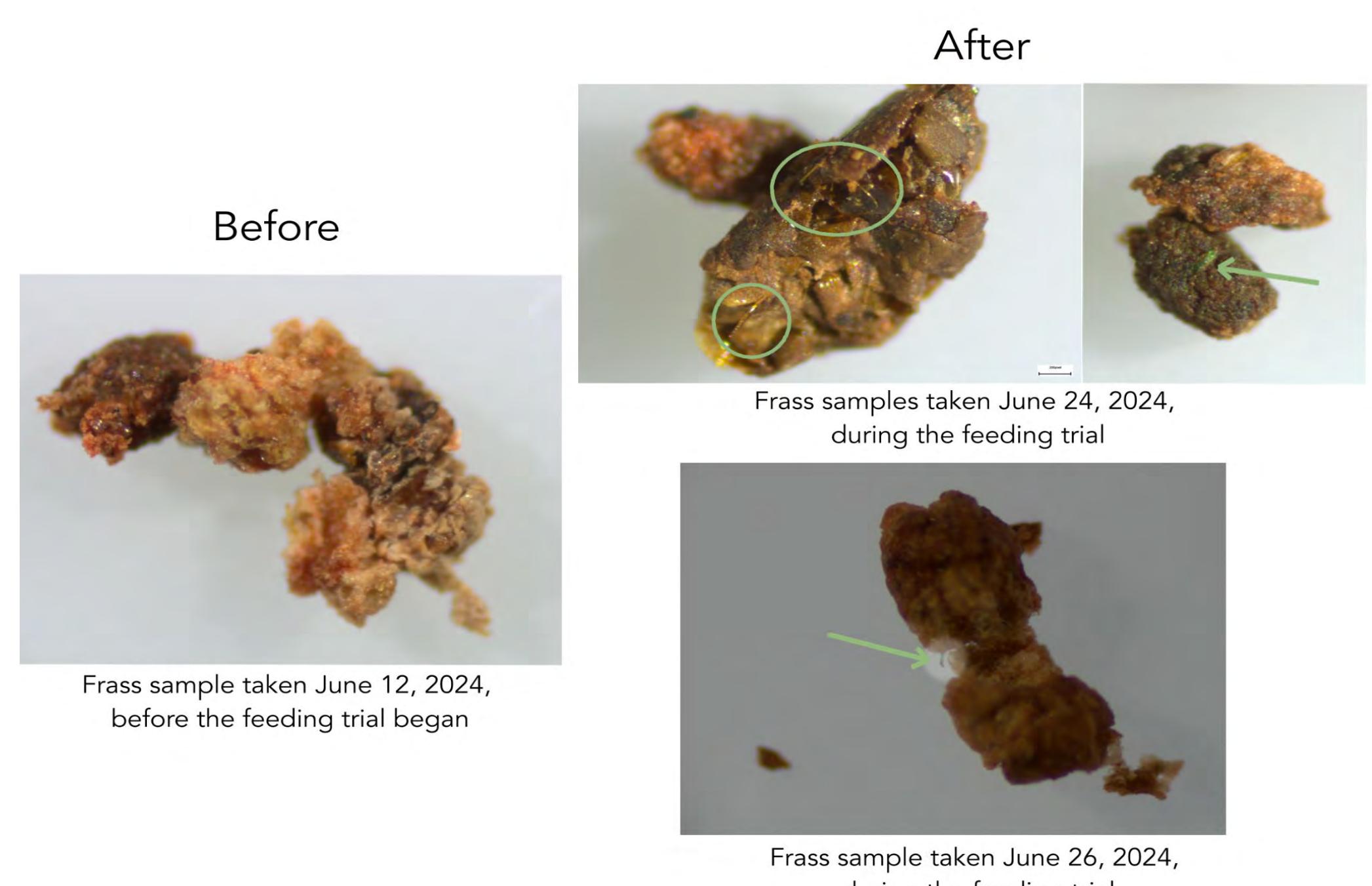
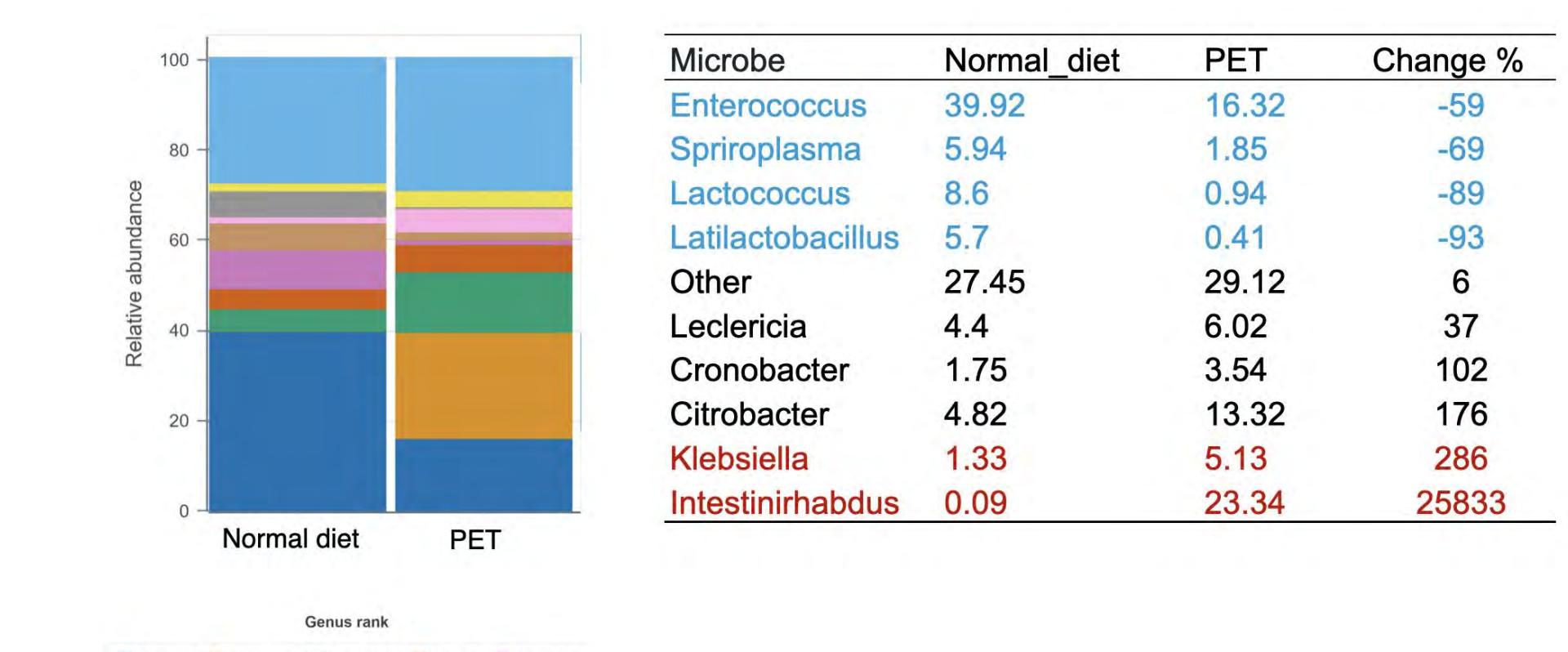


Diagram of the methods and materials used in the research workflow

## Results



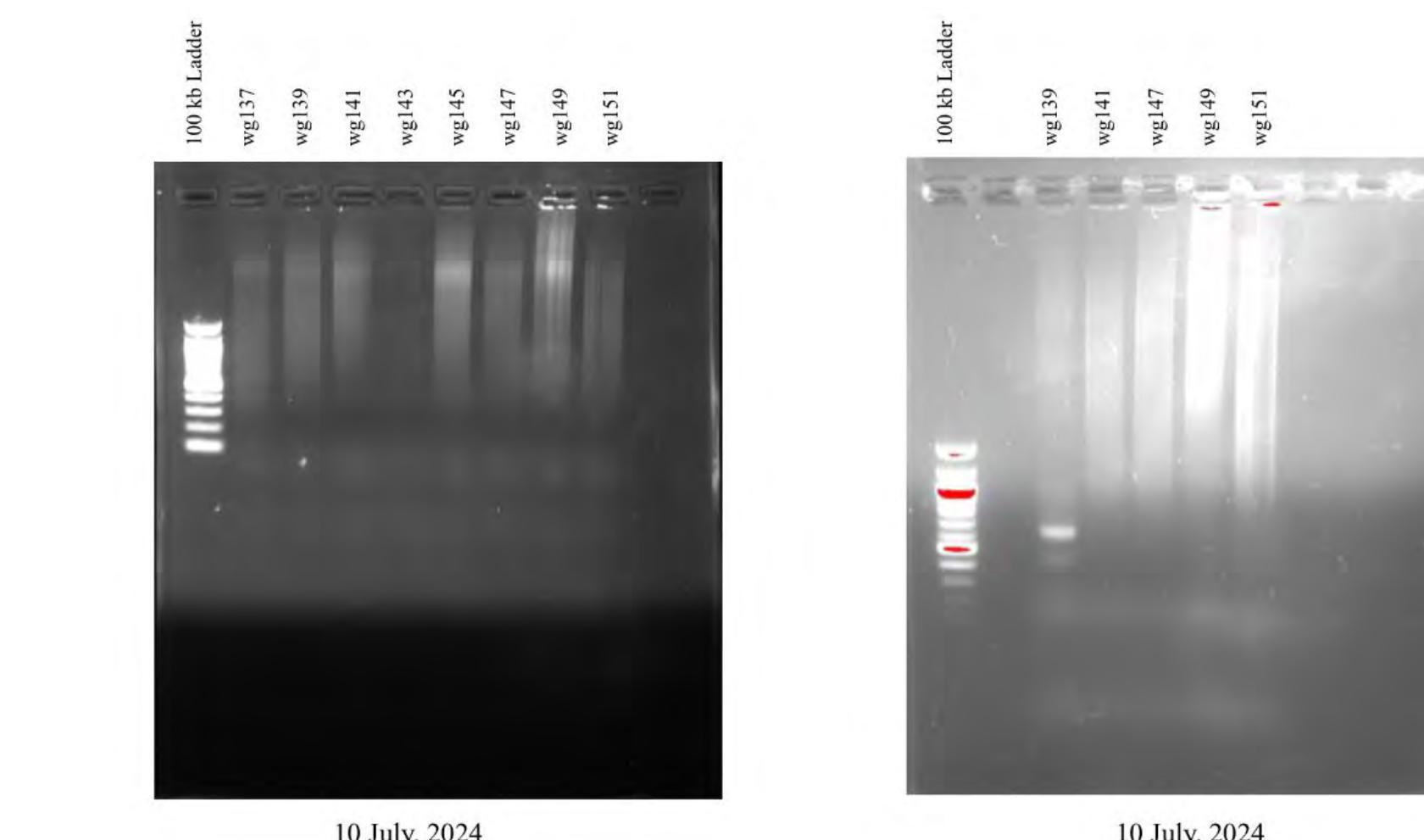
Images of superworm frass and guts taken from a stereo microscope showing evidence of plastic consumption



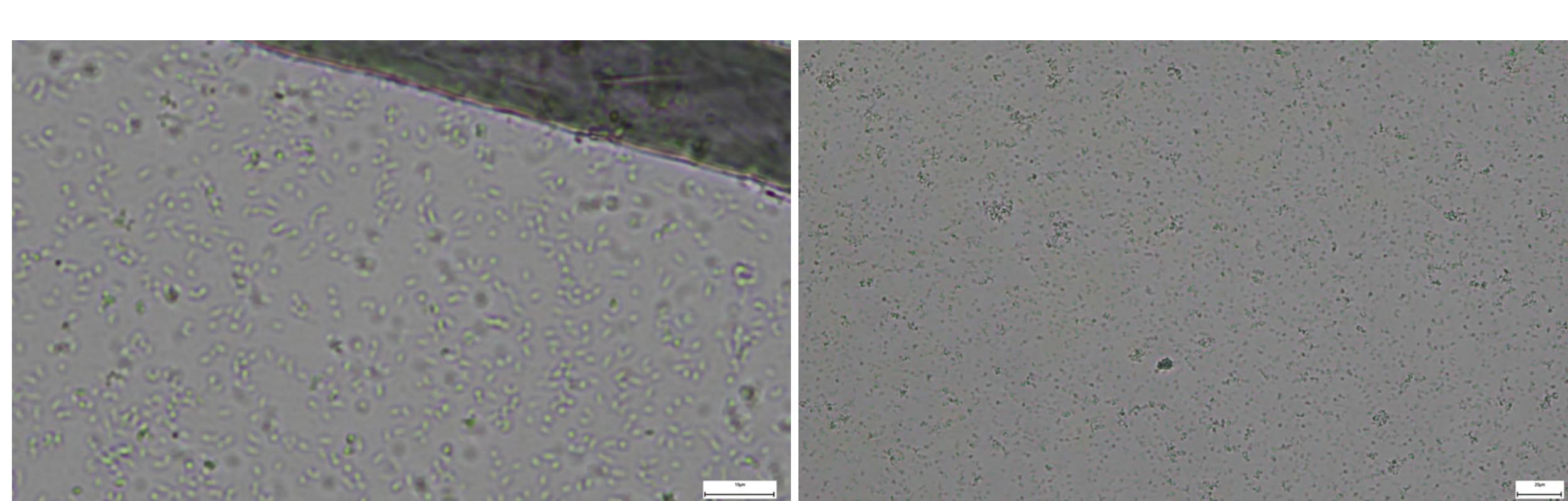
Graph showing relative abundance of bacterial genera from the guts of control superworms and the guts of PET-fed superworms.

Microscope examination of the frass and gut revealed that superworms had consumed PET. Photos taken with a stereo microscope showed plastic fibers and fragments embedded in frass and gut samples, indicating that superworms are capable of digesting PET in the form of polyester thread.

The results from the sequencing report, obtained after a two-week feeding trial, demonstrated the relative abundances of various bacterial genera present in the gut microbiota of superworms from both the control group (fed a normal diet) and the experimental group (fed PET). These findings highlighted notable shifts in microbial composition associated with the altered diet. Most significantly, the genus *Intestinibacter* exhibited a dramatic increase in relative abundance—rising by approximately 25,833%, or over 258-fold—when compared to the control group. Additionally, the genus *Klebsiella* showed a substantial increase of 286%, more than doubling in relative abundance relative to the control group. These pronounced changes suggest that specific bacterial taxa may play a role in the gut's adaptation to plastic-based diets.



Finally, after 1.5 months of selective liquid culturing, the sequencing results of the isolated microbial colonies revealed the presence of a specific bacterial strain. This microorganism was identified as *Stenotrophomonas maltophilia*, a bacterium known for its adaptability and environmental resilience.



## Discussion

Microscopy revealed that superworms are capable of digesting PET supplied as 100% polyester thread during a two-week feeding trial, and sequencing data indicated changes in their gut microbiota. Selective liquid culturing from the guts of polyester-fed superworms was used to pinpoint the microbes responsible for breaking down PET, and *S. maltophilia* was identified. This bacterium has been previously recognized as a potential candidate for breaking down plastics such as LDPE (Selvaraj et al., 2024), PVC (Ye et al., 2024), and PLA (Jeon and Kim, 2013). In a 2023 study, a cutinase-like enzyme from *S. maltophilia* PRS8 was isolated which efficiently hydrolyzed PET at mesophilic temperatures, underscoring its potential for PET degradation (Din et al., 2023). Consequently, further studies may aim to characterize the plastic-degrading enzymes produced by gut microbes in PET-fed superworms, with the long-term goal of applying these enzymes to significantly reduce plastic emissions. Interestingly, Nanopore sequencing of two-week PET-fed superworm guts detected only a single read for *Stenotrophomonas*. A similar observation was made in an earlier study where superworms were fed with plastics such as HDPE, PP, and PS. In that study, *in vitro* incubation of the superworm gut microbiome led to a considerable shift in microbial composition, with *Stenotrophomonas* emerging as a dominant genus (Liu et al., 2024). The differences observed between the short-term feeding trial and long-term culture suggest a self-adaptation mechanism within the superworm gut. Our observations underscore the importance of additional experiments to confirm the findings. Future studies, including repeated growth trials, could verify whether *S. maltophilia* consistently appears in the metagenomic sequences of PET-fed superworm guts.

## Acknowledgments

We thank the following individuals for their guidance and support throughout the research process: Dr. Kasia Dinkeloo, Cade Koenig, Andrea Ordonez, Katherine Del Cairo, Emma Lopez, Kaden Allen, and Rosalyn Canchola. Additional thanks to the H-E-B Grocery Company for providing funding.



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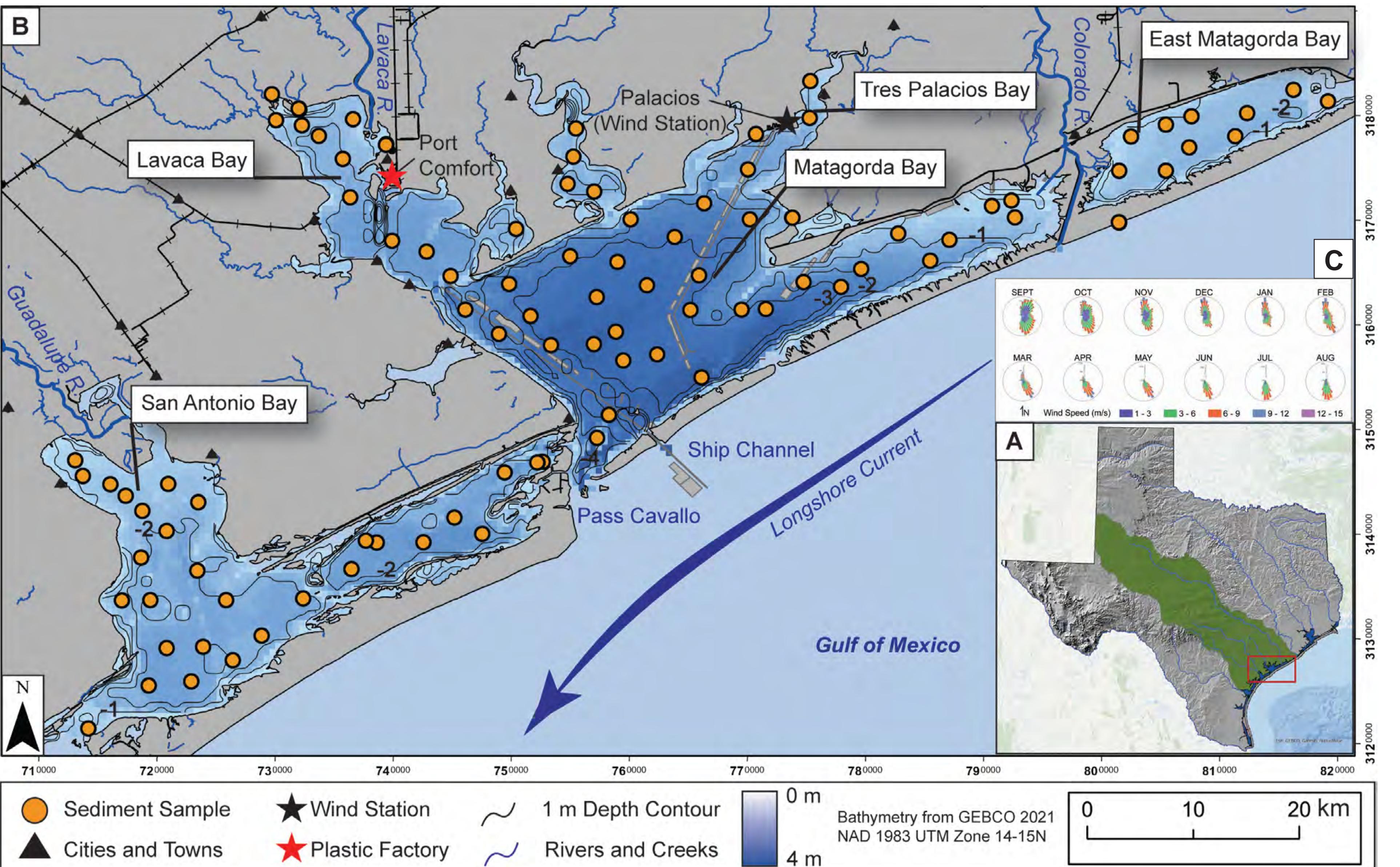
# Dynamics of Marine Litter Post-Hurricane Beryl: Assessing the Ultimate Fate of Flotsam

William Bailey<sup>a</sup>, David Mohrig<sup>a</sup>, Cornel Olariu<sup>a</sup>, Kutalmis Saylam<sup>a,b</sup>

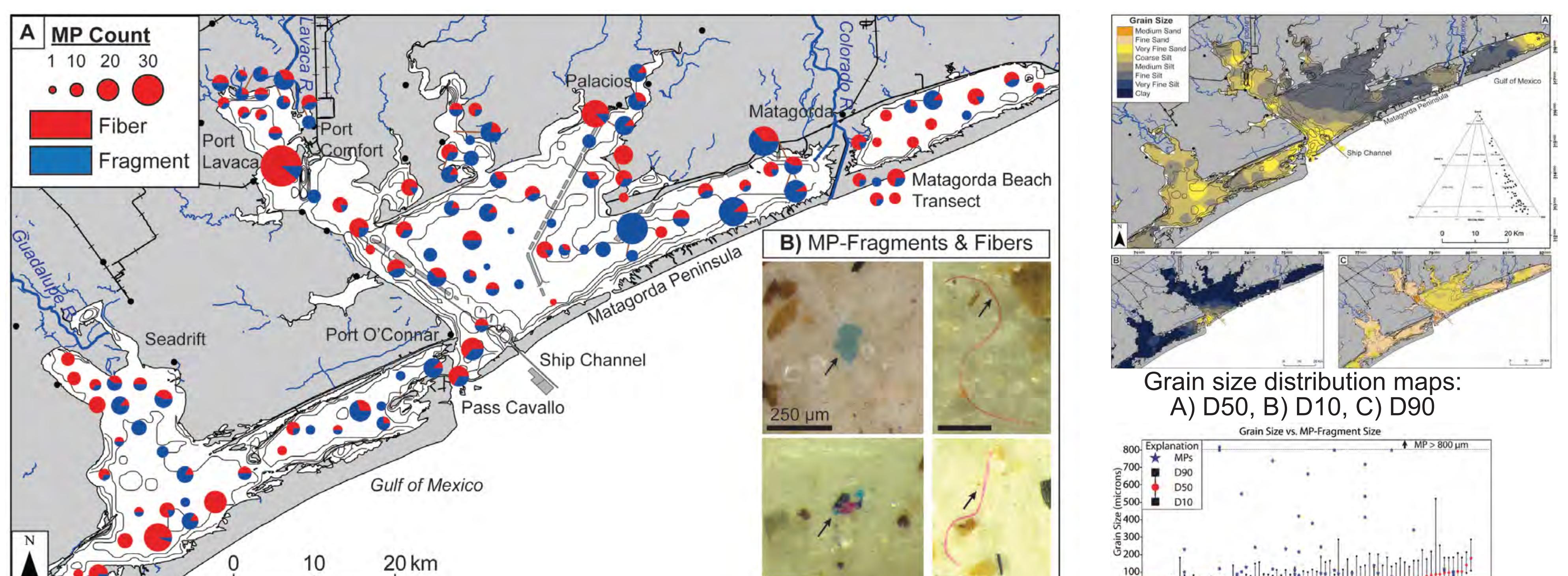
<sup>a</sup>Jackson School of Geosciences | <sup>b</sup>Bureau of Economic Geology | The University of Texas at Austin

## Introduction: Plastics in Coastal Bays and Barriers

- Research of microplastics along the Texas coast to better understand where these pollutants may form hotspots through transport and depositional processes
- First project targeted Texas bays hypothesized to be depocenters for sediment and microplastics
- Second project targeted the barrier island after Hurricane Beryl to better understand where plastics concentrated during storms



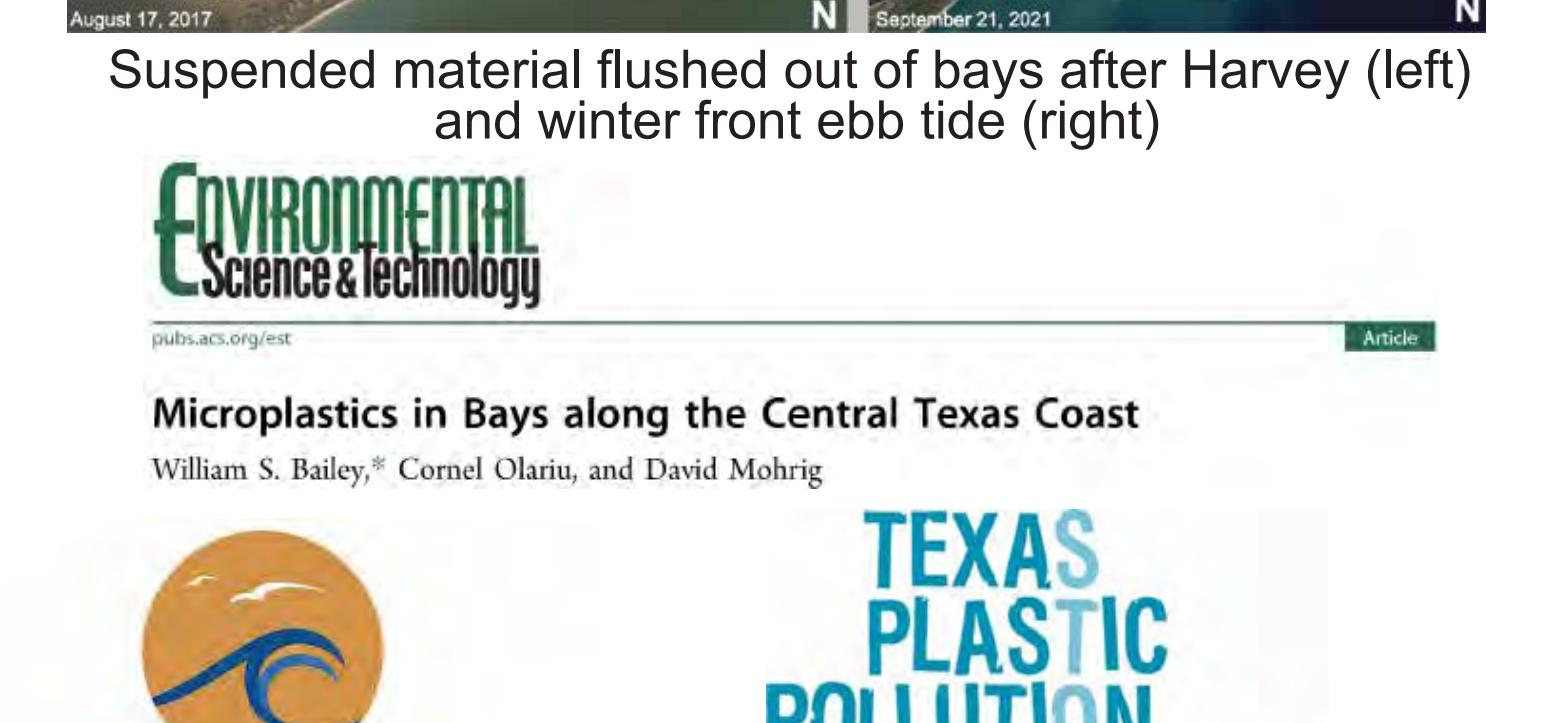
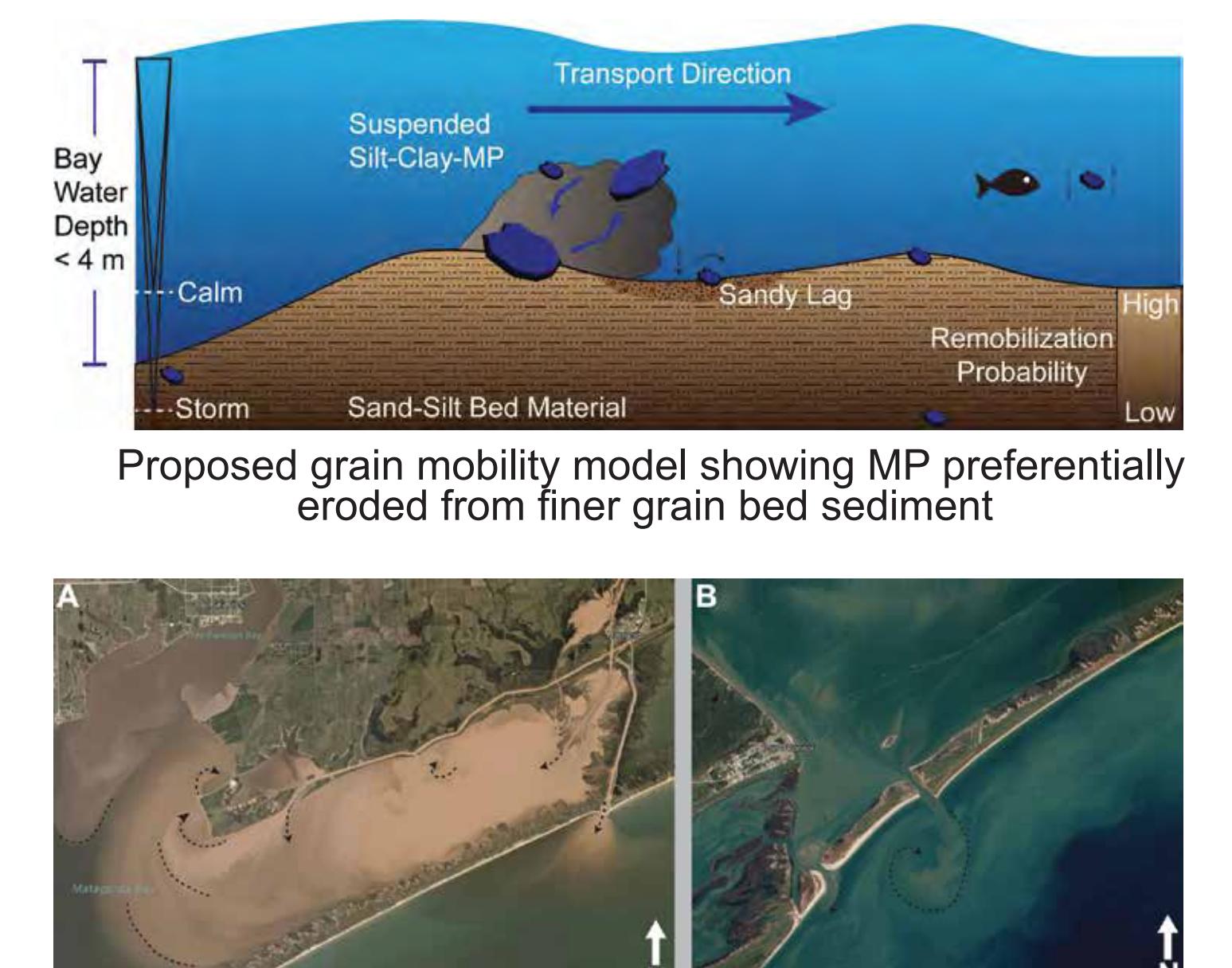
A) Inset map of study area location on Texas Gulf Coast with extent of the river drainage basins discharging into the bays.  
B) Study area with locations of collected sediment samples. Principal rivers and inlets representing permanent marine exchange points.  
A red star indicates location of known plastic point source. C) Average monthly wind roses from Palacios, TX.



A) Distribution map of micro-fibers and -fragments, proportional symbols represent total microplastic concentration. B) Microscopic images illustrating MP examples.

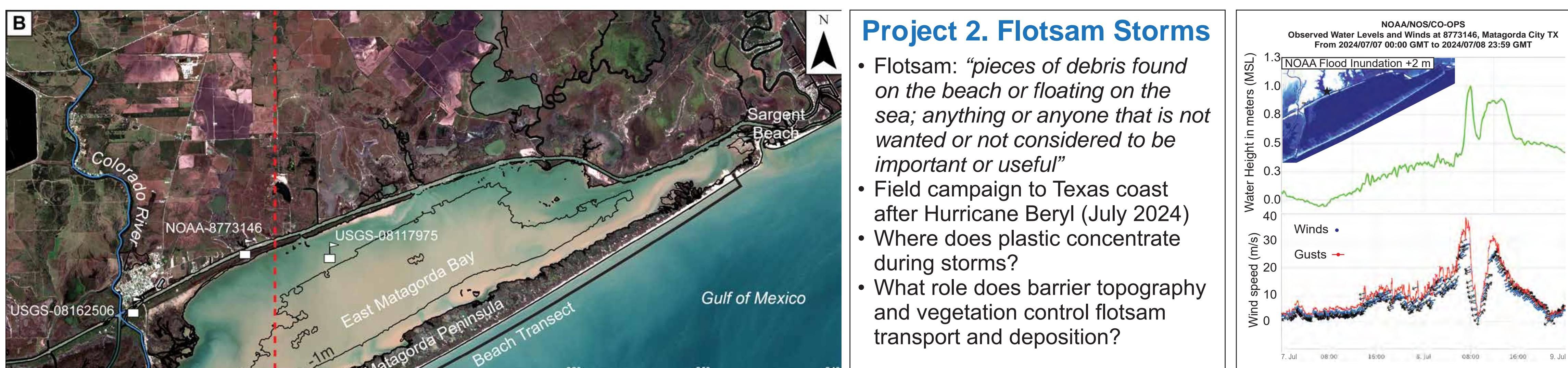
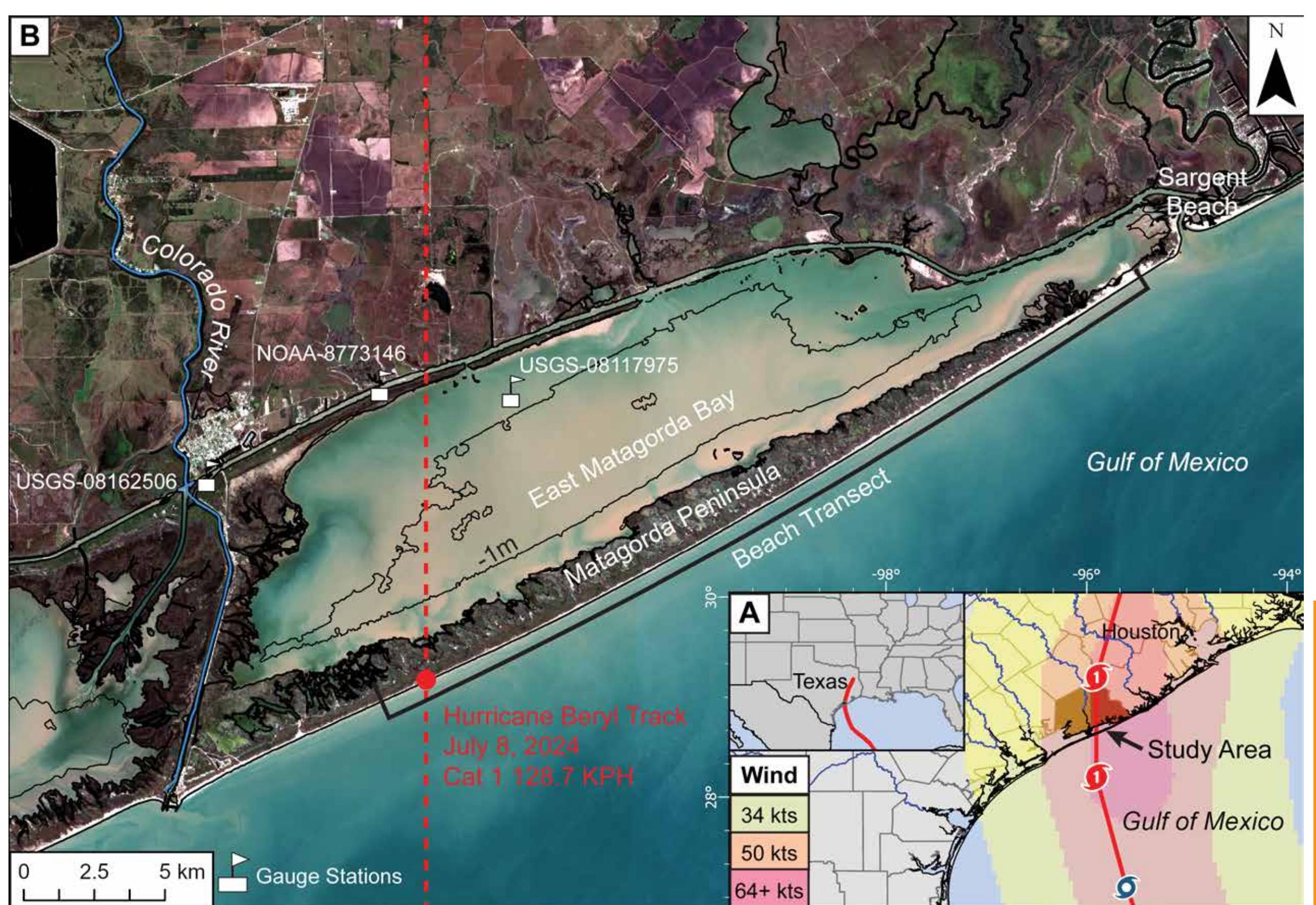
## P1. Bay Microplastics Key Takeaways

- Larger microplastic particles (fragments:  $178 \pm 93 \mu\text{m}$ , fibers: 0.5-2.0 mm) consistently deposited with finer sediments indicating high transportability.
- The high degree of microplastic resuspension into bay waters has significant implications for limiting microplastic accumulation within bay sediments.
- This work provides a baseline for future studies quantifying the roles of wind on residence time of microplastics in coastal environments.



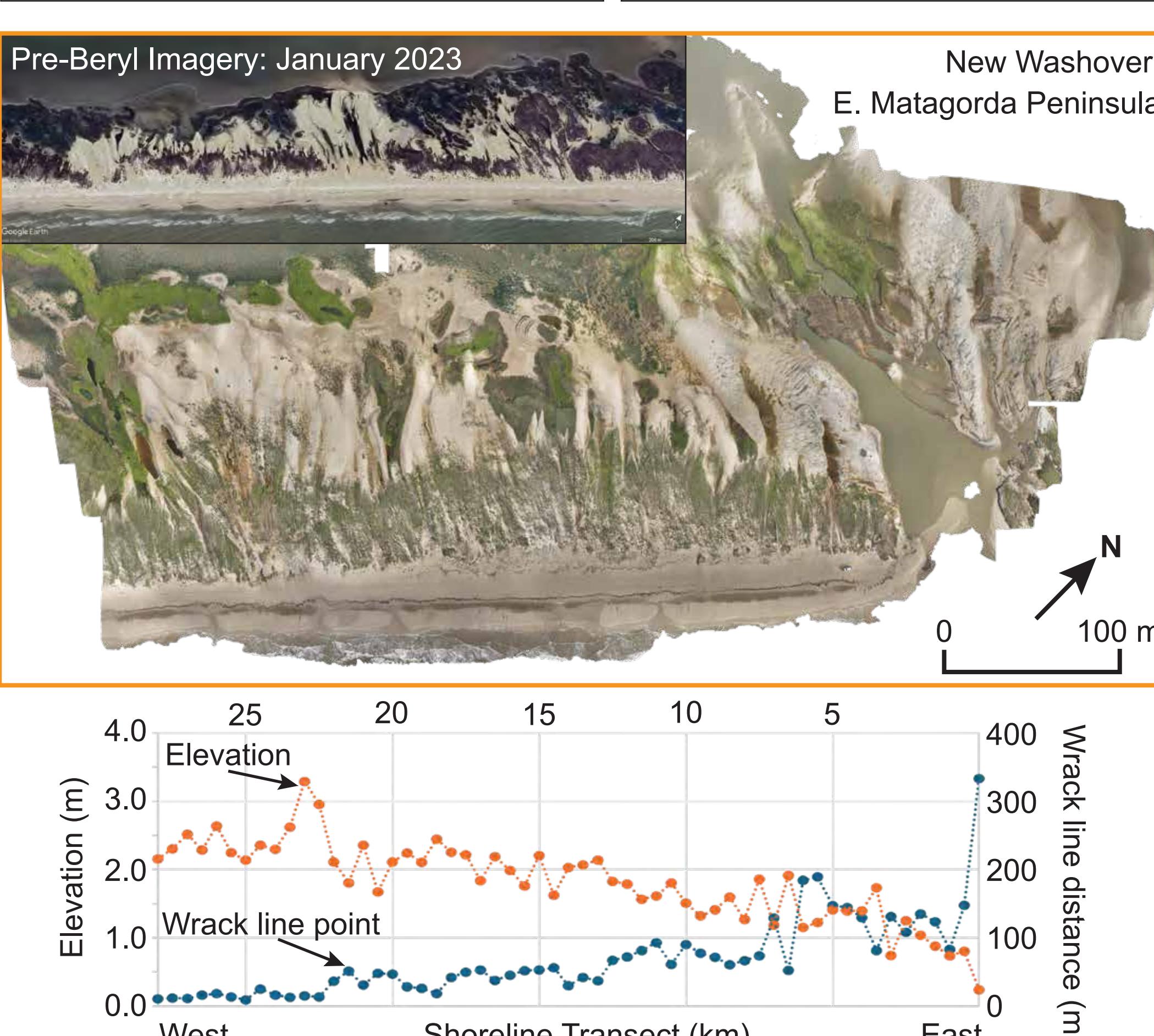
This work was supported by the Matagorda Mitigation Trust and the Jackson School of Geosciences, University of Texas at Austin. We greatly appreciate the Near Surface Observatory at the Bureau of Economic Geology, UT-Austin, for providing access to the pre- & post-storm lidar. We thank Josh Malone for acquiring the drone imagery and field assistance.

Contact: William Bailey | Email: [wsbailey@utexas.edu](mailto:wsbailey@utexas.edu)

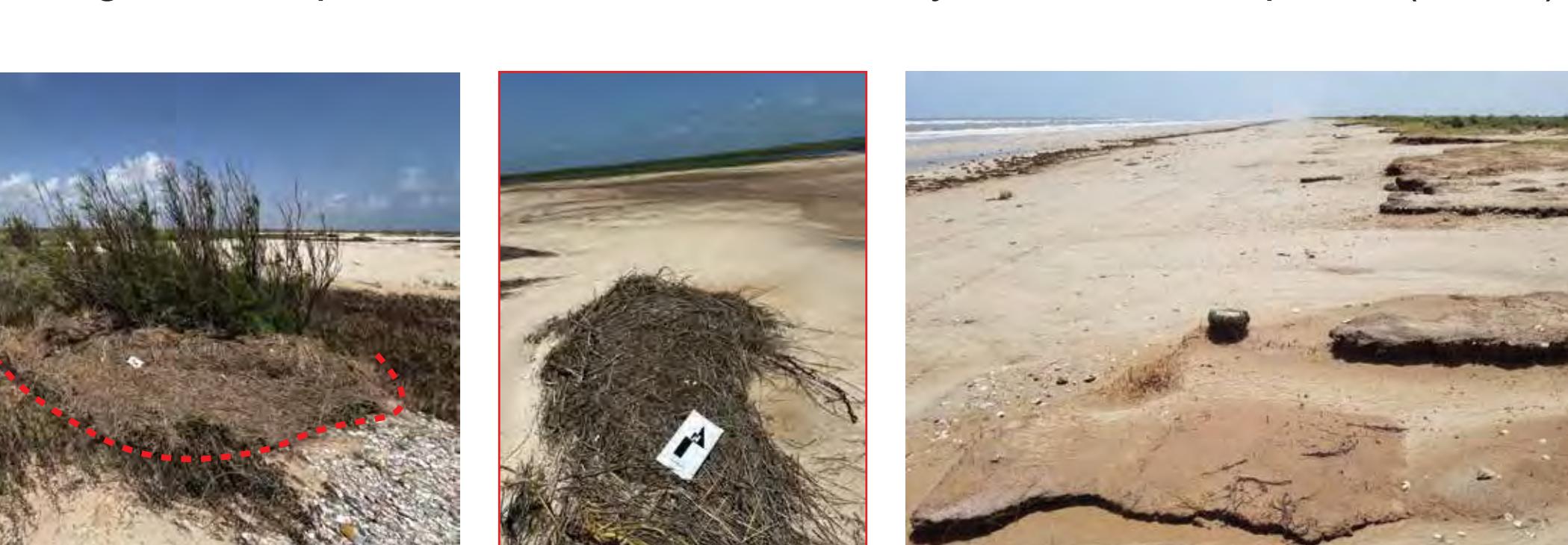


## Project 2. Flotsam Storms

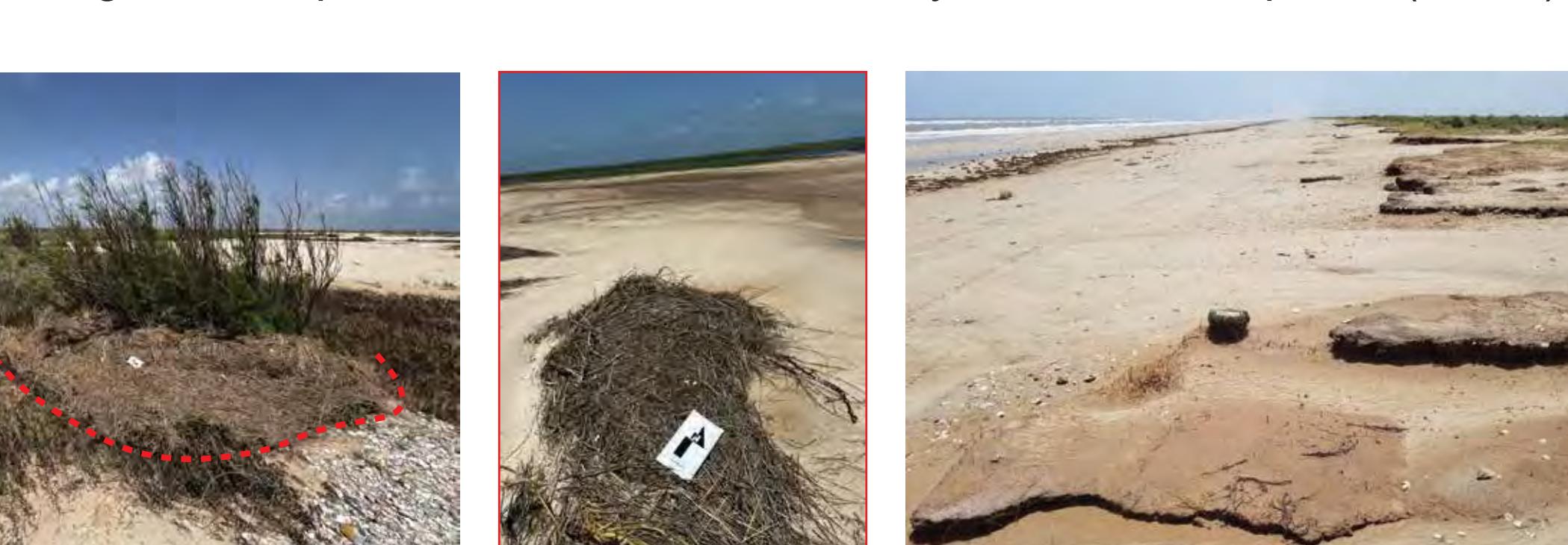
- Flotsam: "pieces of debris found on the beach or floating on the sea; anything or anyone that is not wanted or not considered to be important or useful"
- Field campaign to Texas coast after Hurricane Beryl (July 2024)
- Where does plastic concentrate during storms?
- What role does barrier topography and vegetation control flotsam transport and deposition?



Ponds acting as natural "decanters"



New washovers

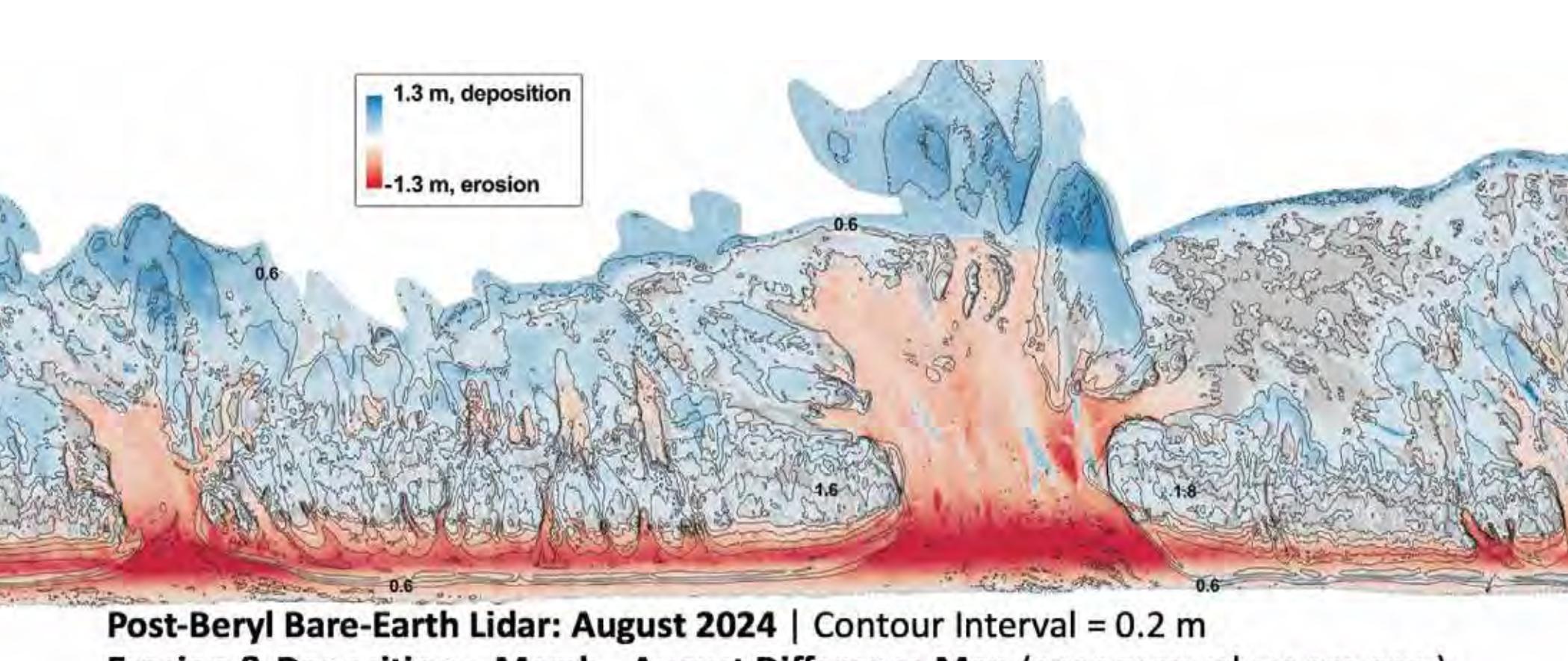


New washover fans eastern end of beach transect (above) with isolated plastic "rafts" in organic-rich patches in otherwise relatively clean sand deposits (below)



Plastic-filled flotsam (red lines) plastered along seaward edges of vegetation and exposed Hocene mud evidence of significant erosion (above)

Flotsam deposits associated with dune topography (left)



Post-Beryl Bare-Earth Lidar: August 2024 | Contour Interval = 0.2 m

Erosion & Deposition = March - August Difference Map (near-zero values are gray)

## P2. Results & Discussion

- Flotsam may be quantified using first return lidar surveys to illustrate depositional hotspots after storms.
- New overwash fans indicate large quantities of debris sourced to the back-barrier and bay sediments, as well as return channels exporting material to the Gulf.
- Given the ability of storms to erode material from bays and shorelines, the Gulf of Mexico is likely the ultimate fate for coastal pollutants.

## Why Should We Care?

- We dump plastic (and microplastics) into the rivers and coastal waters.
- While our pollutants are seemingly washed away to the ocean, storms reintroduce our unwanted debris (flotsam) to our coasts choking us back.
- The answer to the flotsam problem depends on topography / bathymetry of the coast, frequency of storms, or simply, the sedimentary dynamics of the coast, thus requiring more studies.

# Effects of PET Microfiber Exposure on Mating Behavior, Foraging Behavior, and Cognitive Flexibility in *Gambusia affinis*

Jingping Gruber, Adrienne Lihou, Rivers Hartzell, Maxximus G. Ramsaroop, Molly Cummings

The University of Texas at Austin



The University of Texas at Austin  
Department of Integrative Biology  
College of Natural Sciences

## Introduction

Plastic production has increased dramatically in the last century, causing many adverse environmental impacts (Li et al., 2024). Although plastics are notable for their longevity, factors such as photodegradation, abrasion, and erosion can lead to fragmentation (Geyer et al., 2020 & Prüst et al., 2020). These fragments, known as microplastics (MPs, plastics <5mm in diameter), vary in shape, size, polymer type, and other traits. Polyethylene terephthalate (PET) microfibers are among the most common and persistent forms in aquatic systems (Reblein et al., 2021).

Various organisms ingest MPs, which can cause adverse effects on behavior and cognition (Ma et al., 2019 & Jacquin et al., 2020). These behavioral/cognitive impacts can create feedback loops, perpetuating individual exposure and bioaccumulation of plastics and leading to population impacts that traverse evolutionary time spans by creating adaptive or maladaptive responses (Jacquin et al., 2020).

Despite their importance, there is a substantial lack of experiments studying environmentally relevant MP concentrations, and PET microfibers are seldom used despite their prevalence (Geyer et al., 2022 & Cunningham & Sigwart, 2019). To optimize standardization and ecological relevance in this study, *G. affinis* will be exposed to high and environmentally relevant concentrations of 42 $\mu$ m PET MPs.

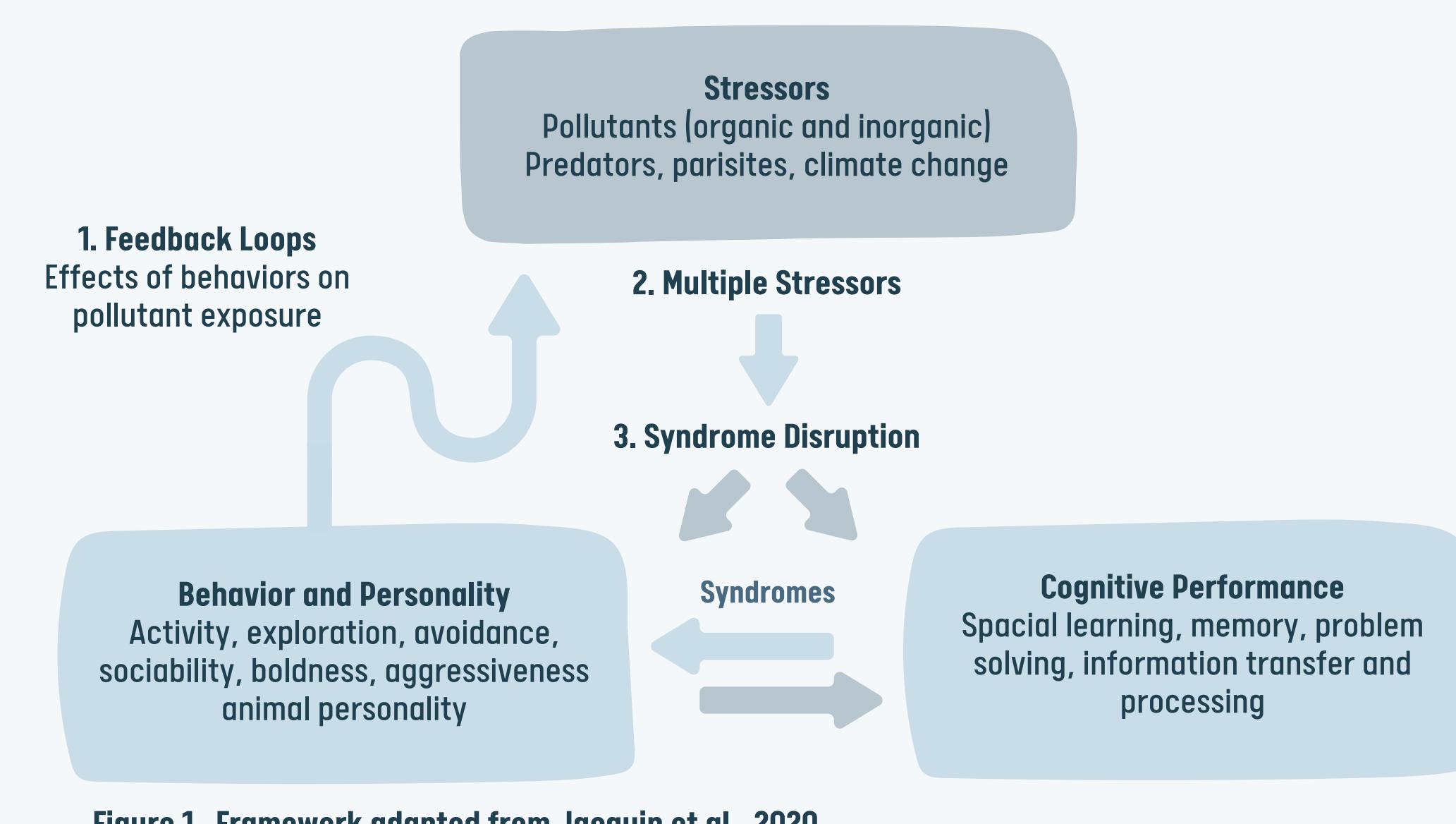


Figure 1. Framework adapted from Jacquin et al., 2020

## Hypothesis

This study aims to identify the effects of PET microfiber exposure on male mating behavior, foraging behavior, and cognitive flexibility in *G. affinis*. We hypothesize that exposure to microplastics will decrease cognitive flexibility, increase feeding motivation, and decrease mating motivation.

## Methods

Figures 2-3: Nile Red-stained MPs under UV and *G. affinis* Illustration

**Microfiber Production:** PET fibers were generated via tissue histology techniques (Knauss et al., 2021). Microfibers were wound onto a spindle, embedded in paraffin, sectioned via microtome at 42  $\mu$ m increments, processed with xylene, ethanol, and acetone, then stained with nile red for recapture.

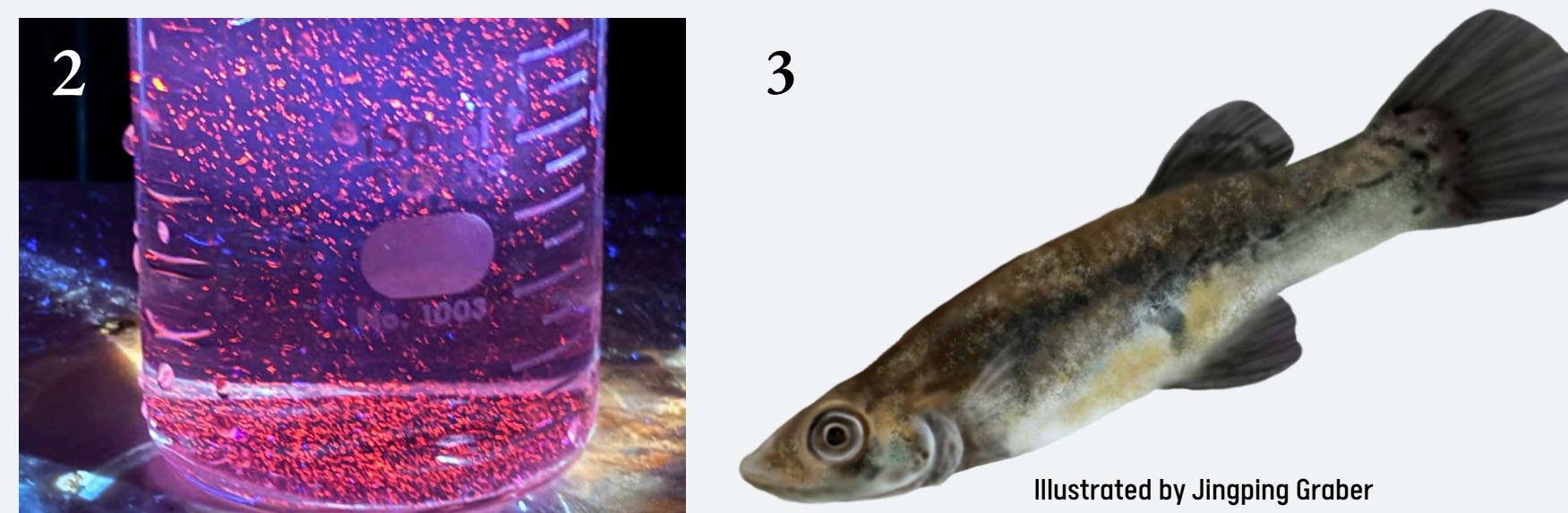
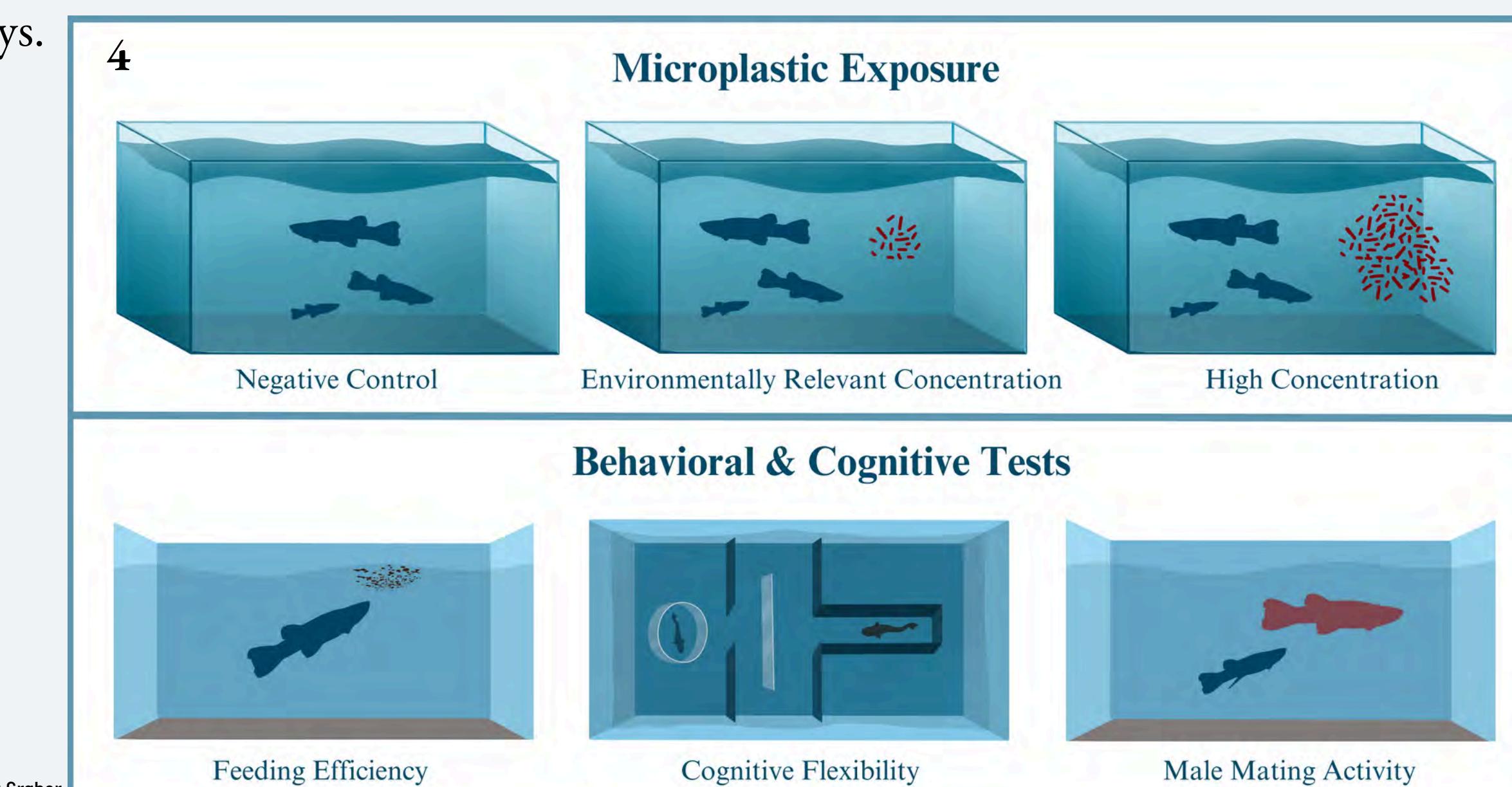


Figure 4: Exposure Treatments & Behavioral Assays

Fish were collected from Brackenridge Field Laboratory, Austin, Texas. Static aquaria were dosed with negative control (0 MP/L), low (10 MP/L), or high (1,000 MP/L) concentrations for 14 days.

### Behavioral & Cognitive Tests:

- Foraging Behavior** (n=76): Fish were starved for 24 hours, then provided 0.005g of dry food and recorded.
- Cognitive Flexibility** (n=124): Fish were placed in an assay tank with a plexiglass obstacle positioned in front of a social reward. After 5 minutes of habituation, navigation of the assay was recorded.
- Male Mating Activity** (n=44): After 48 hours of isolation, males were introduced to an unfamiliar female. Interactions were recorded.



## Results

Figure 5: Cognitive flexibility assay solve time by treatment

Individuals exposed to low MP concentrations presented significantly greater solve-time than individuals from the control group. ANOVA  $p = 0.022$ ,  $t = 1.460$ ,  $n = 124$

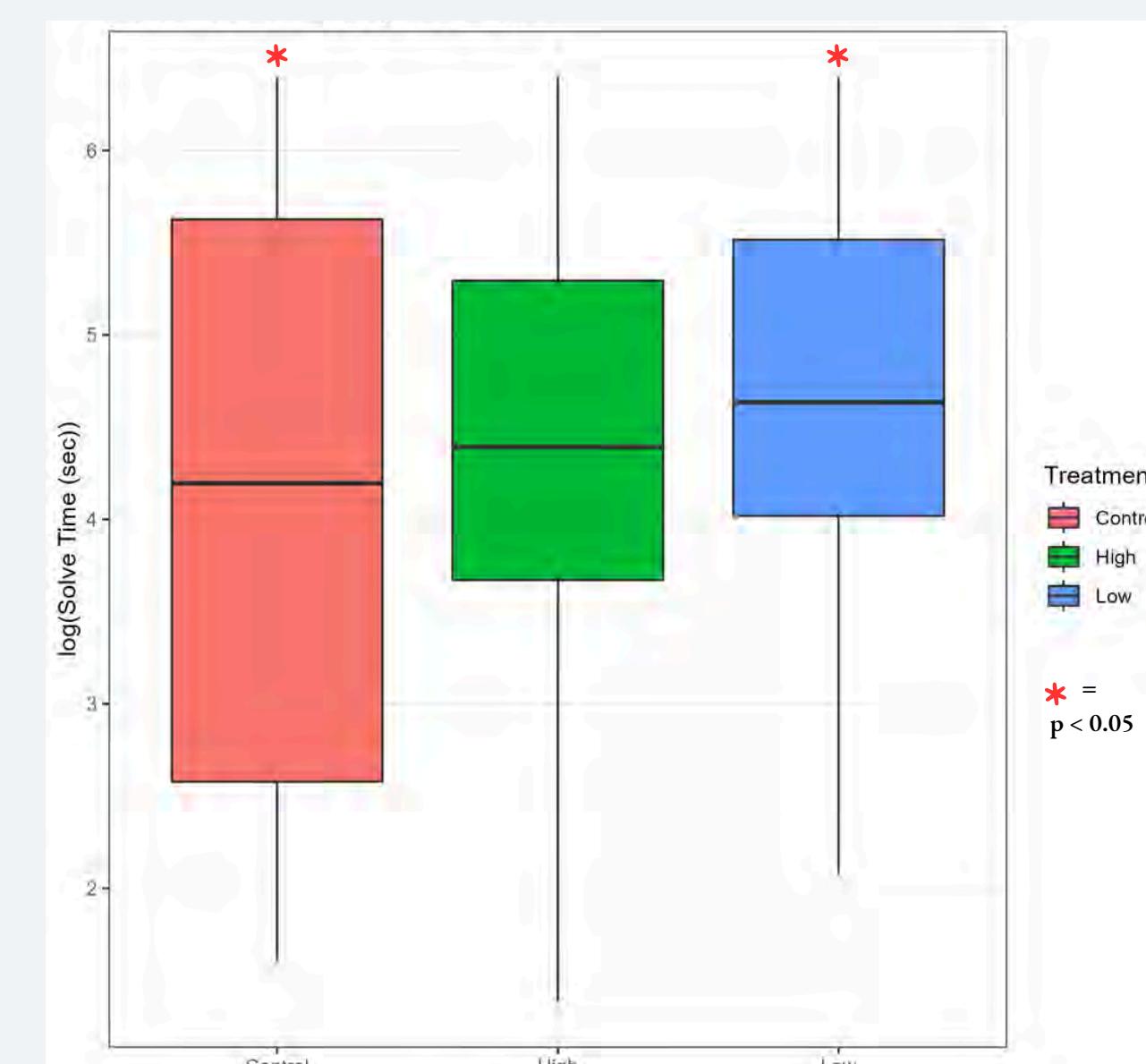


Figure 6: Cognitive flexibility assay solve time by treatment and sex

Exposure to low MP concentrations increases solve time in males, and no such effect is seen in females. Post-hoc Tukey test  $p = 0.0391$ ,  $t = 2.545$ ,  $n = 124$ .

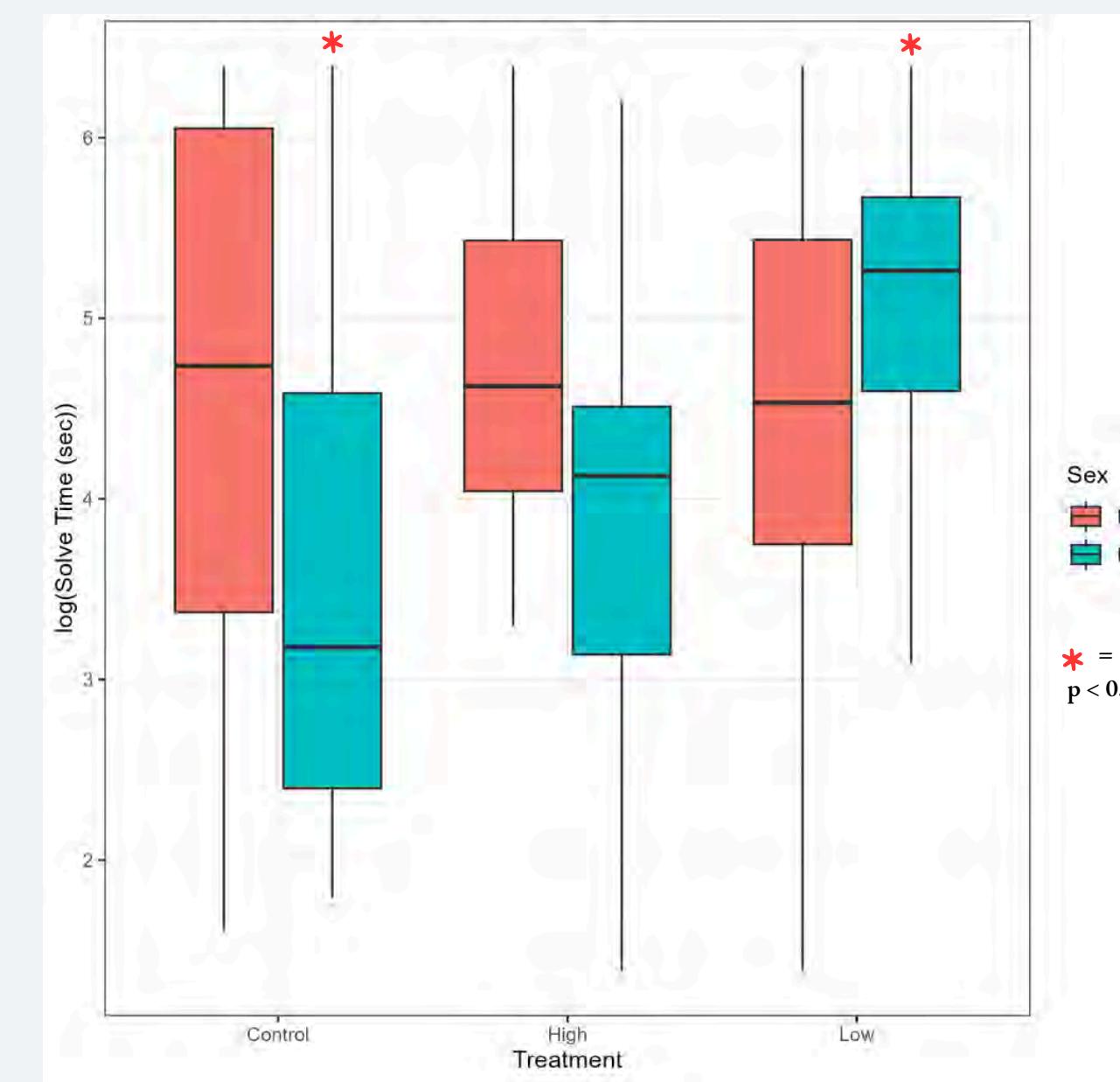


Figure 7: Mating behavior assay chasing occurrences by treatment

Chasing occurs at the same frequency across all three treatment types. Kruskal-Wallis  $p > 0.05$ ,  $n = 44$ .

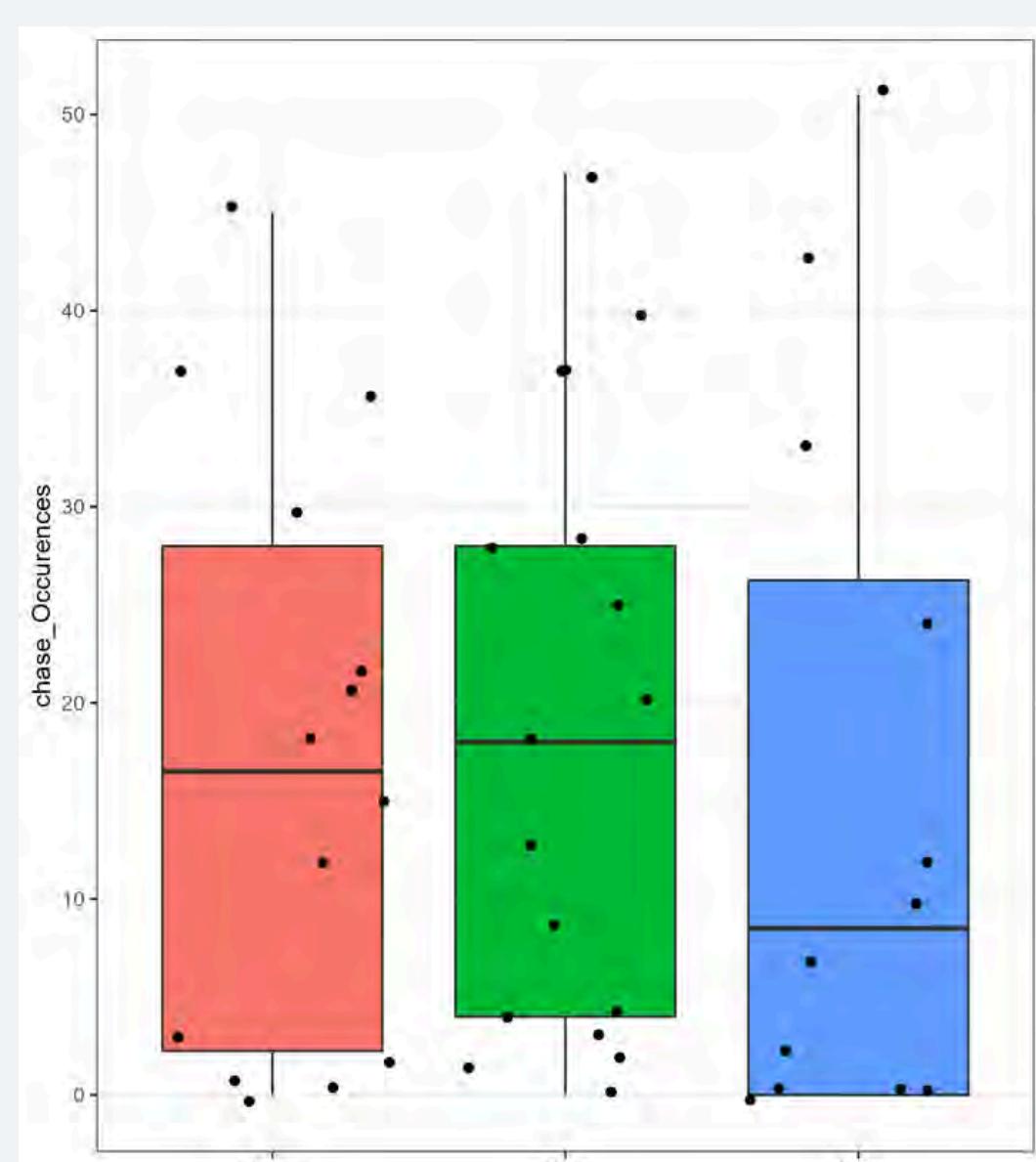
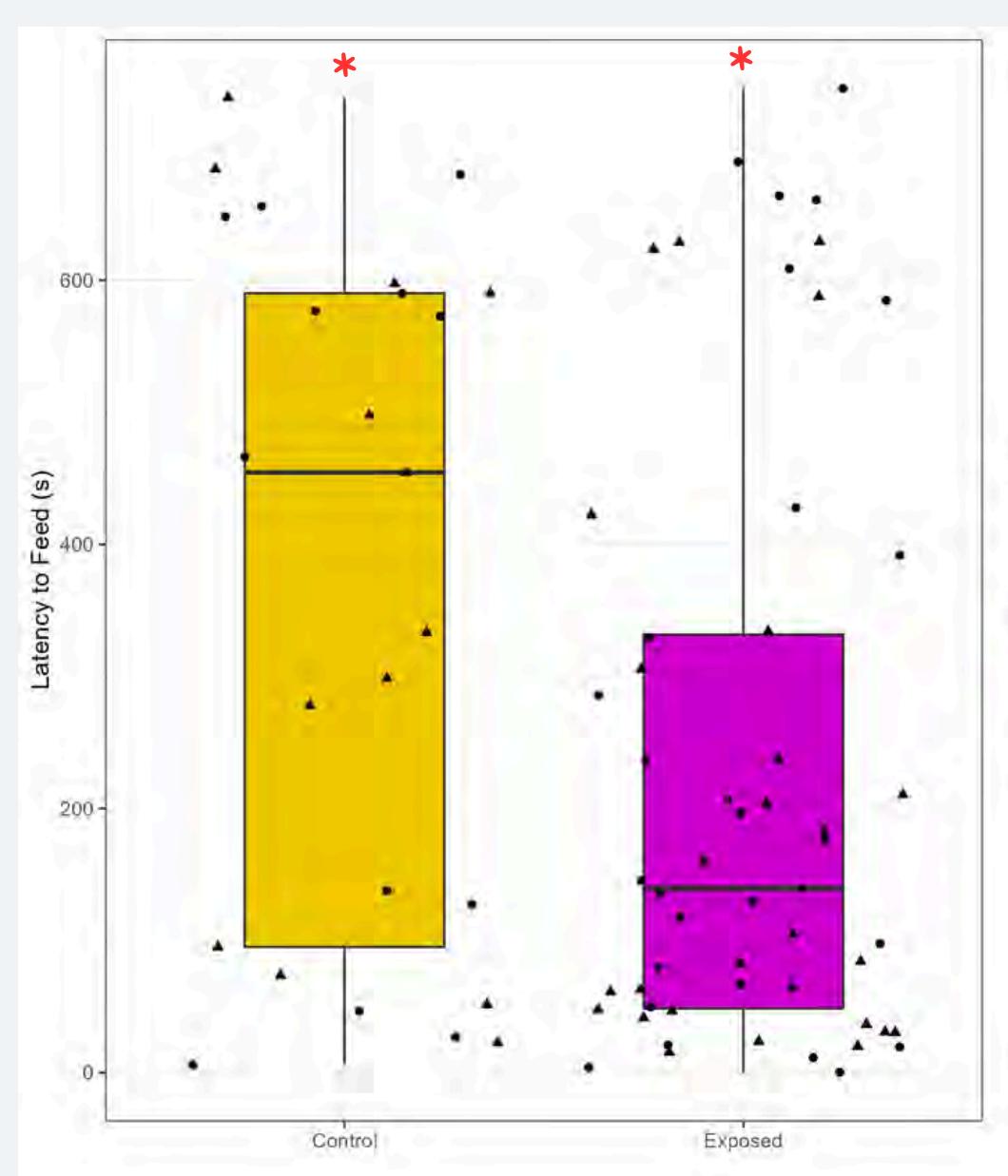


Figure 8: Latency to feed by exposure

Individuals exposed to MPs present shorter feed latency than individuals without MP exposure. Wilcoxon signed rank test  $p = 0.042$ ,  $w = 883$ ,  $n = 76$ .



## Discussion

### Cognitive Flexibility

Solve time between control and low groups significantly differed, with low groups solving slower than controls, indicating that MP exposure impacted problem-solving abilities (Figure 5). This is possible because MPs can pass through the blood-brain barrier and affect their behavior (Mattson et al., 2017). Furthermore, MPs can lead to malnutrition, which results in reduced cognitive function and slower swimming speed (Guerrera et al., 2021 & Yin et al., 2018).

### Mating Behavior

There were no significant differences across groups for chase events occurred, as male mating motivation is innately strong and has little plasticity (Dadda et al., 2008)

### Foraging

MP-exposed fish began feeding earlier than the control fish (Figure 8). This may be due to malnourishment caused by MP accumulation or damage in the intestinal tract which could result in a higher motivation to feed (Guerrera et al., 2021).

### Future Directions

Numbers of MPs present across organs will be determined. This data may provide insights into a biological uptake limit of MPs and indicate why cognitive flexibility does not decrease linearly with increasing MP concentrations.

## Acknowledgements

We would like to thank the Brackenridge Field Laboratory for providing fish for our study, Dr. Molly Cummings for her supervision and access to lab resources, and the University of Texas Integrative Biology Department for funding.

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# Fluorescent detection of Nile Red-stained microplastic uptake in the roots of *Arabidopsis thaliana*

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## Introduction

With plastic synthesis and distribution having increased dramatically in recent years, a closer look at the side-effects of plastic integration into biological contexts has become vital. More specifically, increasing levels of microplastics (MPs) in the rhizosphere, the portion of soil in direct interaction with plant roots, has sparked an uptake in studies exploring plastic-plant root interactions (1). Microfibers (MFs), a subset of MPs, outnumber other MP types, and polyethylene (PET) MFs are among the most common MFs. However, PET MFs are seldom used in exposure studies, indicating a need for further research (2). One location tracking method of micro-scale plastics is the use of fluorescent staining and imaging. This project seeks to develop a method of fluorescent detection that allows for the visualization of polystyrene (PS) microplastic and PET MF uptake in the roots of *Arabidopsis thaliana*. This method's aim is to inform MP and MF uptake ability and location, providing valuable insight into plastic-plant interactions in the soil.

## Methods

Two different methods were developed to visualize the uptake of Nile-Red (NR) stained PS and PET into the roots of *A. thaliana*.

### General workflow

1. Creation of MP Sample
2. Plastic Staining
3. Plastic Sterilization
4. Prepare Square Petri Dishes
5. Growth Period
6. Imaging

### PS Microplastic Uptake:

Polystyrene pellets were ground into a fine powder with particle size <5 mm using a coffee grinder. The MP sample was then stained with NR dye. *Arabidopsis thaliana* was planted in MS/2 media spiked with the NR-stained PS and allowed to grow for a little over a month in vertical square petri dishes. Root samples were taken from media and stained with DAPI dye, following the method developed by Stanton et. al. (3). Finally, root samples were imaged under NR and DAPI excitation wavelengths using a Nikon SMZ25 fluorescent stereomicroscope.

### PET Microfiber Uptake:

PET fibers with a diameter of 0.014 mm were wound onto a spindle, embedded in paraffin wax, and sectioned with a microtome at various increments under 0.5 mm. Fibers were chemically isolated from paraffin and stained with NR dye. *Arabidopsis thaliana* was planted in MS/2 media spiked with the NR-stained microfibers and allowed to grow for a little over a month in vertical square petri dishes. Root samples were taken from media and imaged under NR and GFP excitation wavelengths using a Nikon SMZ25 fluorescent stereomicroscope.

## Figures and Results

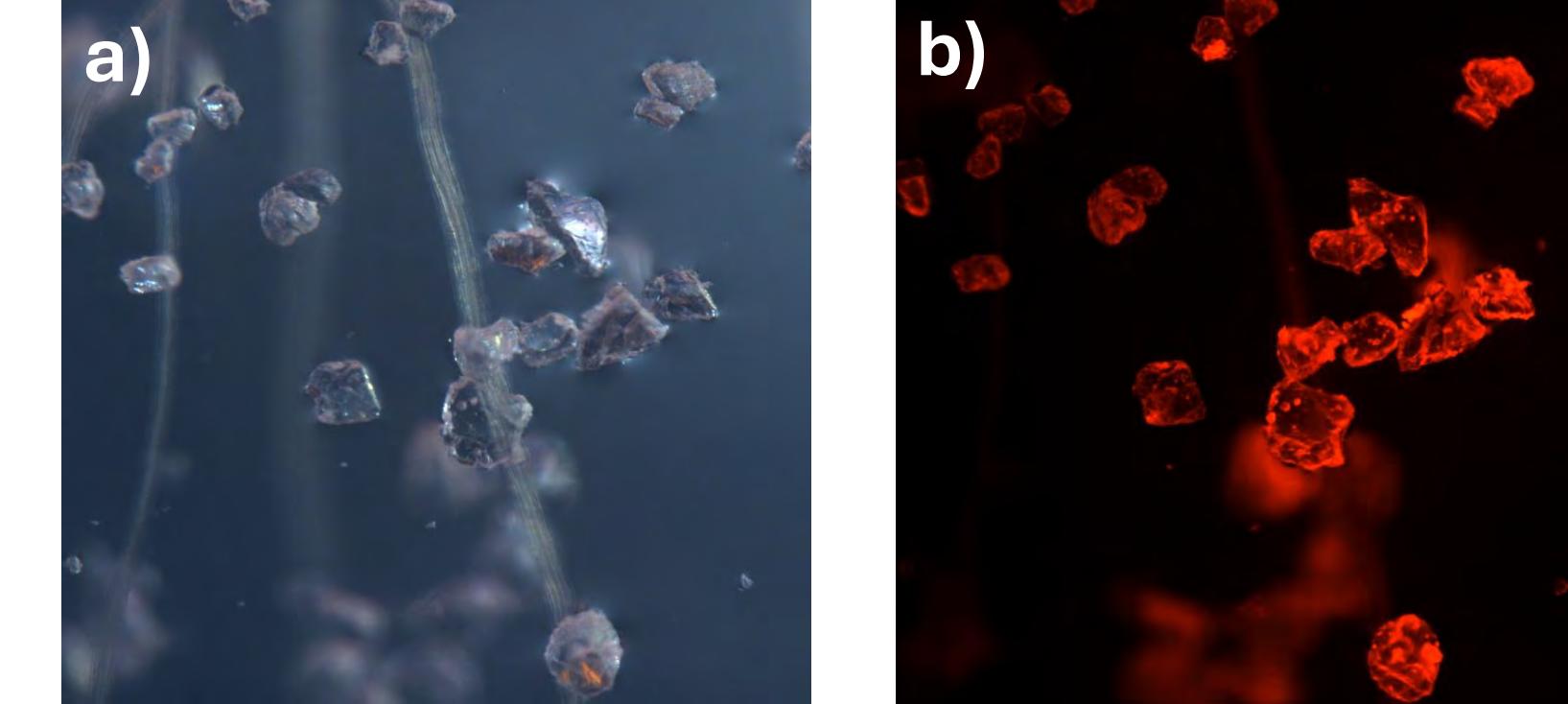


Fig. 1: NR-stained PS oriented around plant root: (a) Brightfield (b) NR-emission

**Fig. 1** showcases the growth conditions in the NR-stained PS spiked MS/2 media. A potential affinity of the plastic for the plant root is demonstrated by the orientation of the NR-stained PS about the root. **Fig. 1. b.** further validates the viability of the method, in that little to no autofluorescence of the root is observed while the stained MPs fluoresce clearly.

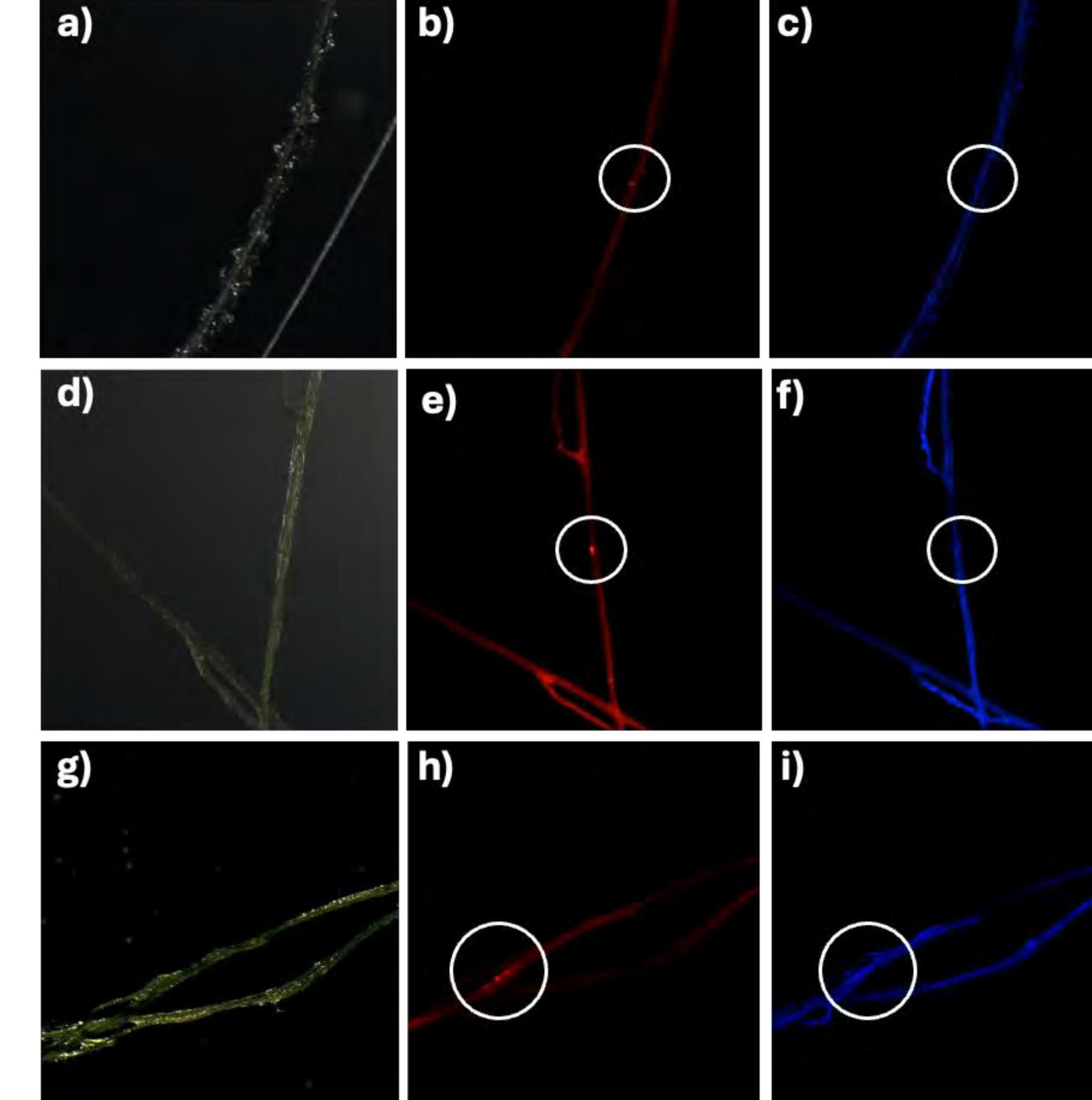


Fig. 2: BF and fluorescent DAPI co-stained *A. thaliana* root samples: (a) Root 1 Brightfield (b) Root 1 NR emission (c) Root 1 DAPI emission (d) Root 2 Brightfield (e) Root 2 NR emission (f) Root 2 DAPI emission (g) Root 3 Brightfield (h) Root 3 NR emission (i) Root 3 DAPI emission

**Fig. 2** demonstrates the identification of possible areas of NR-stained PS microplastic uptake, as visualized by multiple circular regions of intense NR fluorescence that are not similarly intense under DAPI emission. However, due to autofluorescence observed under NR emission, further analytical methods are necessary to produce absolute certainty as to the source of intense fluorescence.

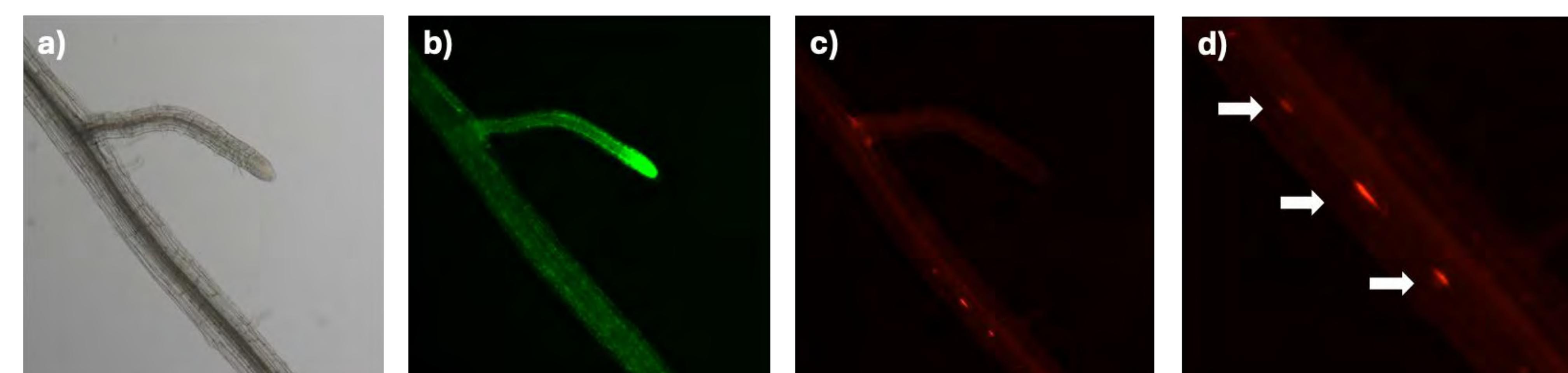


Fig. 3: GFP-expressing *A. thaliana* root imaged: (a) Brightfield (b) GFP emission (c) NR emission (d) Enlarged view of PET MF uptake area under NR emission

**Fig. 3** presents a magnified view of an *A. thaliana* root sample grown in NR-stained MF spiked media. **Fig. 3. d.** identifies multiple regions of potential MF uptake as indicated by the elongated regions of intense fluorescence under the NR excitation wavelength.

**Fig. 4** shows the GFP fluorescence of a root not exposed to experimentally integrated MPs. No fluorescence was observed under NR emission. GFP expression was evident in all other negative controls planted, though with varying intensity and concentrated fluorescence at root tips.

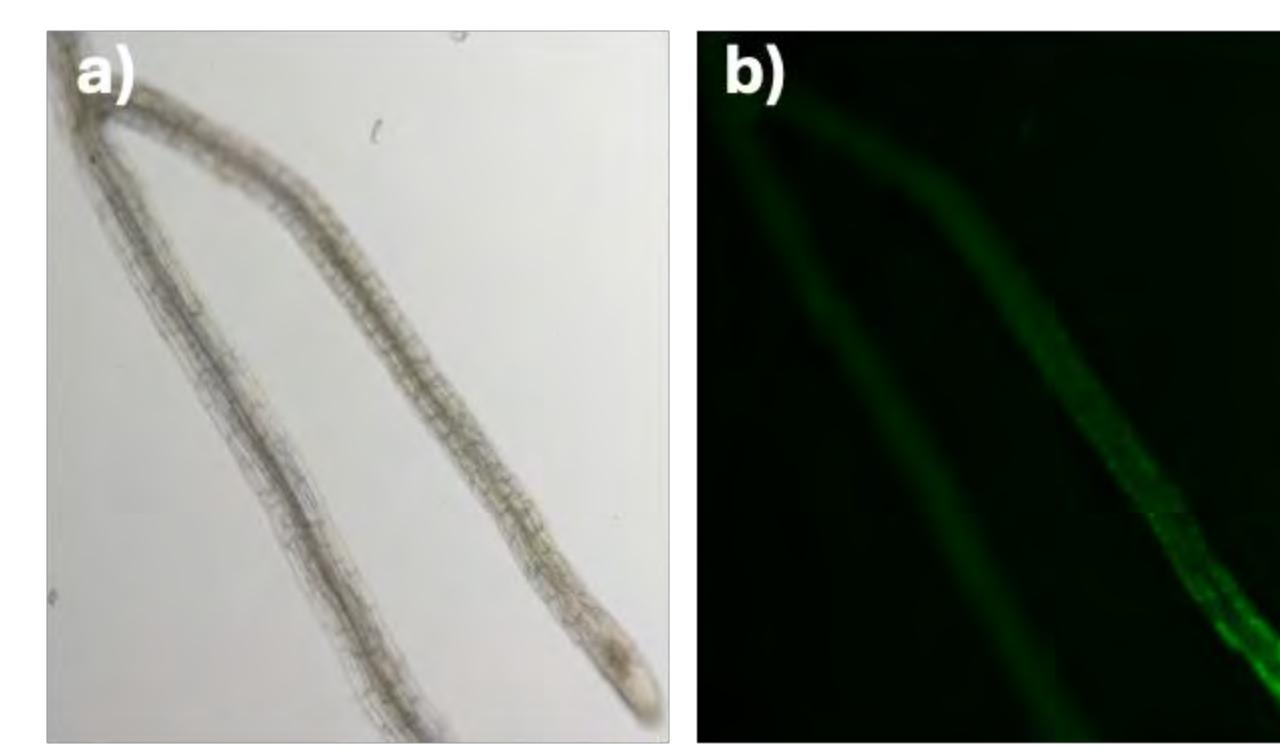


Fig. 4: Negative GFP-expression control root: (a) Brightfield (b) GFP-emission.

## Conclusion + Future Aims

In conclusion, multiple regions of potentially positive MP and MF uptake have been imaged. The introduction of NR-stained PS MPs yielded positive results in the form of multiple areas of circular, high intensity fluorescence in the plant root that were observed only under NR emission. Moreover, when *A. thaliana* was exposed to NR-stained PET MFs, elongated regions of intense fluorescence were observed under NR emission, and not similarly observed under GFP emission. However, due to factors such as the variable autofluorescence of the plant root, further analytical methods are necessary to confidently qualify our results and provide more comprehensive quantitative analysis as to the amount of MP uptake.

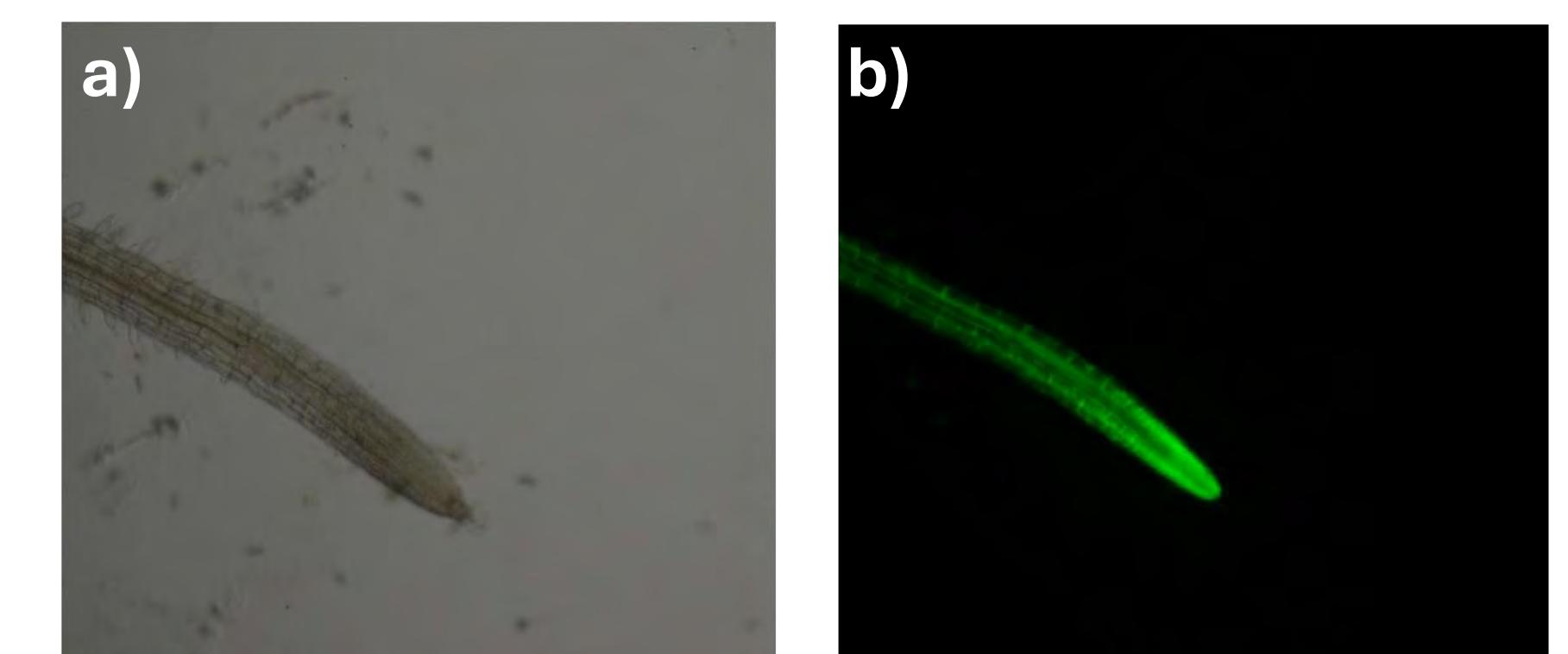


Fig. 5: Negative control *A. thaliana* root: (a) Brightfield (b) GFP emission

Future aims include expanding on the method of PET MF uptake by employing slightly different experimental conditions that aim to combat uncertainty due to autofluorescence. Major factors in this improvement include imaging roots earlier, in order to retain a high amount of GFP and NR fluorescence, as well as exploring both high concentration and ecologically relevant samples. **Fig. 5** shows preliminary negative controls after approximately 15 days of growth, with clear GFP fluorescence. No fluorescence was observed under the NR emission settings. The improved clarity of the negative controls suggests the viability of the revised method.

## Acknowledgments

We would like to thank Dr. Jennifer Brodbelt and Dr. Andrew Ellington for their support of the BioP lab, as well as the Cummings Lab for providing materials for microfiber production.

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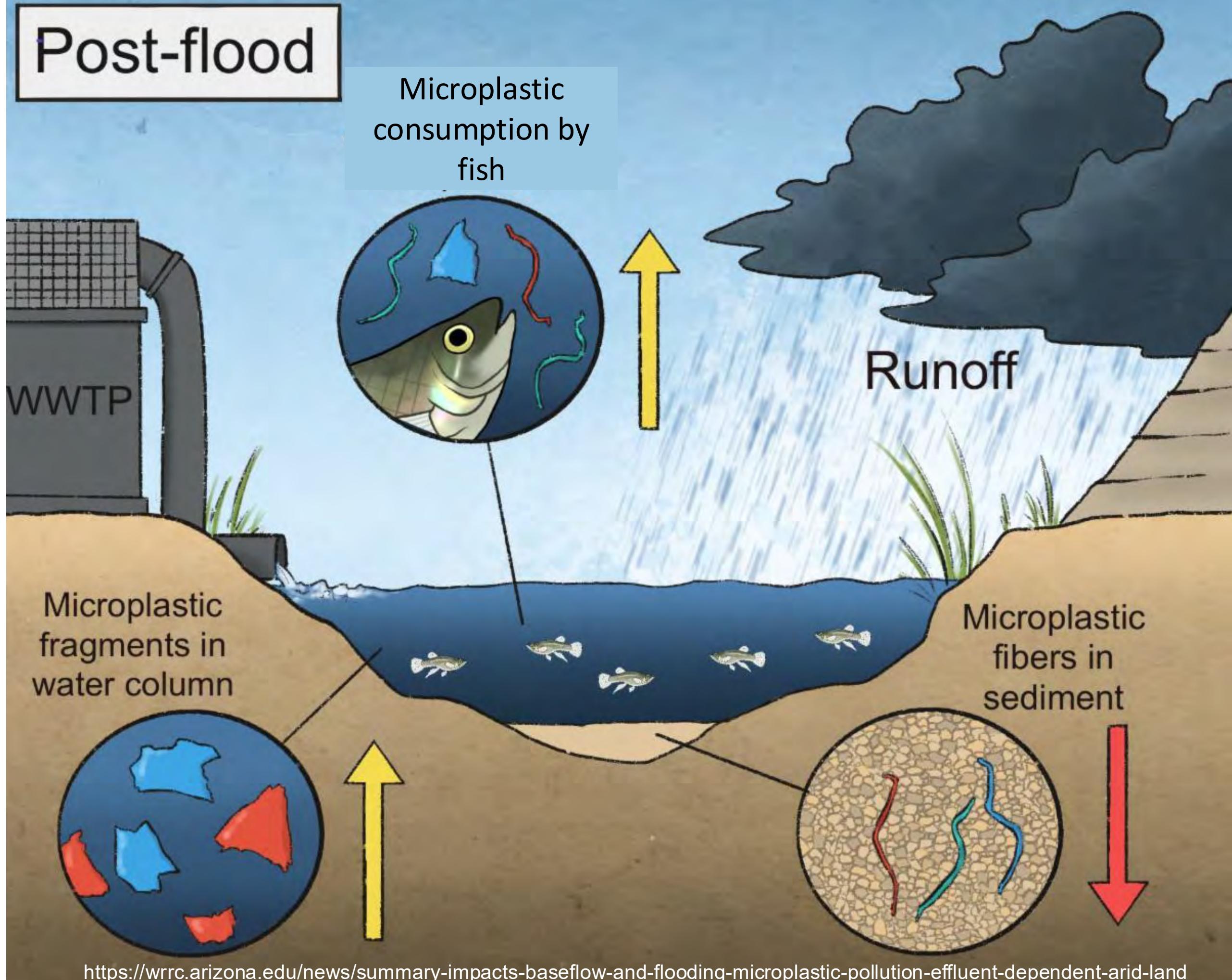
Marufa Akter Upoma, Min Y Pack

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## I. Motivation 1: General sediment transport



## II. Motivation 2: Microplastics in the environment

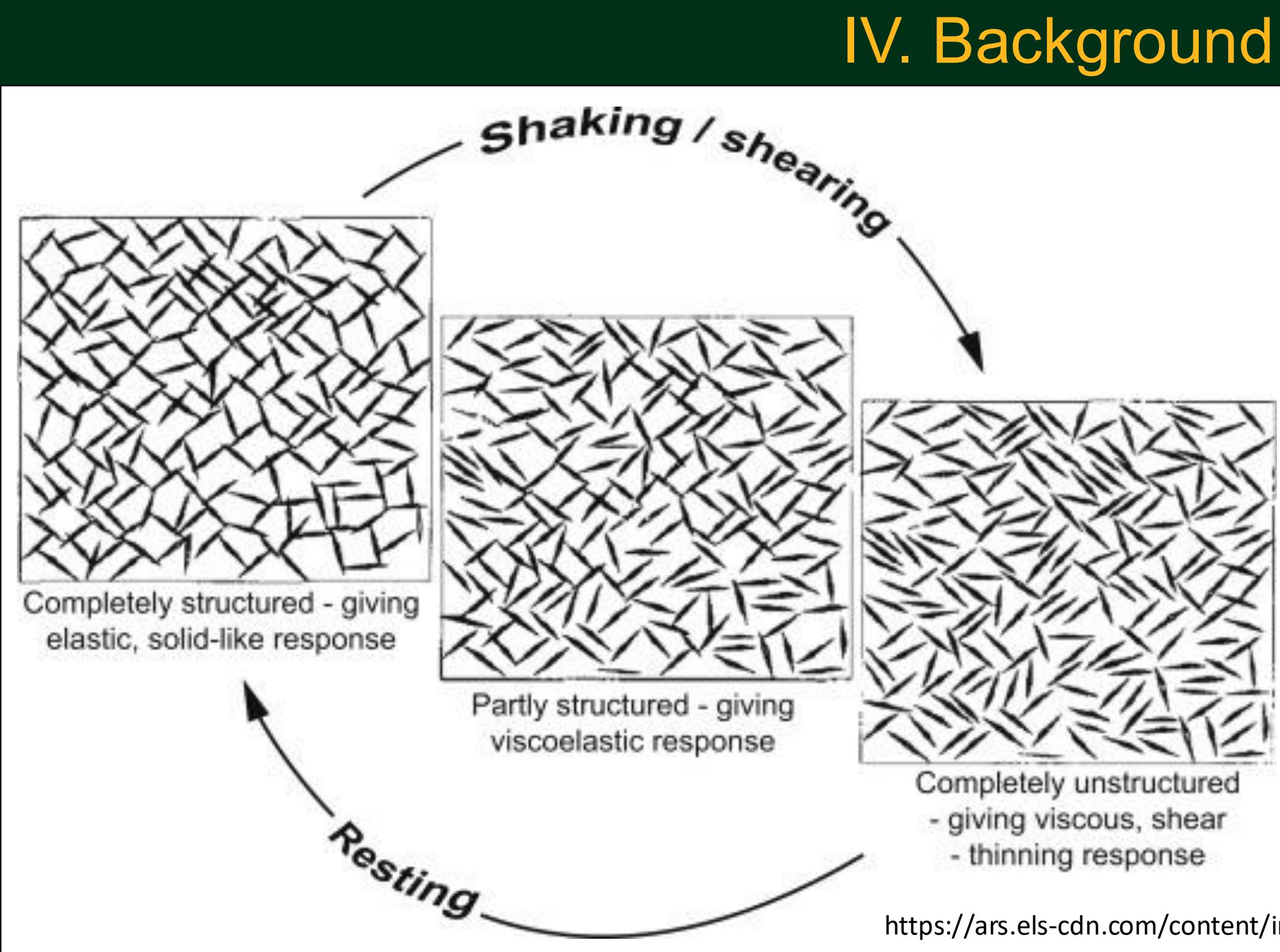


## III. Overview/Burning Question (BQ)

- Wu et al., Environ. Pollut., 2024
  - MPs reduced sediment viscosity and yield stress
  - Potentially increase risk of sediment resuspension
- Wu et al., Sci. Total Environ., 2024
  - Sediment viscosity decreased with MP size and increasing concentration
  - Hypothesized that the reduction in viscosity was due to a decrease in the extracellular polymer substances and the cation exchange capacity of the sediment

BQ: How do MPs affect complex rheological signatures such as thixotropy in sediments?

## IV. Background



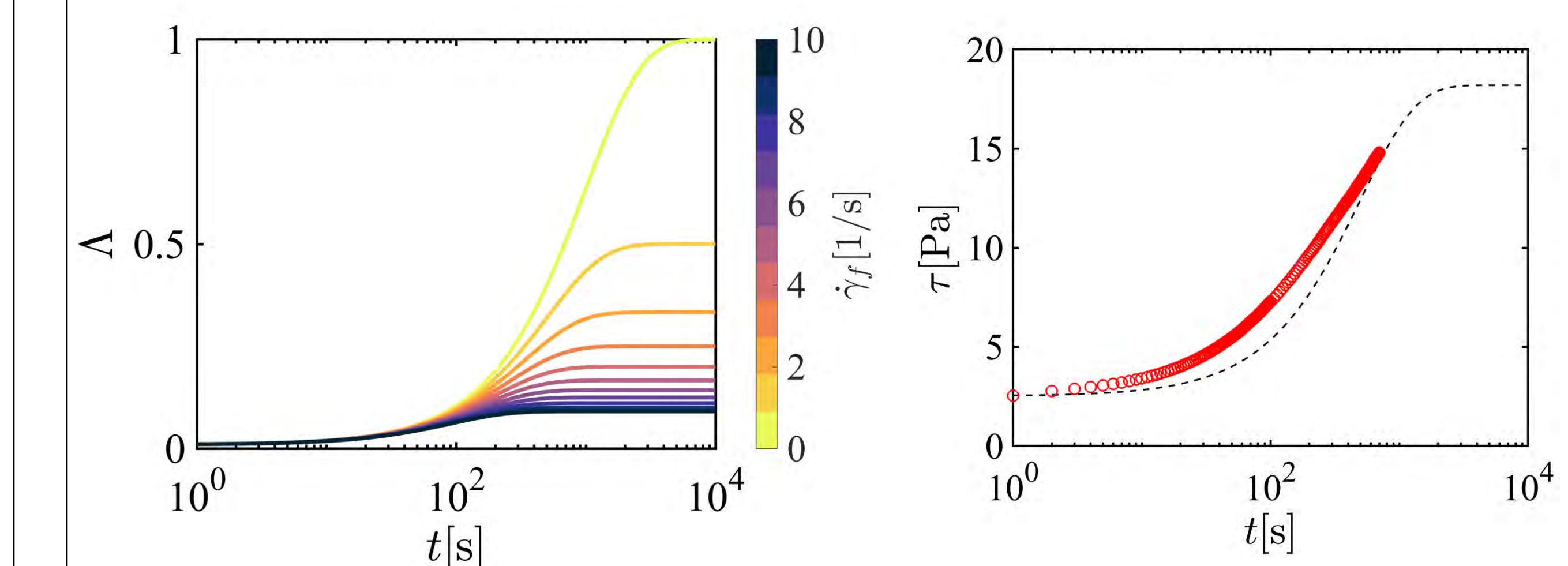
Thixotropy refers to a time-dependent viscosity of non-Newtonian fluids...

Sediments are known to be thixotropic (Shakeel et al., Estuar. Coast. Shelf Sci., 2021), and the presence of microplastics

## VIII. Results: Thixotropic recovery &amp; MPs

Thixotropic kinetic

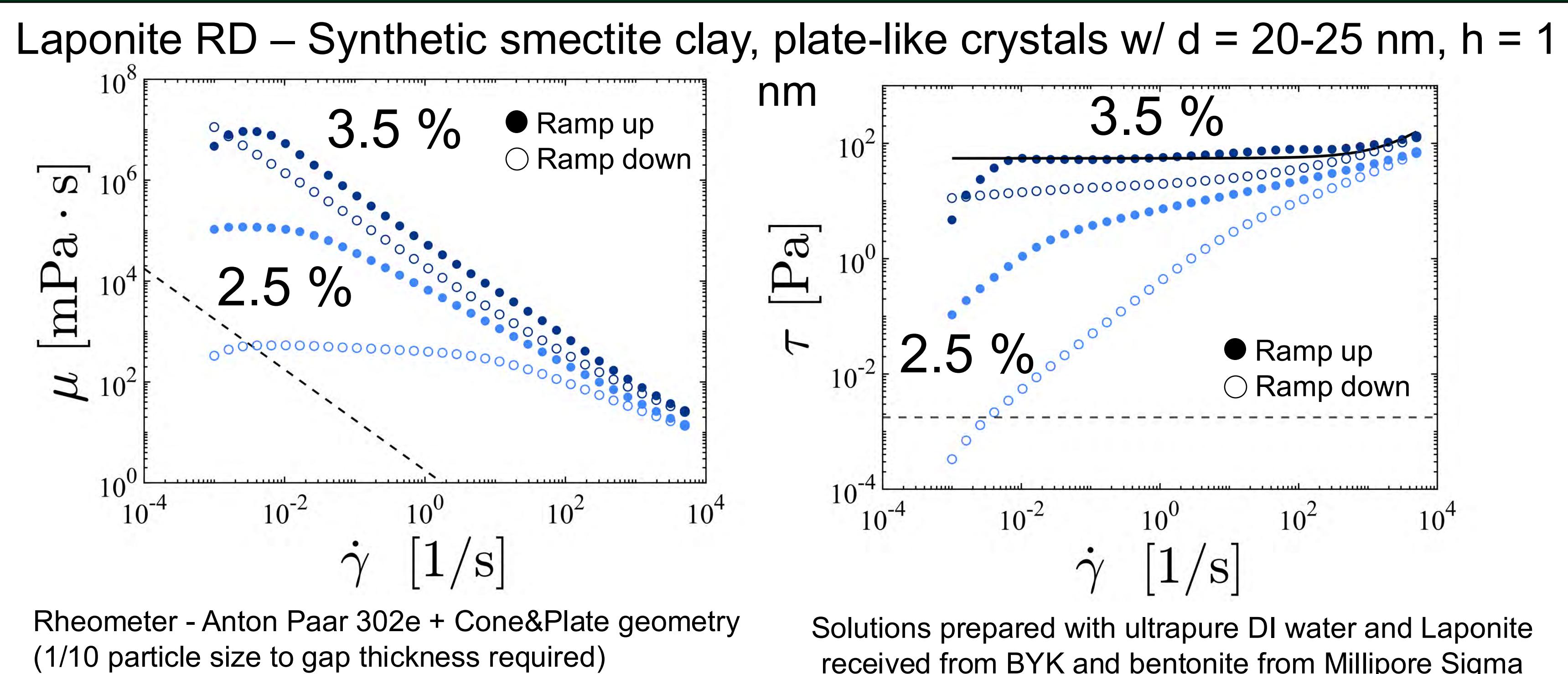
$$\Lambda(t) = k_A \lambda_T + (R_{ss} - k_A \lambda_T) \exp(-t/\lambda_T)$$



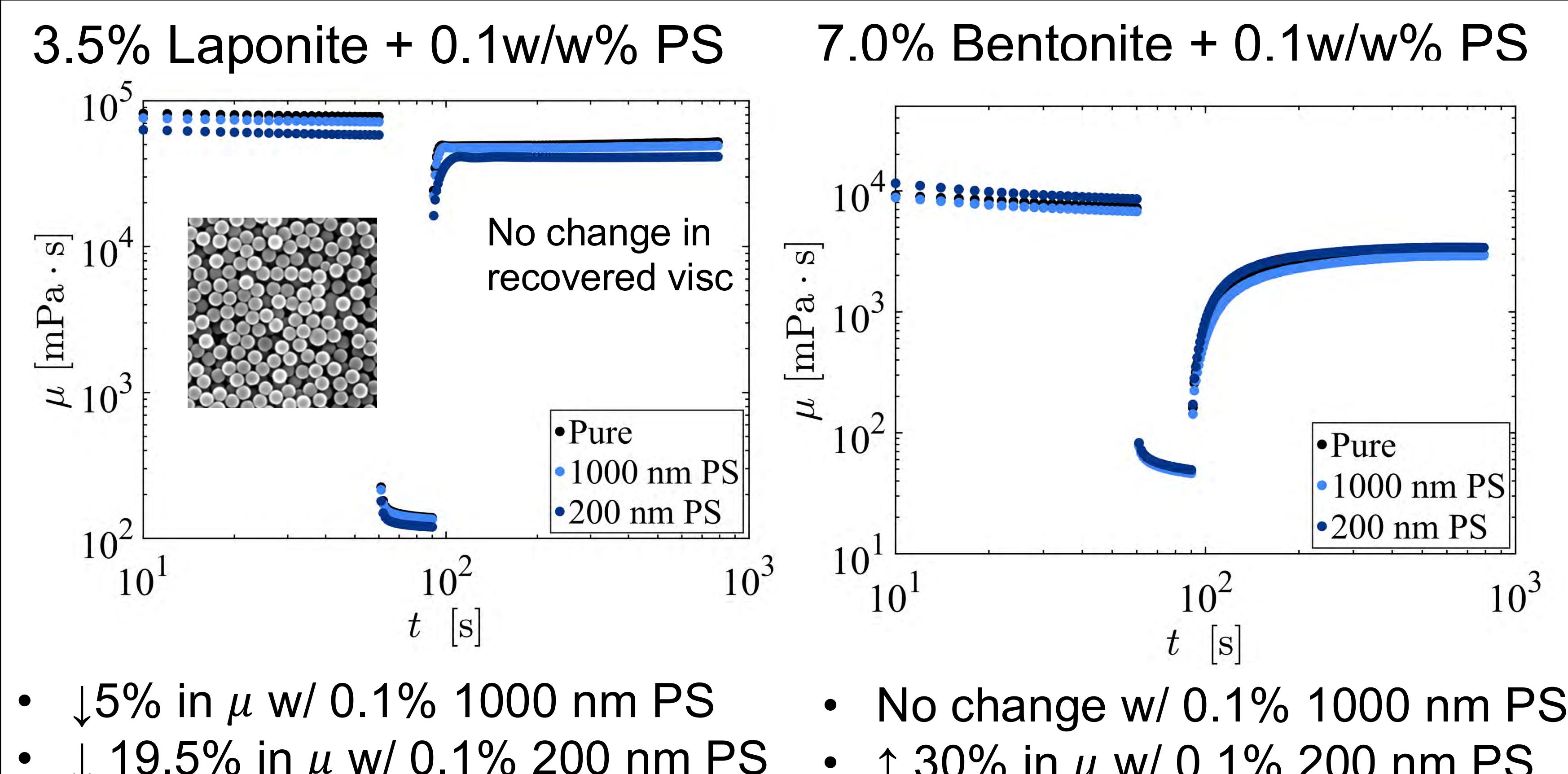
Shear-induced structure breakdown leads to structure recovery and is limited by the final shear rate

**Hypothesis: MPs may affect the aggregation times of sediments influencing their transport dynamics in nature**

## VI. Methods/Results: Pure Laponite



## VII. Results: Clay &amp; Polystyrene (PS) particles



## VIII. Summary

- MPs have a significant influence on the zero-shear viscosity changes to clays in non-trivial ways
- Viscosity dec. with dec. particle size w/ laponite yet viscosity inc. with dec. particle size with bentonite
- The thixotropic recovery rate is greatest for the 1  $\mu$ m particle case for Laponite
- Future work will probe the thixotropic effects across a larger particle size range

Authors acknowledge Baylor University startup funds for the funding of this work as well as Dr. Christie Sayes, Taiwo Ayorinde, Debora Berti for their help with the microplastics, DLS, and SEM

# Making Space for Migratory Birds: An Urban Conservation Program Highlight

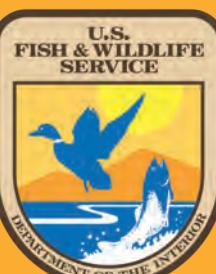
Kiara Carrasco<sup>1</sup>, Chloe Dannenfelser<sup>1</sup>, Liz Virg<sup>1</sup>, and Nancy Brown<sup>2</sup>

<sup>1</sup>American Bird Conservancy, <sup>2</sup>U.S. Fish and Wildlife Service



**SPLASH**  
STOPPING PLASTICS AND  
LITTER ALONG SHORELINES

**American Bird  
Conservancy**



## BACKGROUND

Due to its unique position between two major migratory bird flyways and its diverse range of ecosystems, the Houston-Galveston region plays a crucial role in supporting migratory birds. The region faces challenges from a growing human population, with one major concern being the accumulation of trash. This pollution degrades habitats, poses ingestion and entanglement risks, and threatens the overall health of coastal and urban ecosystems. With over 7 million residents in the greater metropolitan area, it is essential to create and maintain bird-friendly spaces to support the millions of migratory birds that pass through each year.



Photo by Natalia Kuzmina for American Bird Conservancy

## URBAN BIRD TREATY PROGRAM

The **Urban Bird Treaty** is a collaborative partnership program between U.S. cities and the U.S. Fish and Wildlife Service with the goal of protecting urban bird populations.

The treaty was signed in Houston on April 18, 2003.

The four main program focus areas include:

- 1 Habitat Conservation
- 2 Community Engagement
- 3 Hazard Reduction
- 4 Grant Program

American Bird Conservancy received funding in 2023 through the NFWF Five Star and Urban Waters Restoration Program.



Photo by Liz Virg

## CURRICULUM HIGHLIGHT

### Migratory Bird Board Game

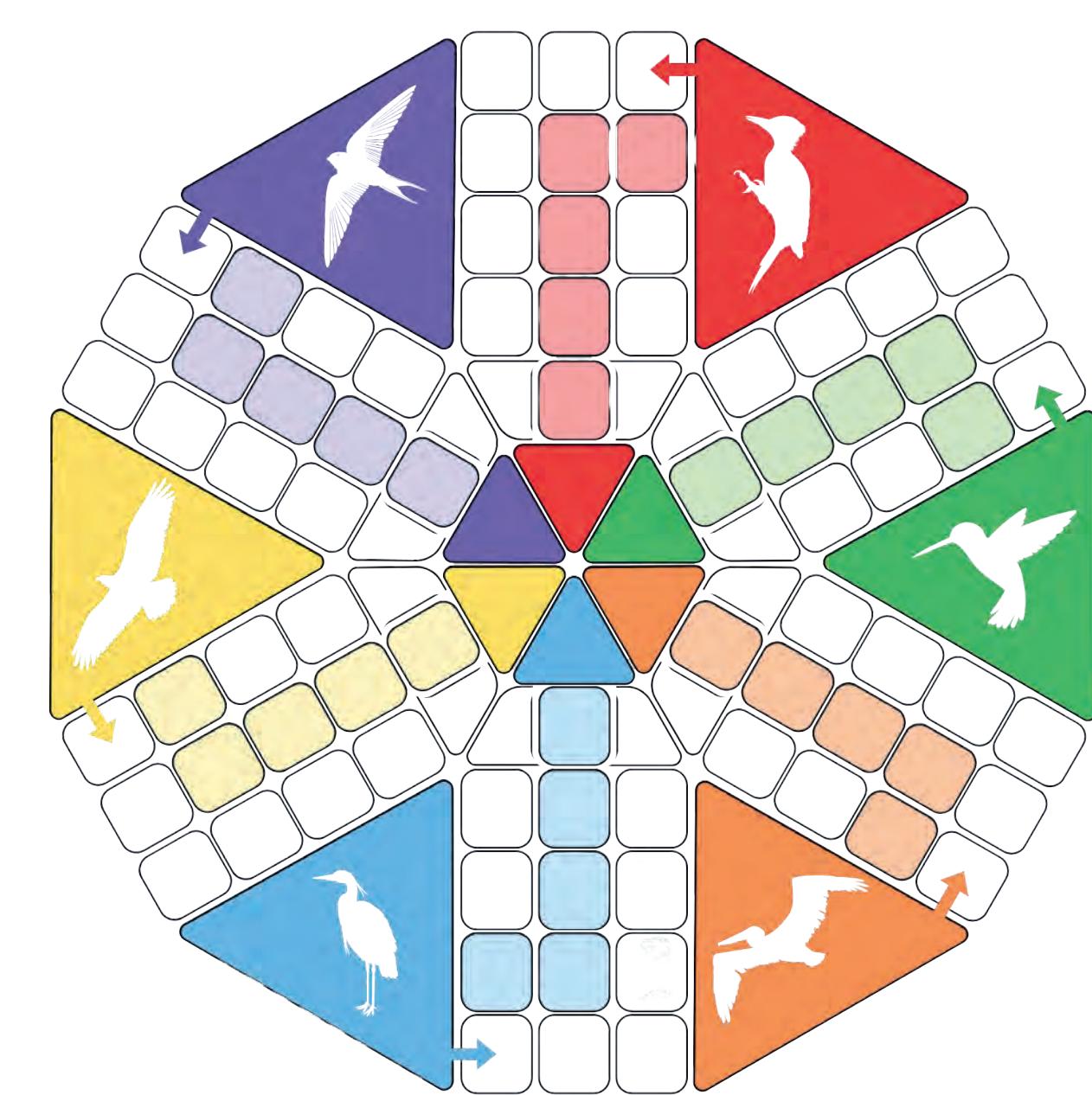


Photo by Liz Virg

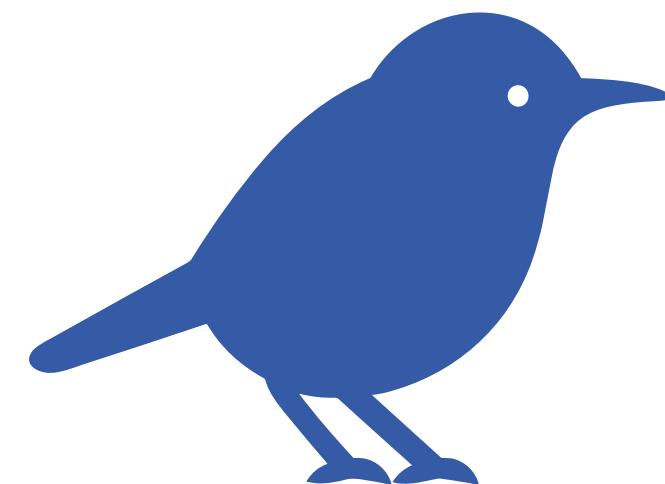
## ABC PROJECT OBJECTIVES AND CURRENT OUTCOMES



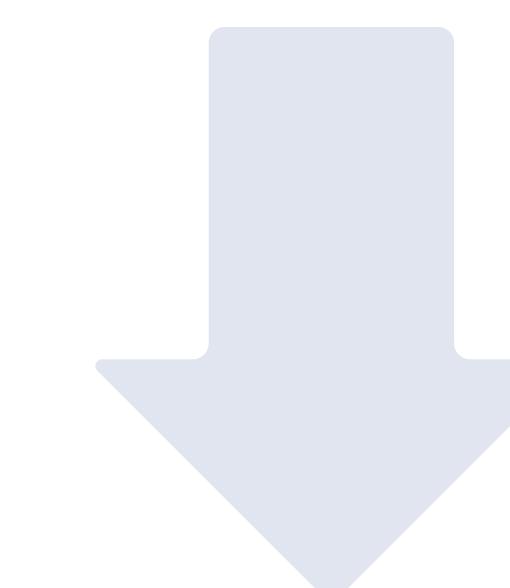
- Hire Conservation Fellow for migratory bird-related education and outreach programming.



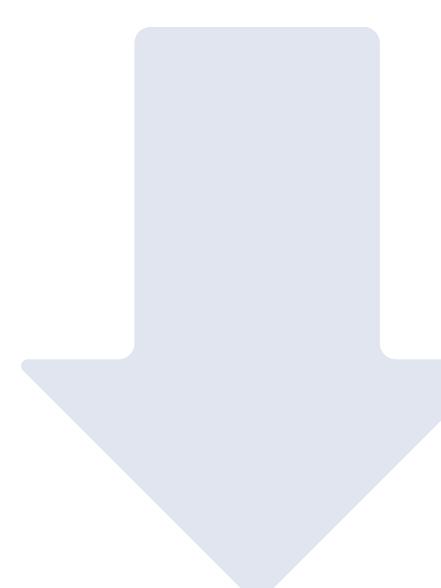
- Host beach and bayou cleanups to remove trash and restore habitat.



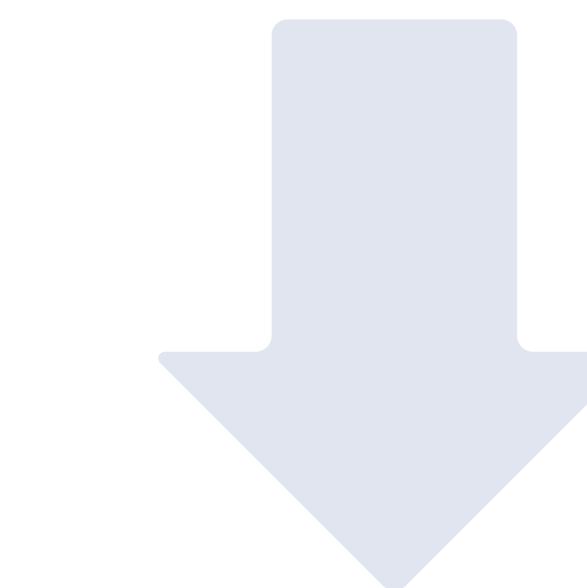
- Coordinate fall and spring bioblitz.
- Develop bird-focused curriculum for students.



- 76 cleanup volunteers.
- 138 students engaged.
- 404 reached from outreach events.



- 2383 lbs of trash removed.
- 38 acres of habitat restored.



- Upcoming bioblitz on May 10, 2025 in celebration of World Migratory Bird Day.

## LEARN MORE

### Urban Bird Treaty Houston



### Education



### Cleanups



### Bioblitz



### Stay connected to SPLASH



## ACKNOWLEDGEMENTS

This work was supported by the National Fish and Wildlife Foundation Five Star and Urban Waters grant program with guidance from U.S. Fish and Wildlife Service. The authors gratefully acknowledge the contributions of the American Bird Conservancy and Gulf Coast Bird Observatory SPLASH program staff.

# Microbial Marvels: Investigating Dubia Roach Microbiota in Relation to Polyethylene Biodegradation

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BioProspecting 2.0 Stream, Freshman Research Initiative, The University of Texas at Austin



The University of Texas at Austin  
Freshman Research Initiative  
College of Natural Sciences



## Background

Our planet is being embalmed in plastic, and nature is suffocating under its overwhelming weight. To date, over 8.3 billion metric tons of plastic have been produced, but less than 10% of this amount has actually been recycled.<sup>2</sup> As plastic waste continues to infiltrate Earth's ecosystems, researchers are increasingly focusing on biologically mediated methods of plastic degradation as a potential solution to this growing crisis.

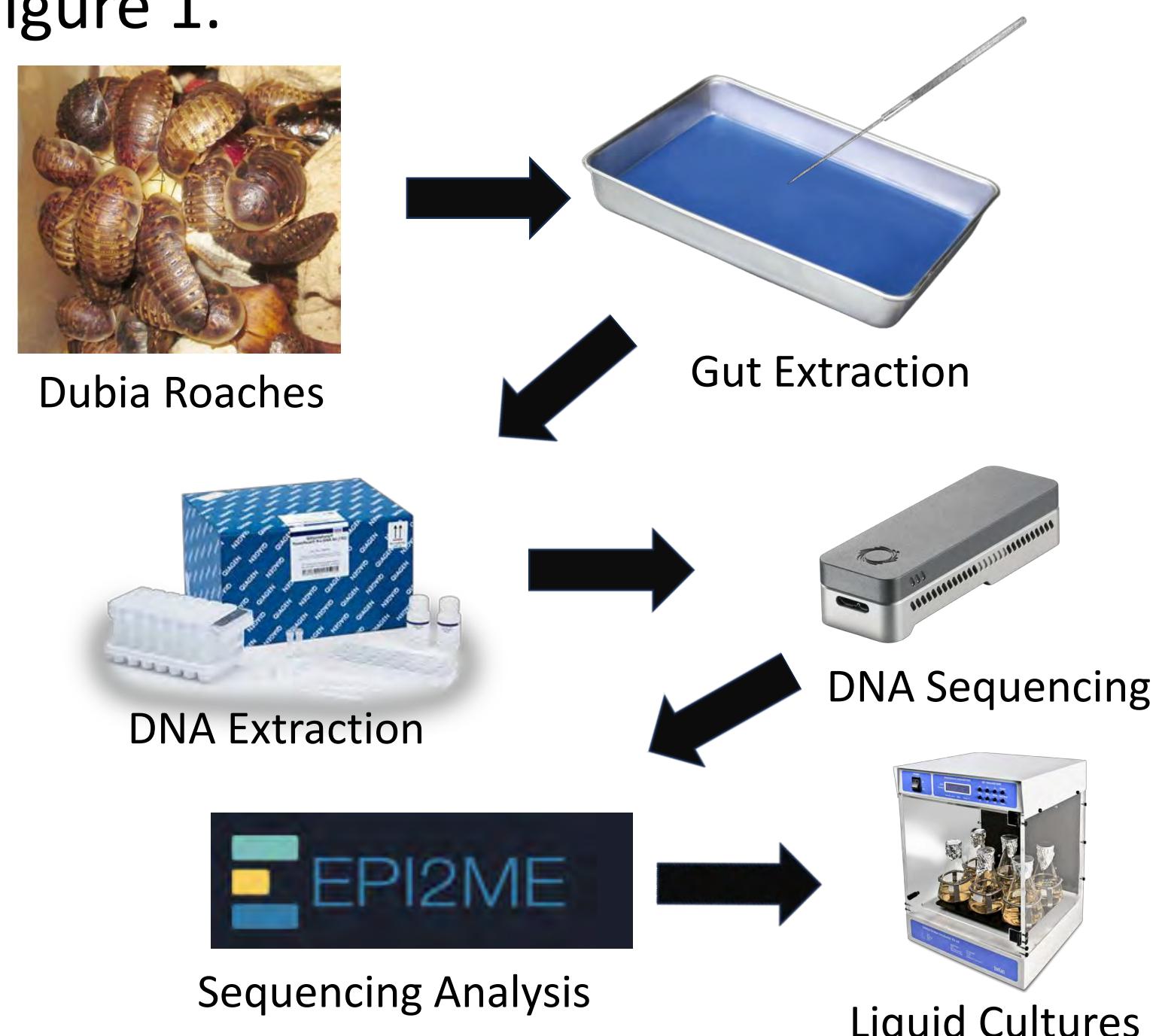
Among the many organisms being studied for their role in plastic biodegradation, several insect species have emerged as promising candidates, with *Zophobas morio* leading much of the current research.<sup>5</sup> Although this species has shown significant potential, studies involving other hexapod invertebrates remain limited. This is particularly true for species in the order *Blattodea*, which includes both cockroaches and termites.

This project aims to highlight the biodegradation potential of *Blaptica dubia*. A recent study suggests that these cockroaches might have the ability to degrade polystyrene, but beyond this, very little is known about their role in the breakdown of plastics in general. The goal of this research is to demonstrate that *Blaptica dubia* can ingest and biodegrade polyethylene, as well as to discover how this impacts the gut microbiota composition of the species.



## Methods

Figure 1.



## Dubia Roaches

*Blaptica dubia*, or more popularly known as the "dubia roach," is a medium-sized roach species native to several countries in South America. This species is very popular in the exotic pet trade and has become a staple feeder insect due to its hardiness, nutrition, and how prolific it is.<sup>1</sup> Going through incomplete metamorphosis, nymphs of this species go through several instars until eventually reaching sexual maturity. The species itself is ovoviparous, with adults being between 1-2 inches in length. Sexual dimorphism is also observed in dubia roaches, as males exhibit long wings, a characteristic not seen in females.<sup>1</sup>

Widely overlooked in terms of their biodegradation capabilities, one recent study suggests that these roaches harbor a unique gut microbiome capable of degrading plastic. Although that study focused entirely on the biodegradation of polystyrene, it does serve to lay a foundation for the bioprospecting research for this species as a whole.<sup>4</sup>



Figure 2.

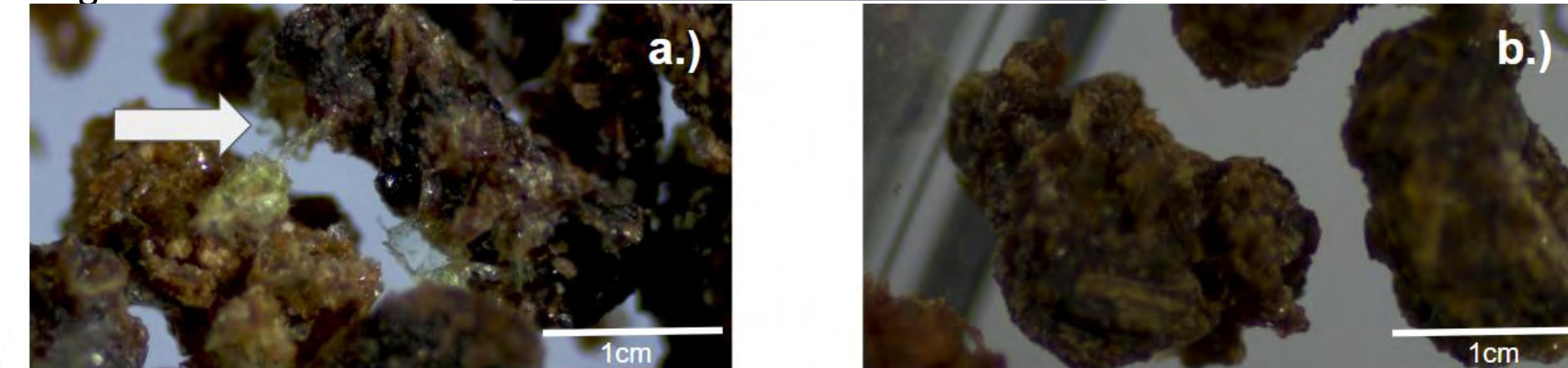


Figure 3.

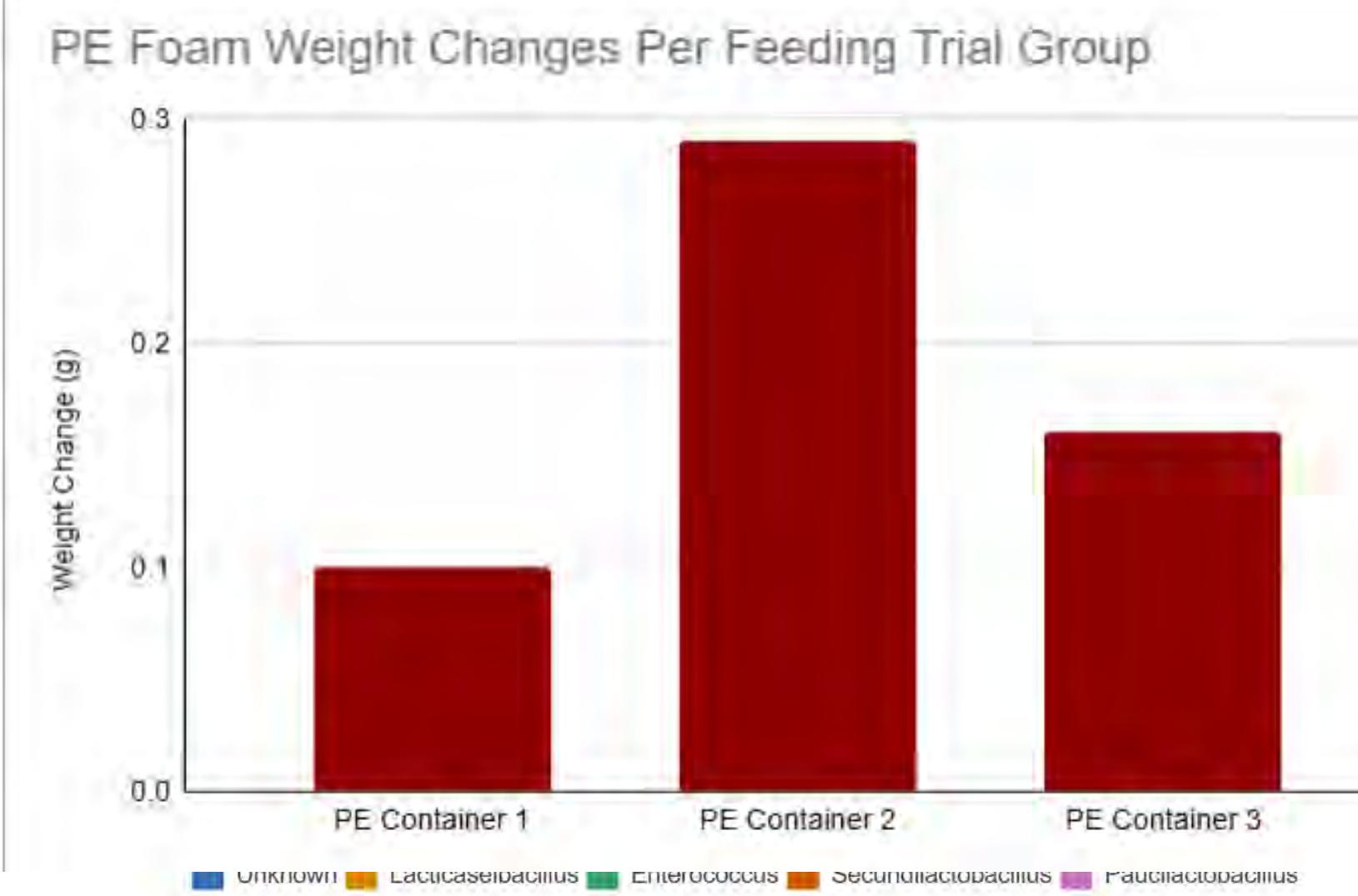
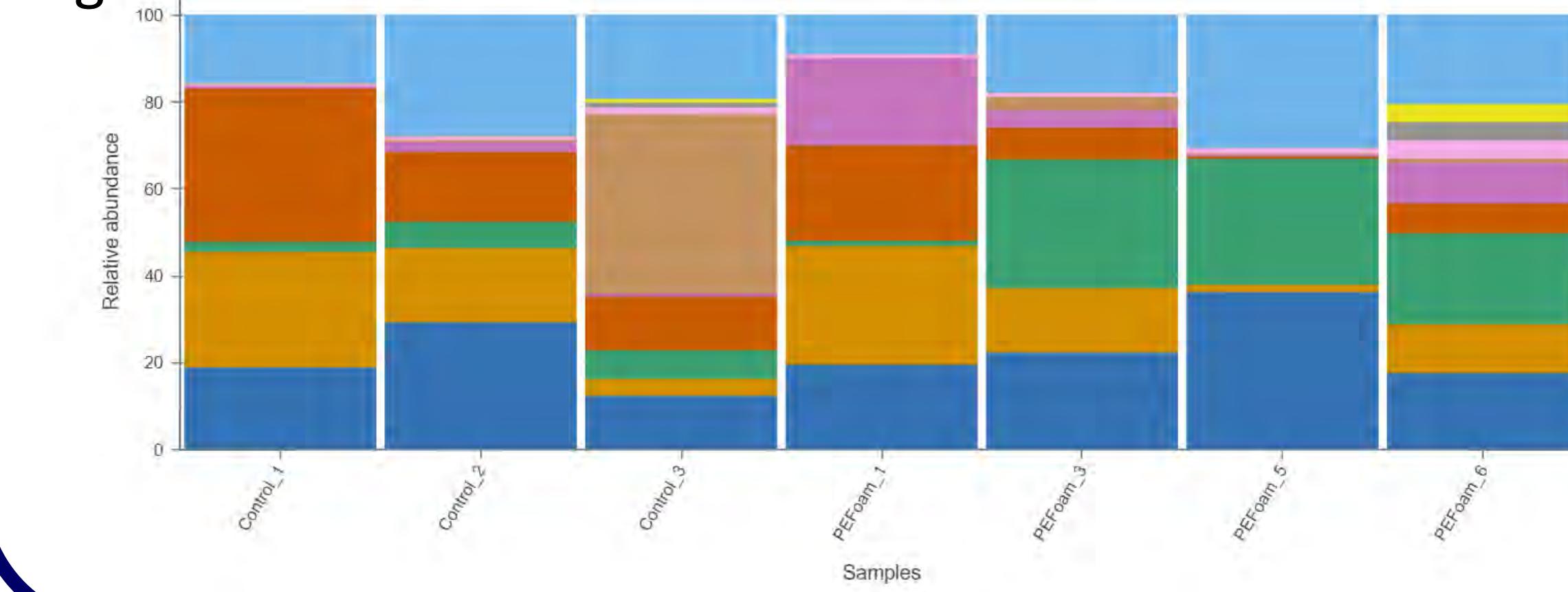


Figure 4.



## Analysis and Future Direction

Once the guts were extracted from the dubia roaches used in the feeding trials, the DNA was extracted and sequenced using Oxford Nanopore Tech's Minion and EPI2ME software (Figure 1). In our analysis, we observed that the cockroach frass contained pieces of a foam-like material (seen in figure 2a.). This indicates the ingestion of polyethylene by said roaches. This is a unique characteristic that is not seen in normal frass (control frass pictured in figure 2b.). From this data, as well as the weight changes observed in the PE foam pre- vs. post-feeding trials (figure 3), we can indeed conclude that these roaches will ingest polyethylene foam.

To analyze the impact of such a diet on these roaches, we ran metagenomic sequencing using Oxford Nanopore Tech's Minion sequencer and analyzed the data through EPIT2ME software. Through sequencing, and as seen in figure 4, we found that the representation of *Enterococcus spp.* increased by 25% in pre- vs. post-feeding trials. This is an interesting observation, as some species in this genus have previously been studied for their ability to thrive in polyethylene-rich environments.<sup>3</sup> Considering this and the moderately decent mean 13.5 Q-score (figure 5), this finding suggests that the PE-based diet of the cockroaches may be setting up the conditions for the growth of these specific microbes.

As a future direction for this study, we plan to isolate and characterize strains of microbes from the guts of these roaches. This will be accomplished through selective LDPE liquid cultures. By isolating these bacteria, the microbes can be studied under more controlled conditions. From here, a much more thorough analysis of the microbes themselves will be performed. Furthermore, it would also prove useful to repeat the entire experiment under the same conditions to confirm the consistency of the results. This approach would serve to validate the conclusions from this study and provide a foundation for future research into this species as a whole.

## Acknowledgements

I would like to thank H-E-B for making this research possible. I would also like to thank user *OxDionysus* from Arachnoboards.com for providing the post that gave inspiration for this study.



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# Non-Plastic Solutions for Oyster Reef Restoration: Efficacy and Environmental Impacts of Novel Restoration Materials

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## Introduction

- Oyster reefs are declining globally<sup>1</sup> with 62.6% lost in Mosquito Lagoon, Florida (ML) since 1943.<sup>2</sup>
- Plastic restoration materials may shed microplastics (MP) into waterways, leading to interest in non-plastic alternatives.<sup>3</sup>
- MPs that are ingested can be fatal to small organisms by blocking their digestive tract.<sup>4</sup>
- Efficacy and microparticle shedding in non-plastic restoration materials is poorly understood.



### Research Questions

- Do non-plastic restoration materials shed microparticles?
- How durable are non-plastic restoration materials?

## Methods

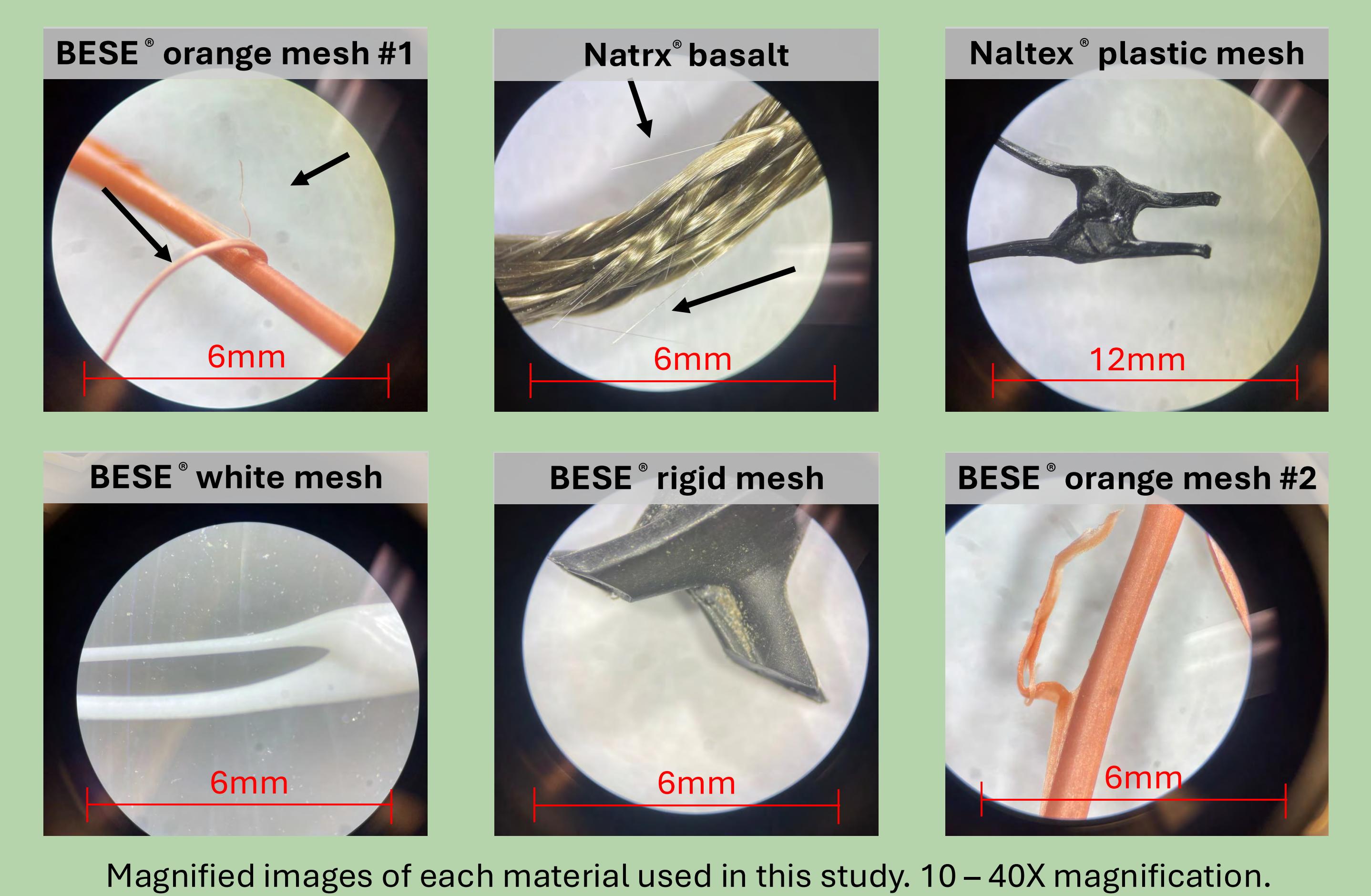
### Laboratory Experiment

We tested four types of BESE® biopolymer mesh and pre-soaked and unsoaked Natrx® basalt bags, using Naltex® plastic mesh and flasks containing only water as controls.

- Squares (5 x 5 cm) of each material were placed in 150mL Erlenmeyer flasks of 30 ppt artificial seawater on a shaker table to simulate field conditions, with five replicates for each material.
- Contents of the flasks were vacuum filtered every four weeks. Flasks were then refilled with new water.
- Microparticles were counted and measured on gridded filter paper using a microscope at 40x magnification.
- Differences between treatments were assessed using Kruskal-Wallis tests and Wilcoxon signed-rank tests.

### Field Data Collection

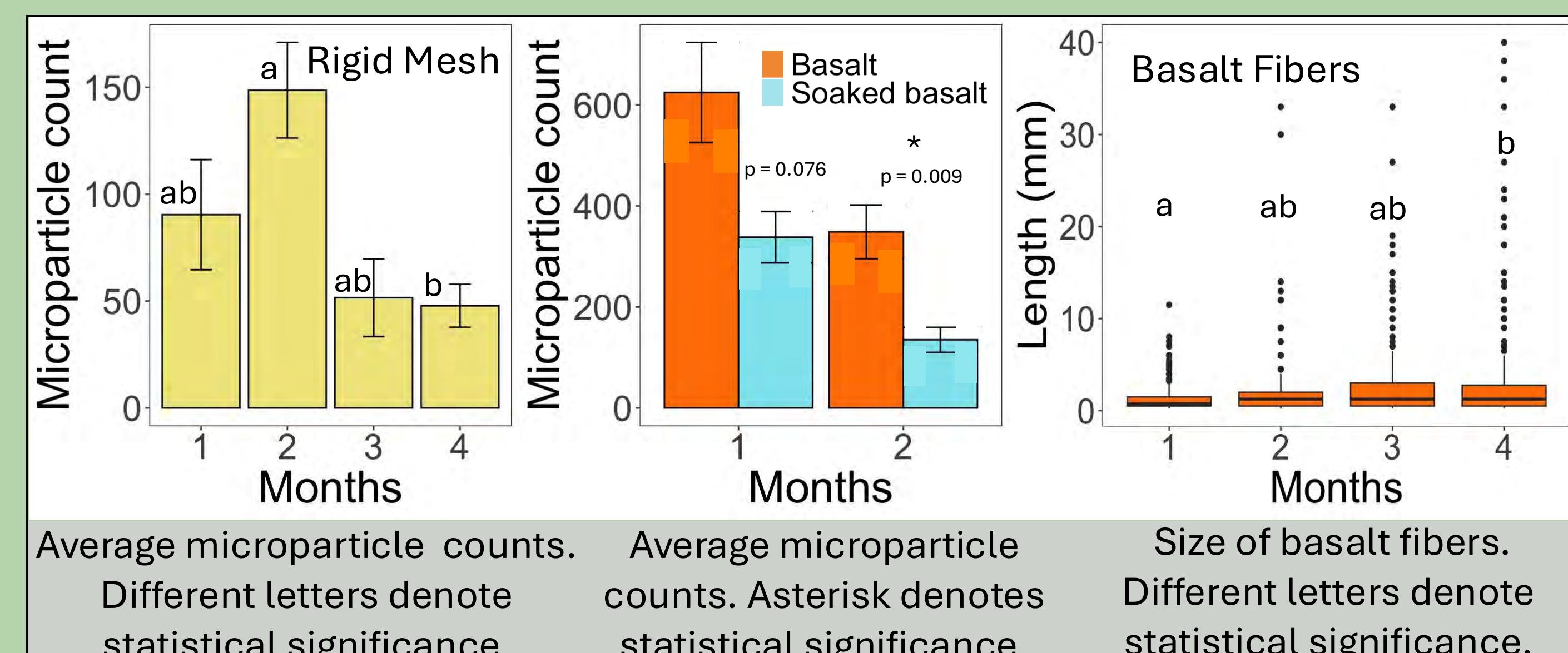
We deployed the above materials on oyster reefs in May 2024 and examined them after seven months to measure durability in materials by measuring number and dimensions of tears.



## Preliminary Results

### Laboratory Results

- Most materials stopped shedding particles after one month, but Natrx® basalt bags and BESE® rigid mesh continued to shed throughout the study.
- Basalt microfibers significantly increased in length over time.



### Field Results

- All materials experienced some damage after deployment.
- BESE® white mesh and BESE® rigid mesh had the largest tears.

Summary of damage per shell bag after seven months in field. Numbers presented are mean ( $\pm$  SE). White mesh bags completely disintegrated after seven months.

Material	Number of tears	Tear length (mm)
Orange mesh 1 (n = 30)	$4.6 \pm 0.50$	$90.5 \pm 18.55$
Orange mesh 2 (n = 30)	$3.6 \pm 0.43$	$87 \pm 17.83$
White mesh (n = 30)	NA	NA
Rigid mesh (n = 5)	$0.8 \pm 0.15$	$268.3 \pm 112.12$
Basalt (n = 25)	$1.4 \pm 0.37$	$56.1 \pm 7.34$
Plastic mesh (n = 30)	$1.8 \pm 0.28$	$31.9 \pm 4.63$

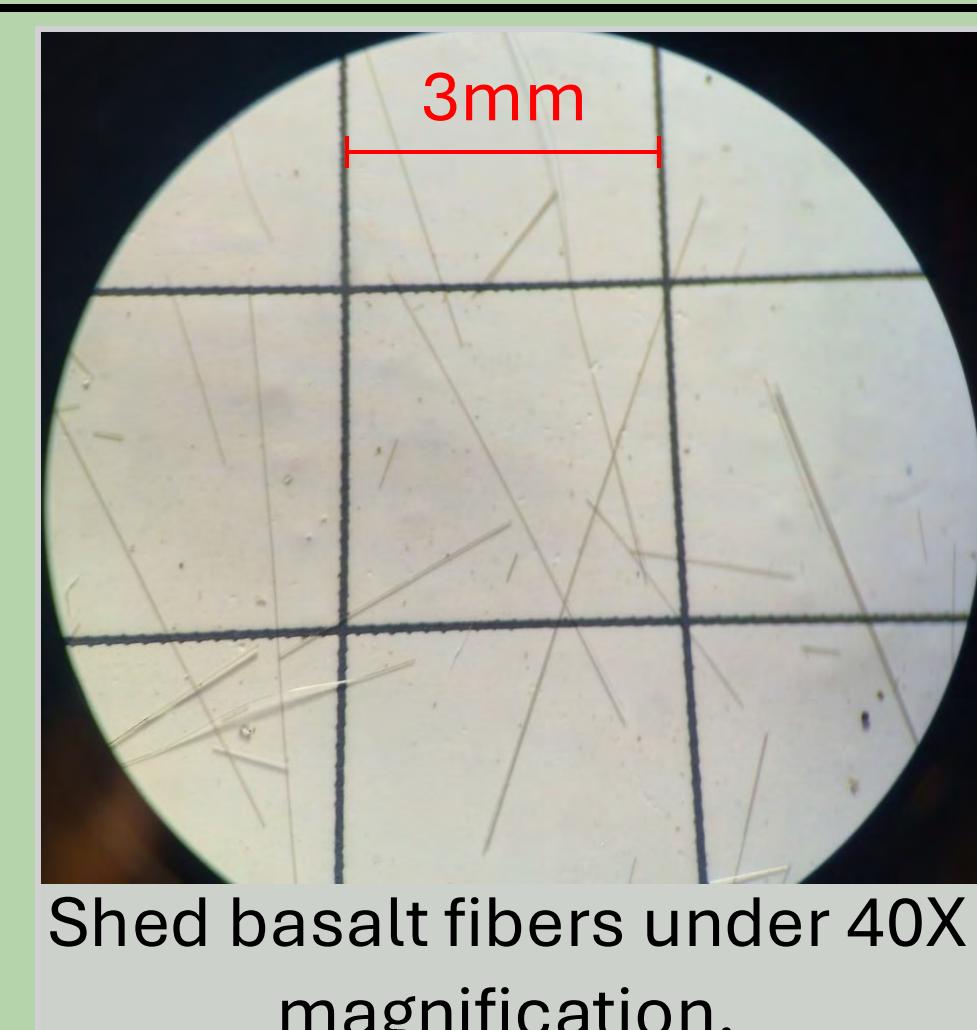
## Discussion

- Materials that shed microparticles should be carefully studied to prevent replacing plastic with another harmful substance.
- White mesh performed well in laboratory trials but failed in field deployment. **This result emphasizes the importance of examining both environmental impacts and material integrity in novel restoration materials.**

Material	Free of Microparticles?	Durable in field?
Orange mesh 1	Yes	Yes
Orange mesh 2	Yes	Yes
White mesh	Yes	No
Rigid mesh	No	No
Basalt	No	Yes

### Natr x Basalt Bags:

- Fibers were shed in high volumes throughout the lab experiment.
- Basalt fibers are not understood and may persist in waterways.
- The consequences of basalt microfiber ingestion by aquatic organisms are unknown.



Data are preliminary and will continue to be collected for 12 months before final analysis. Additional chemical analyses are also underway.

## Acknowledgements

We would like to thank all restoration partners and volunteers. This project is funded by the NOAA/SeaGrant Marine Debris Challenge grant and UCF.

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- Nitsch et al., 2021. *Sustainability* 13: 7415.

# Plastic-free restored habitats: Reducing plastic pollution in community- based restoration of oyster reefs

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## Importance

- Unsustainable harvest practices and environmental changes have reduced oyster reef distribution globally
- Habitat restoration plays a key role in rebuilding degraded reefs, often by deploying substrate into coastal waters to facilitate larval recruitment of oysters



Figure 1. Placing bagged shell at a community-based restoration event (Photo Credit: TAMU-CC MarComm)

## Problem

- Small scale restoration efforts often rely on materials that contribute to plastic pollution
- Plastic mesh offers affordability, versatility, and durability

## Project Goals

- Determine the efficacy of using plastic-free alternatives to restore intertidal oyster reefs
- Quantify the unintended consequences of using plastic materials in restoration
- Conduct community outreach, education, and dissemination of project findings

## Approach

### Materials

- NatrX basalt mesh
- BESE biopolymer mesh
- Cement-infused jute rings
- Polyethylene plastic mesh

### Deployment

- Fill bags with 12 L of recycled oyster shell
- 6 reefs deployed in both TX and FL
- 10 reps of each material per reef
- Quarterly sampling for 2 years
- Decomposition trials in triplicate adjacent to each reef



Figure 2. Litterbag with float to assess decomposition (Photo Credit: Lisa Chambers)

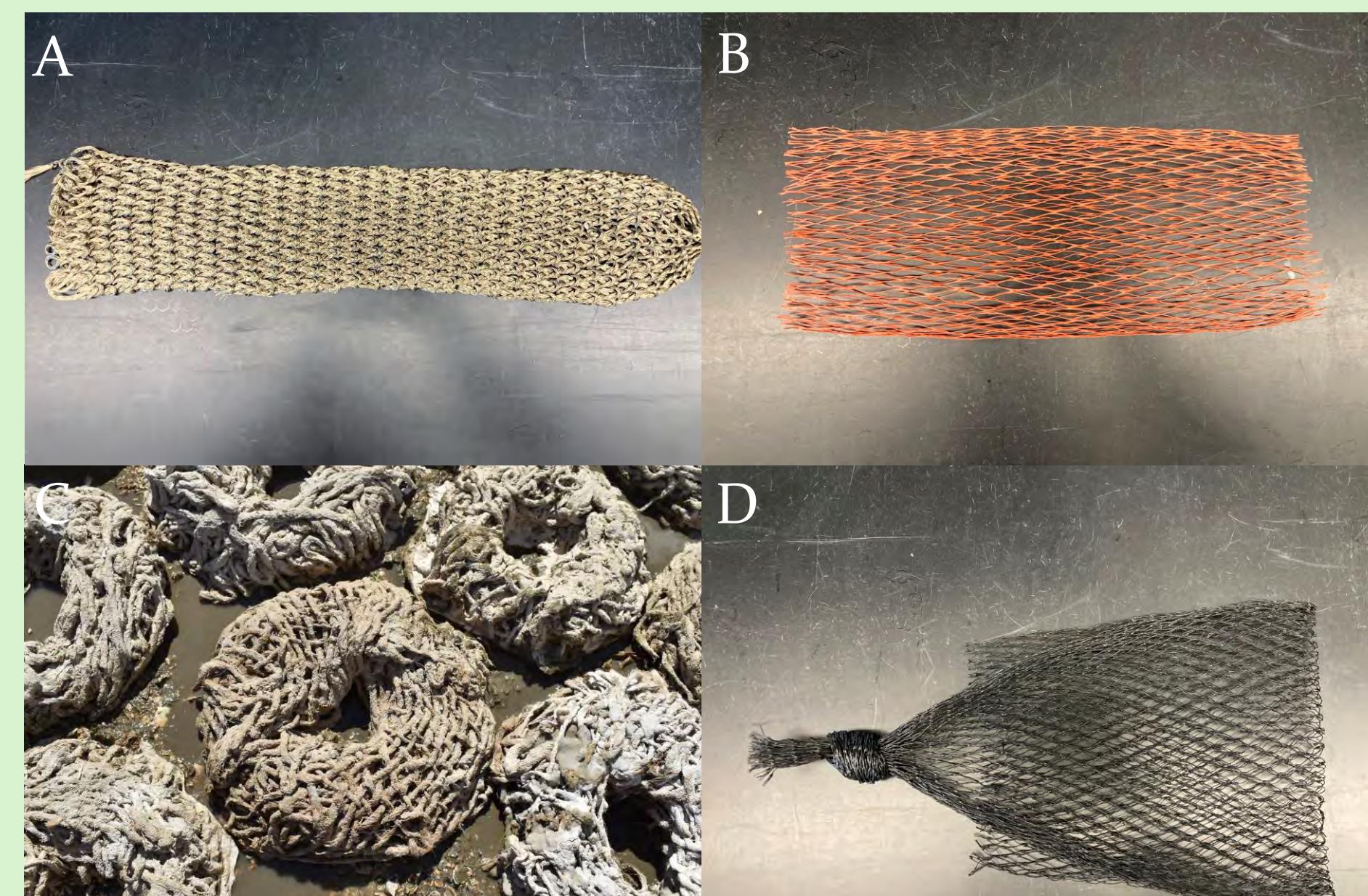


Figure 3. Restoration materials (A) NatrX basalt mesh, (B) BESE biopolymer mesh, (C) cement-infused jute ring, and (D) polyethylene plastic mesh (Photo Credit: TAMU-CC staff, Lisa Chambers)



Figure 4. Creating cement-infused jute rings (Photo Credit: Linda Walters)

## Novel materials tested in both Texas and Florida to assess usability and longevity

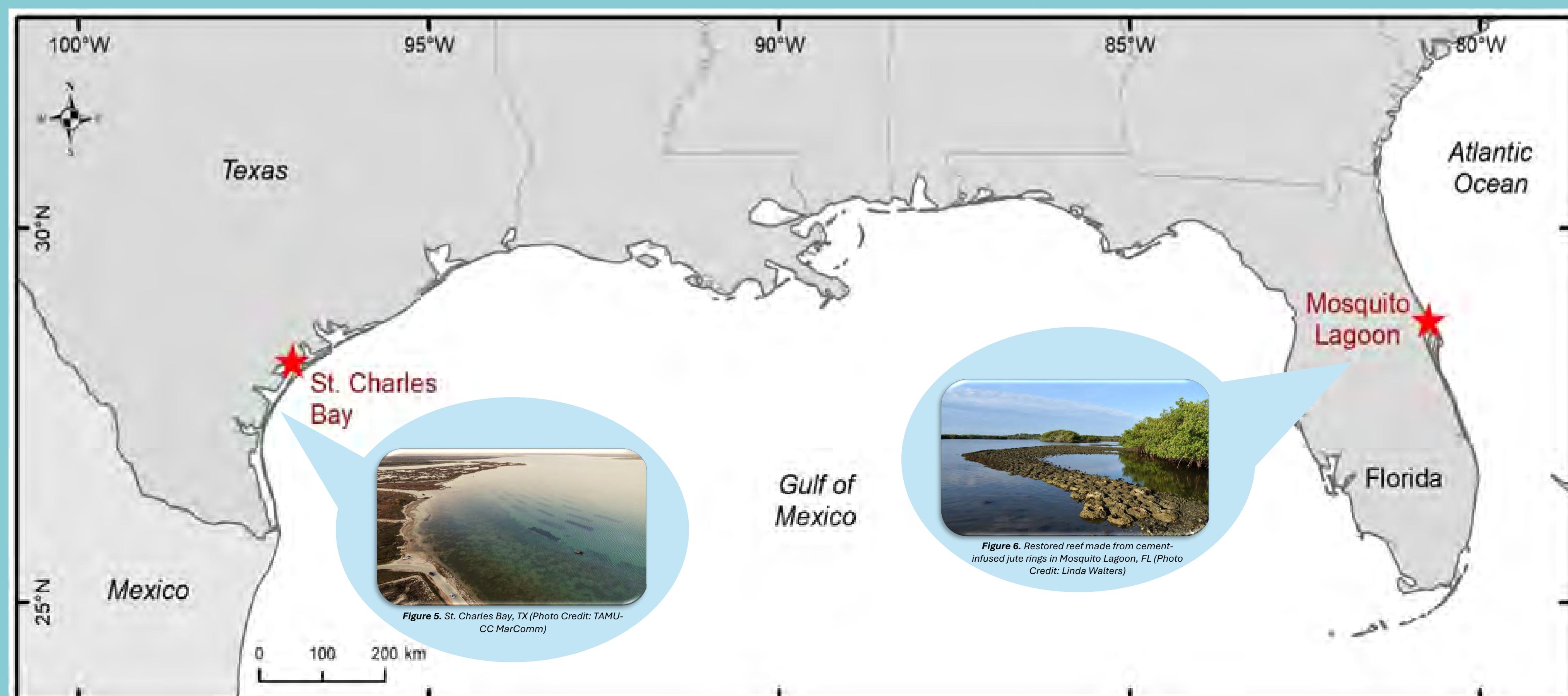


Figure 7. Project Locations

### Restoration Efficacy

- Oyster abundance and size
- Faunal community
  - Encrusting fauna
  - Motile fauna
- Bag rips and tears
- Benefit-cost ratios

### Material Degradation

- Decomposition trials
- Chemical analysis
  - Carbon
  - Nitrogen
  - Phosphorous
  - Heavy metals
- Micro- and nanoplastics

### Community Engagement

- Engage with community groups
  - Unidos en STEM Teen Cafes
  - Voices of the Colonias
  - The Arc of Indian River County
- Community restoration events
- Material volunteer-friendliness



Figure 8. Photos from previous community engagement by the project team: (A) Voices of the Colonias, (B) Unidos en STEM Teen Cafes, (C) The Arc of Indian River County, and (D) School outreach event. (Photo Credit: TAMU-CC staff, Linda Walters)

## Anticipated Benefits and Impacts

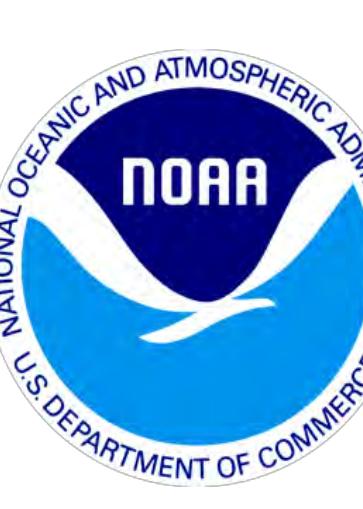
- Determine best practices
- Reduce plastic debris
- Community engagement
- Informed management
- Enhance ecosystem resilience



Figure 9. Volunteers at shell bagging event (Photo Credit: Kiese and Co)

## Acknowledgements

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# THE NURDLEOME

## Identification and characterization of microbes found on gulf coast nurdles

Author: Vibha Annaswamy | Research Educator: Dr. Kasia Dinkeloo  
University of Texas at Austin | Freshman Research Initiative | Bioprospecting 2.0

FIND THE MICROBES

FIND THE DEGRADERS

FIND THE SOLUTIONS

### Why Nurdles?

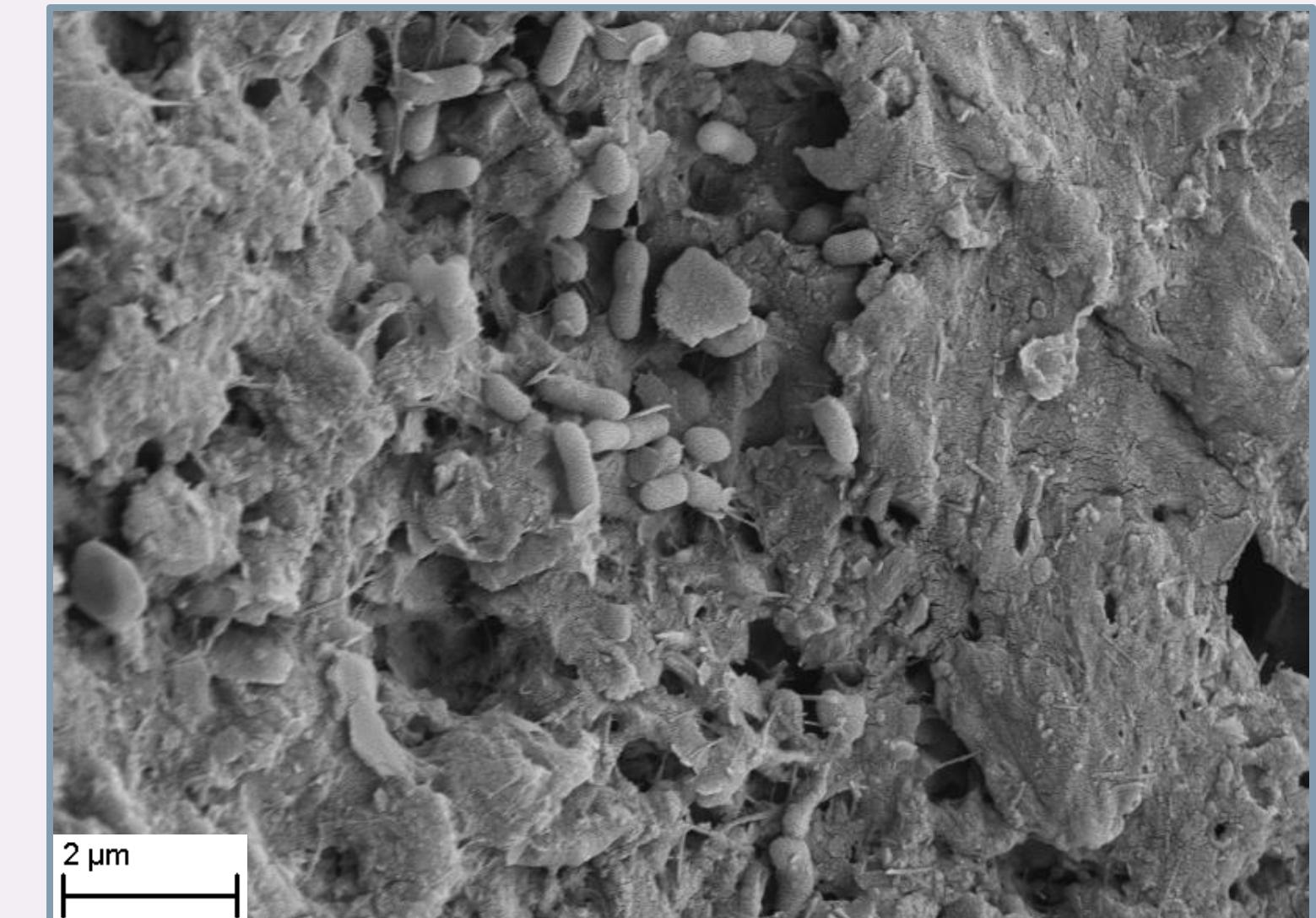
Nurdles (figures 1-2) are small plastic pellets that are used to manufacture various plastic goods. As they are often littered during transport, they can be found washed up on beach shores from runoffs or ship spills. They are one of the largest sources of micropollutants in the ocean and can be fatal to marine life. Not only do various organisms suffocate on the small pieces, but nurdles can even attract toxic chemicals to their surfaces that are released when ingested.<sup>1</sup> Since these pellets are resilient and buoyant, they serve as ideal surfaces for the formation of biofilm and accumulation of various microbes (figures 3-4). This colonization process is referred to as "plastic rafting".<sup>2</sup> It is theorized that the microbes are using the nurdles as an energy source, therefore having the ability to break down plastic. By identifying the full diversity of these microbes, we can study potential plastic-degrading species and eventually bioprospect the enzymes that facilitate this process for larger scale plastic degradation.



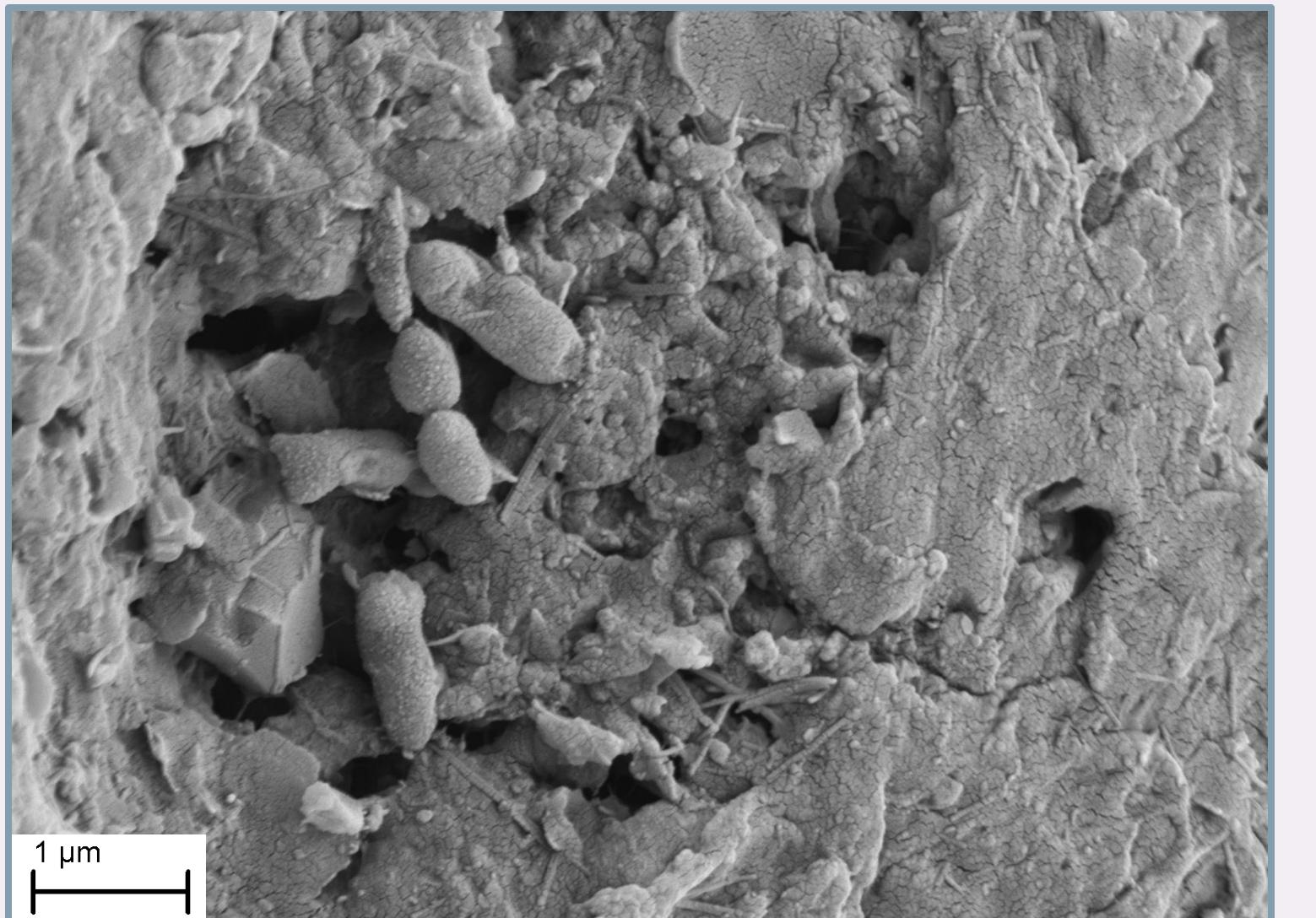
Figure 1: Photograph of a nurdle taken at Bob Hall Pier.



Figure 2: Stereomicroscope image of bulk nurdles collected from Port Aransas.



Figures 3-4: SEM images of coastal nurdles using enhanced prep to preserve bacteria.



### Targeted Metagenomic Sequencing

#### Methods

Nurdles collected from Bob Hall Pier in the spring of 2024 were processed using targeted metagenomic sequencing. For this process, we extracted DNA directly from bulk collections of nurdles to limit interference with their original microbiome. We also extracted DNA from a sand sample in order to compare the bacterial diversity associated with nurdles to that of the surrounding environment. Through library prep and sequencing, we used metagenomics to study the entire composition of microbial species present in samples of bulk nurdles while minimizing potential culture bias.

#### Results

Figures 6-7 display the most abundant genera identified in DNA samples extracted from bulk collections of coastal nurdles and from the sand. It is clear that the microbes residing on nurdles are distinct from those in the surrounding environment, revealing that the nurdle microbiome is unique to these plastics.

Genus	Number of Reads in Nurdles	Number of Reads in Sand
Sulfitobacter	5704	688
Planococcus	5104	9
Psychrobacter	4771	9
Salinimonas	4733	6
Limnobacter	110	16108

Figure 6: Average number of sequencing reads across nurdle samples as compared to the sand. The most abundant genera have been included in this table.

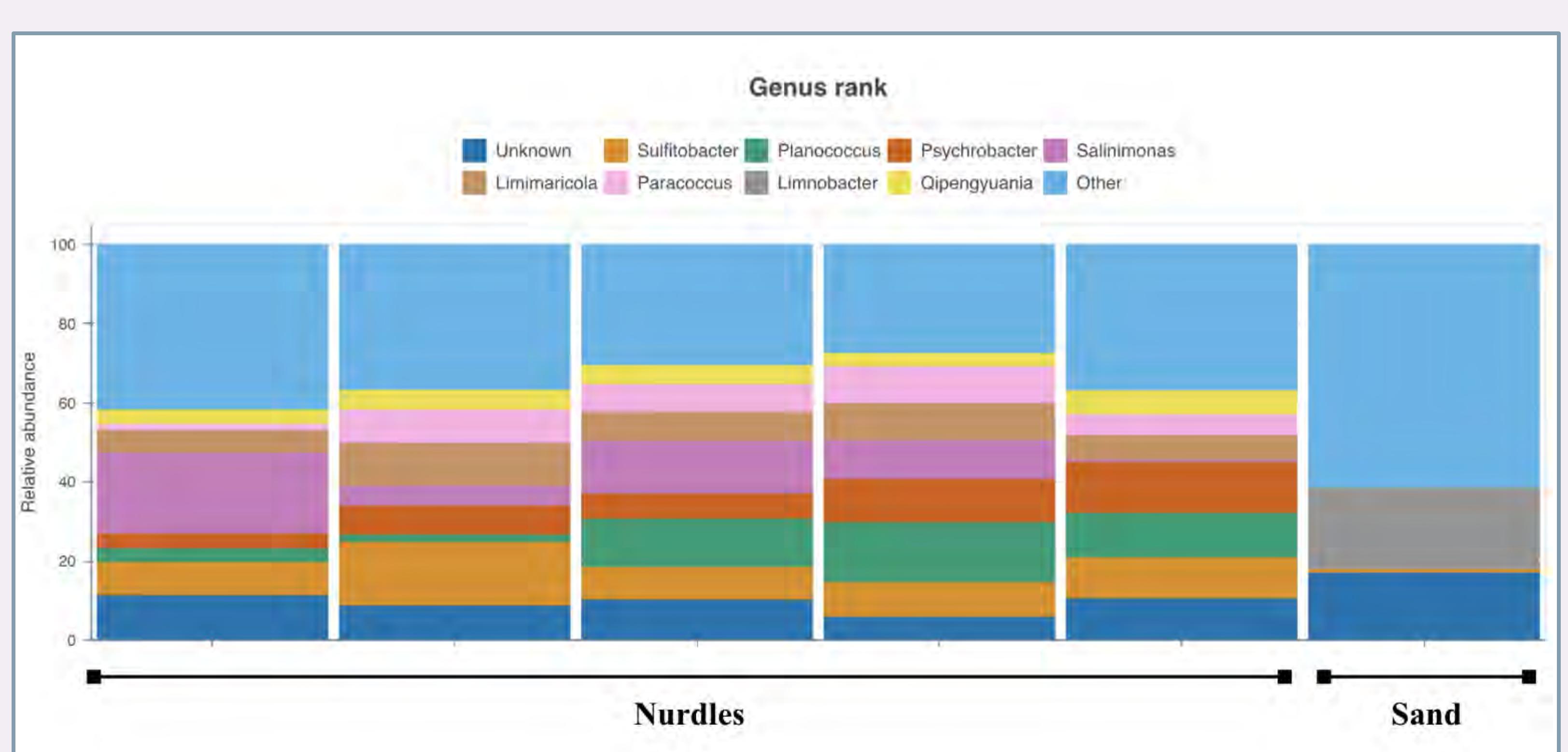
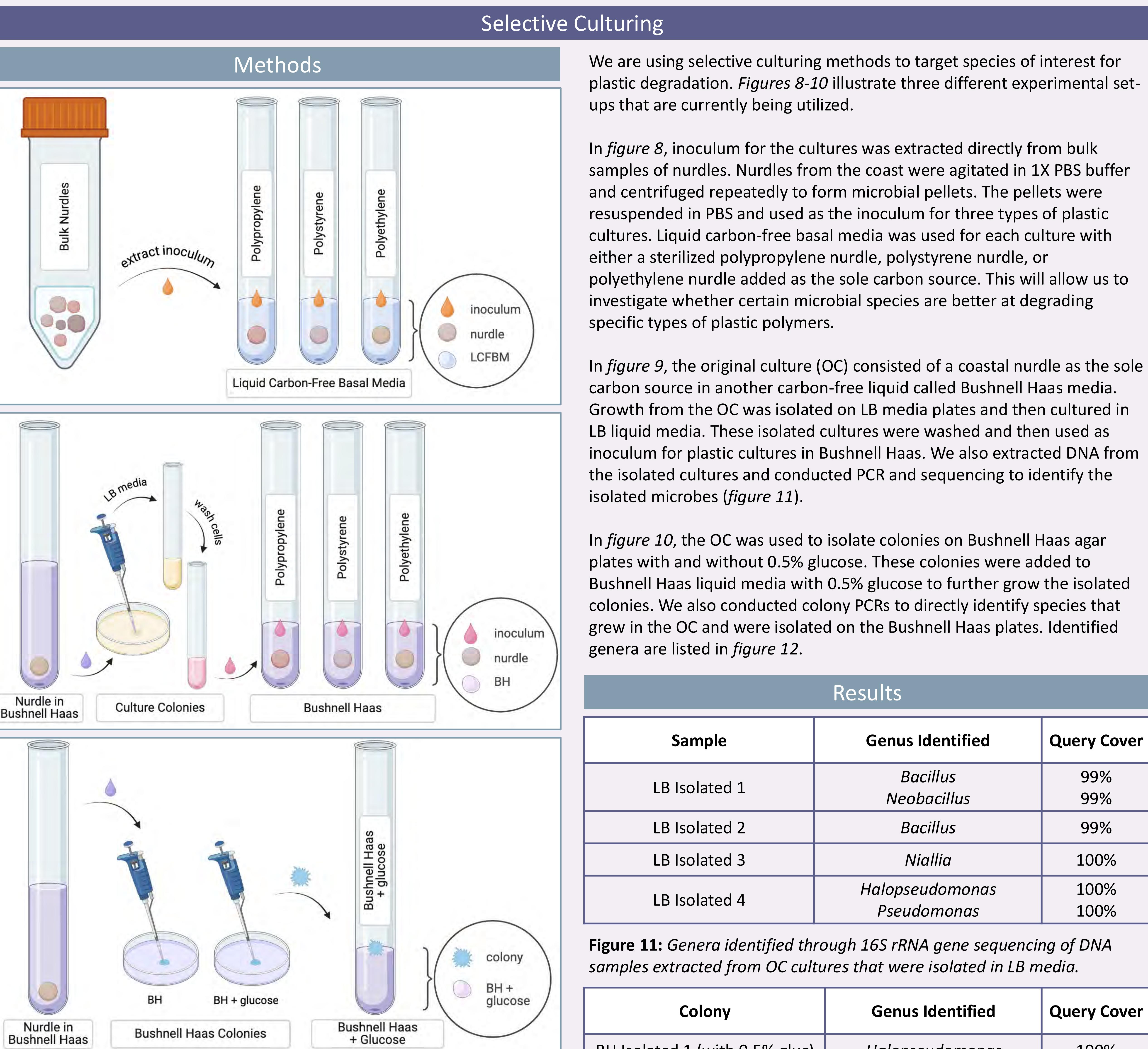


Figure 7: Relative abundances of genera identified in DNA extracted from coastal nurdles and the surrounding sand. Sequencing data was analyzed using Minimap2.



### Selective Culturing

We are using selective culturing methods to target species of interest for plastic degradation. Figures 8-10 illustrate three different experimental setups that are currently being utilized.

In figure 8, inoculum for the cultures was extracted directly from bulk samples of nurdles. Nurdles from the coast were agitated in 1X PBS buffer and centrifuged repeatedly to form microbial pellets. The pellets were resuspended in PBS and used as the inoculum for three types of plastic cultures. Liquid carbon-free basal media was used for each culture with either a sterilized polypropylene nurdle, polystyrene nurdle, or polyethylene nurdle added as the sole carbon source. This will allow us to investigate whether certain microbial species are better at degrading specific types of plastic polymers.

In figure 9, the original culture (OC) consisted of a coastal nurdle as the sole carbon source in another carbon-free liquid called Bushnell Haas media. Growth from the OC was isolated on LB media plates and then cultured in LB liquid media. These isolated cultures were washed and then used as inoculum for plastic cultures in Bushnell Haas. We also extracted DNA from the isolated cultures and conducted PCR and sequencing to identify the isolated microbes (figure 11).

In figure 10, the OC was used to isolate colonies on Bushnell Haas agar plates with and without 0.5% glucose. These colonies were added to Bushnell Haas liquid media with 0.5% glucose to further grow the isolated colonies. We also conducted colony PCRs to directly identify species that grew in the OC and were isolated on the Bushnell Haas plates. Identified genera are listed in figure 12.

### Results

Sample	Genus Identified	Query Cover
LB Isolated 1	<i>Bacillus</i> <i>Neobacillus</i>	99% 99%
LB Isolated 2	<i>Bacillus</i>	99%
LB Isolated 3	<i>Niallia</i>	100%
LB Isolated 4	<i>Halopseudomonas</i> <i>Pseudomonas</i>	100% 100%

Figure 11: Genera identified through 16S rRNA gene sequencing of DNA samples extracted from OC cultures that were isolated in LB media.

Colony	Genus Identified	Query Cover
BH Isolated 1 (with 0.5% gluc)	<i>Halopseudomonas</i>	100%
BH Isolated 2 (with 0.5% gluc)	<i>Burkholderia</i> <i>Pseudomonas</i>	100% 100%
BH Isolated 3 (with 0.5% gluc)	<i>Halomonas</i>	100%
BH Isolated 4 (no gluc)	<i>Burkholderia</i> <i>Pseudomonas</i> <i>Halopseudomonas</i>	100% 100% 100%

Figure 12: Genera identified through 16S rRNA gene sequencing of OC colonies that were isolated on Bushnell Haas plates.

### Future Questions

Do certain microbes degrade specific types of plastic polymers? What enzymatic pathways allow them to do so? Can these mechanisms be applied to large-scale plastic degradation?

### Acknowledgements

Thank you to the Freshman Research Initiative and our stream's research educator, Dr. Kasia Dinkeloo. Thank you to UT Austin's Office of Undergraduate Research awarding fellowship funds to this project. Additional thanks to electron microscopy specialist, Michelle Mikesh, for assisting with the use of SEM. And of course, I would like to acknowledge the nurdles that so graciously host microbes for us to study.

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Figure 1: Copepod with microfibers

# Threads of Change: Zooplankton Community Shifts in Response to Fiber Disturbances

Authors: Caitlyn Lankford, Heaven Thompson, Ashton Fisher, Addison Lehew, Dr. Mary Kay Johnston



## Introduction



Fig 2: Shoal Creek sampling site between César Chávez (1<sup>st</sup>) and 2<sup>nd</sup> St.

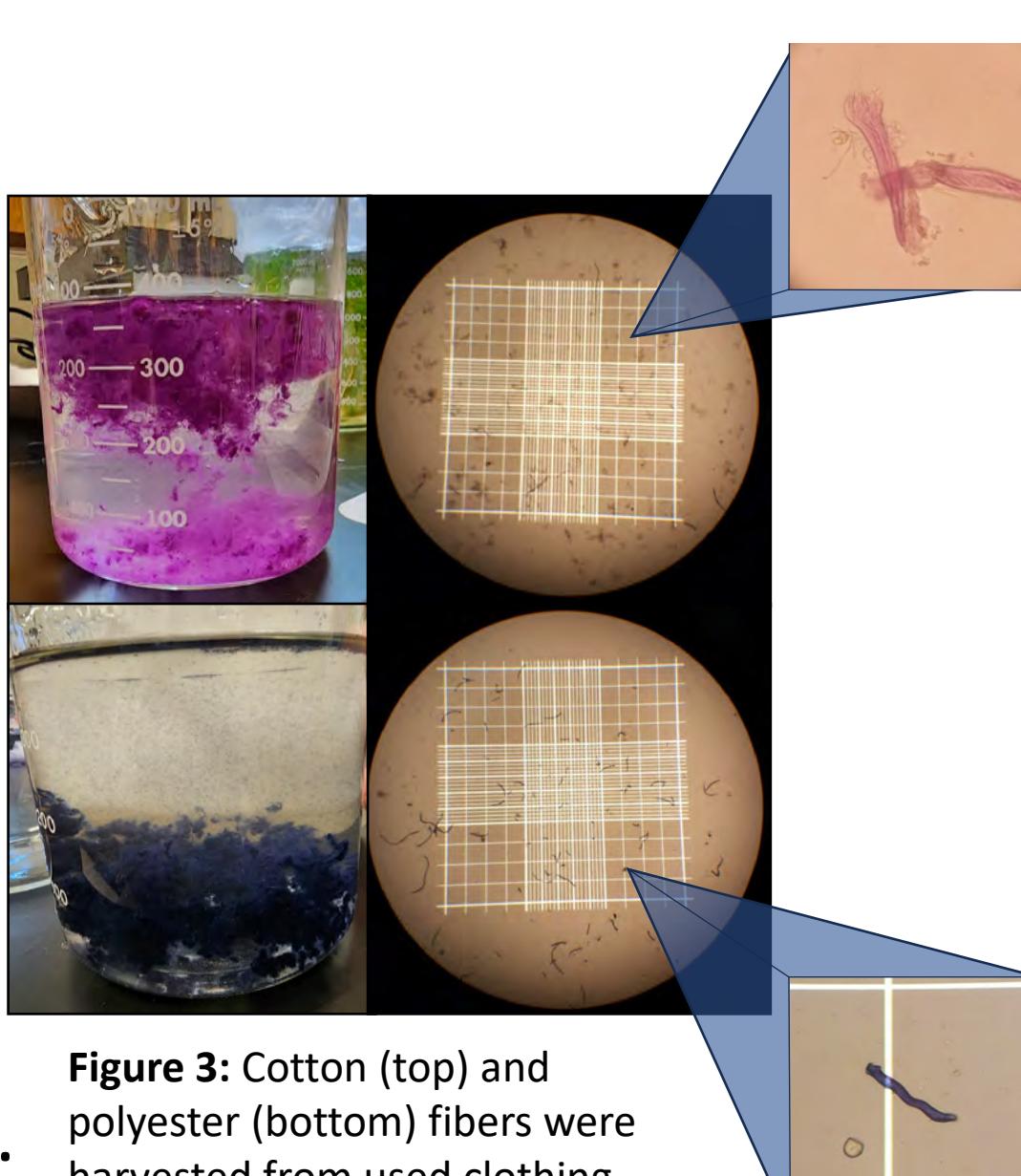
Microplastics, particularly synthetic fibers like polyester, are a growing concern in urban waterways. They can disrupt aquatic ecosystems by altering zooplankton feeding, reproduction, community dynamics, or destabilizing food webs and nutrient cycling (Rochman et al., 2015; Setälä et al., 2013). This study explores microplastic impacts through two approaches: controlled microcosm experiments assessing zooplankton responses to synthetic and natural fibers, and a field survey of microplastic concentrations across two urban creeks in Austin, Texas.

We hypothesized that polyester fibers would negatively affect zooplankton species richness, diversity, and community composition, with greater impacts in fishless systems due to altered predation dynamics. We expected higher microplastic concentrations downstream in urban creeks, reflecting urban runoff accumulation.

## Methods

### Microcosm Experiment

Zooplankton from fish and fishless ecosystems were exposed to polyester fibers, cotton fibers, or control conditions across 48 microcosms. Fibers (36–80  $\mu\text{m}$ ) were introduced at  $9 \times 10^6$  fibers/mL. Microcosms were maintained under a 12-hour light cycle for three weeks and regularly fed *chlorella vulgaris*. We assessed species richness, diversity, and community composition.



	Control	Cotton	Polyester
Fish	4	4	4
Fishless	4	4	4

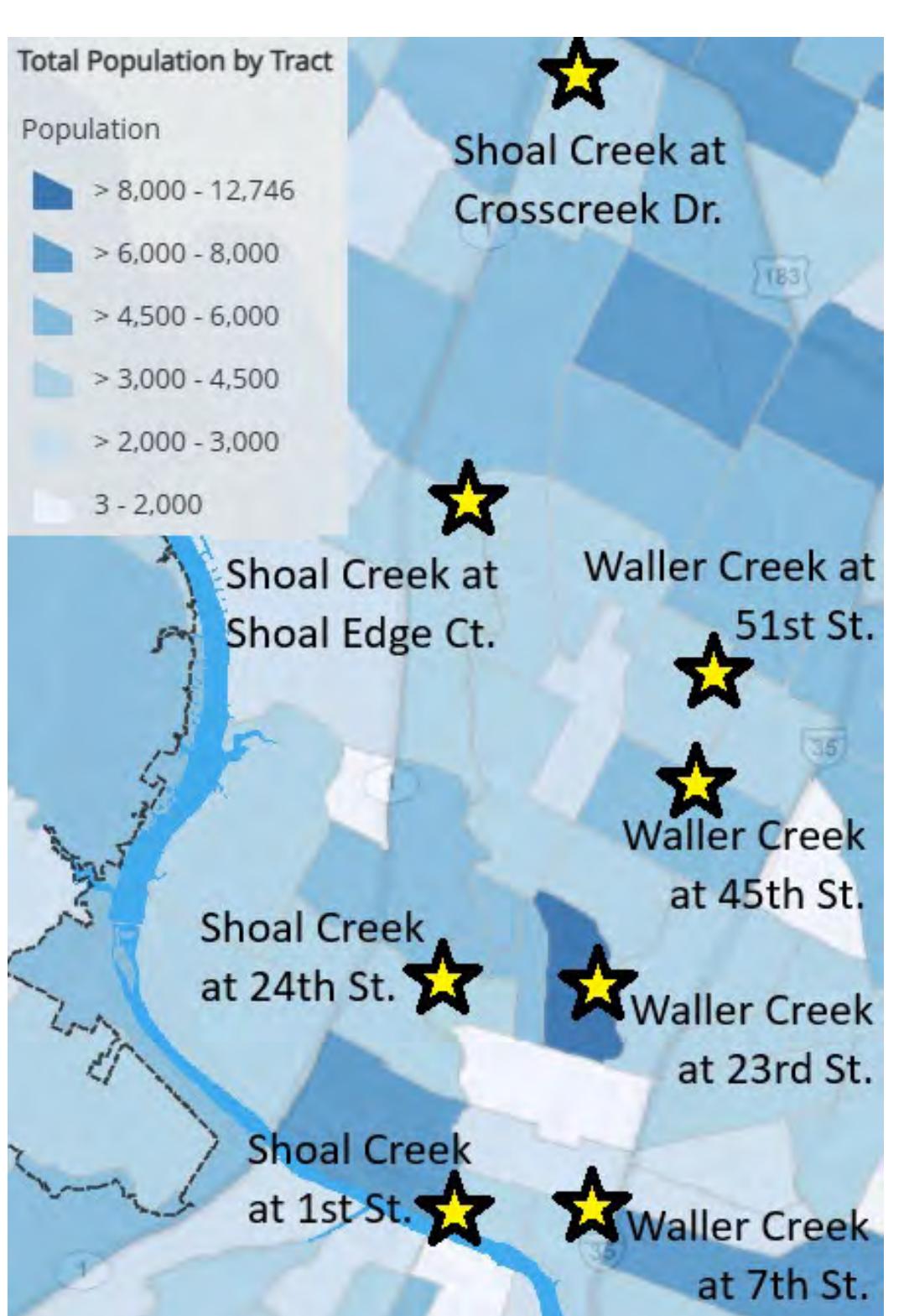


Figure 4: Population density by census tract in Austin, TX, overlaid with microplastic sampling sites along Shoal Creek and Waller Creek. Darker shades indicate higher population density. Data source: City of Austin (n.d.), Austin Demographics Hub..

### Microplastic Survey

Vertical samples of the water column were collected from four sites along Shoal Creek and Waller Creek, aligned with the City of Austin's Environmental Integrity Index (EII) locations. At each site, three 200 mL samples were vacuum-filtered through a 0.45  $\mu\text{m}$  membrane filter.

Microplastics were visually identified under a dissecting microscope and categorized by type (fiber or fragment) and color (red, white, blue, green, black).

Figure 4: Population density by census tract in Austin, TX, overlaid with microplastic sampling sites along Shoal Creek and Waller Creek. Darker shades indicate higher population density. Data source: City of Austin (n.d.), Austin Demographics Hub..

### Microcosm Experiment

Zooplankton communities were primarily shaped by ecosystem type, with fishless systems supporting higher abundance and diversity. Fiber presence had subtle, taxon-specific effects. Copepods increased in cotton treatments and decreased with polyester. In contrast, rotifers remained unaffected by fiber type, thriving in fishless environments where predation was absent. These findings highlight that while overall community structure remained stable, specific taxa like copepods may act as indicators of microplastic impacts due to their adaptable feeding strategies and ecological importance.

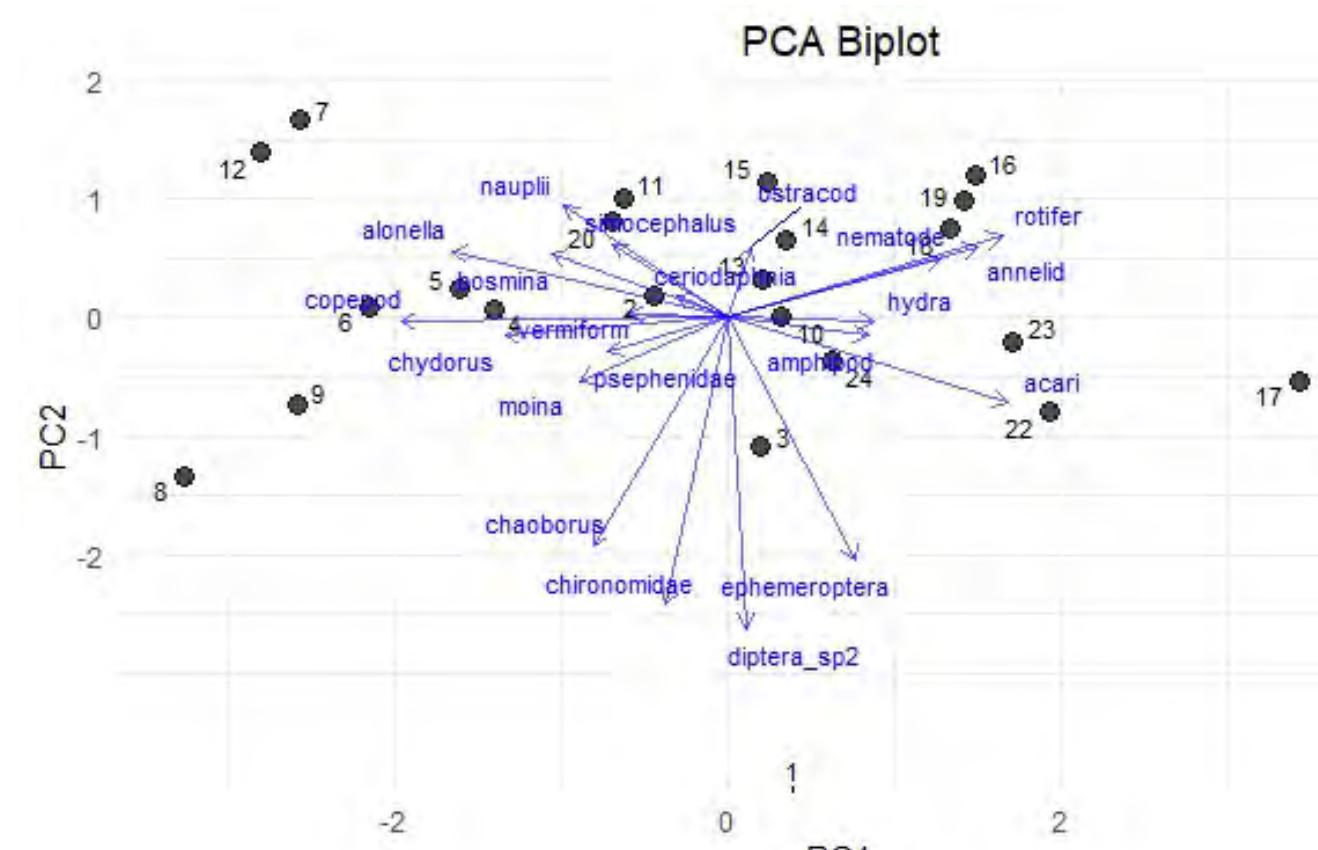


Figure 5: PCA biplot showing zooplankton community variation across fiber treatments and ecosystem types. PC1 explains 18.3% and PC2 12.9% of the variance. Copepods, chironomids, and rotifers were key drivers. Ecosystem type significantly influenced PC1 ( $F_{2,18} = 28.90$ ,  $p < 0.001$ ), while fiber effects were context-dependent ( $F_{2,18} = 3.35$ ,  $p = 0.058$ ).

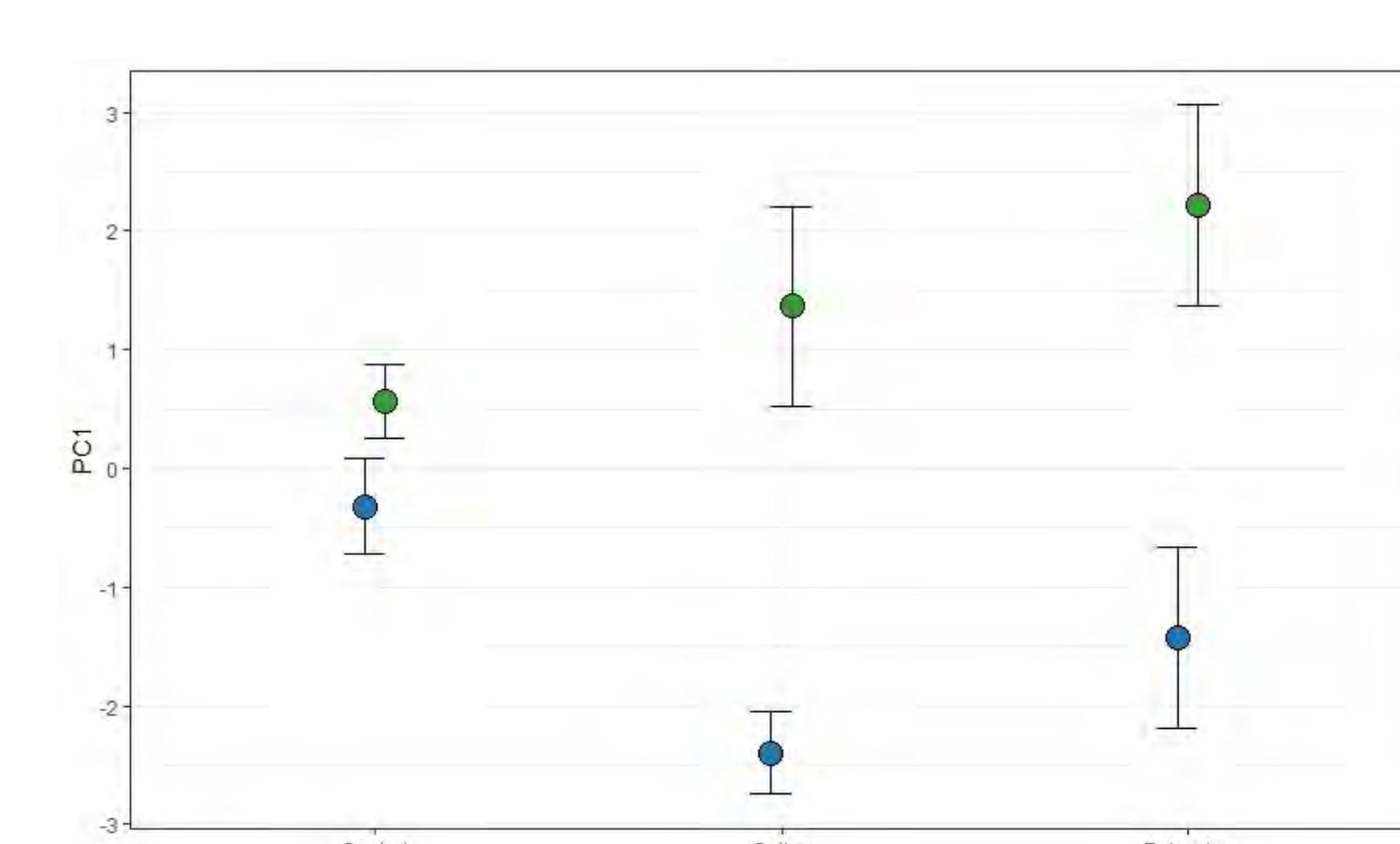


Figure 6: PC1 scores of aquatic communities under different fiber treatments (Control, Cotton, Polyester) for Fish (blue) and Fishless (green) ecosystems. Error bars show standard error. A significant interaction between fiber and community type ( $F_{2,18} = 5.82$ ,  $p = 0.011$ ) was observed. Polyester caused the largest divergence, with fishless systems consistently scoring higher on PC1.

## Results

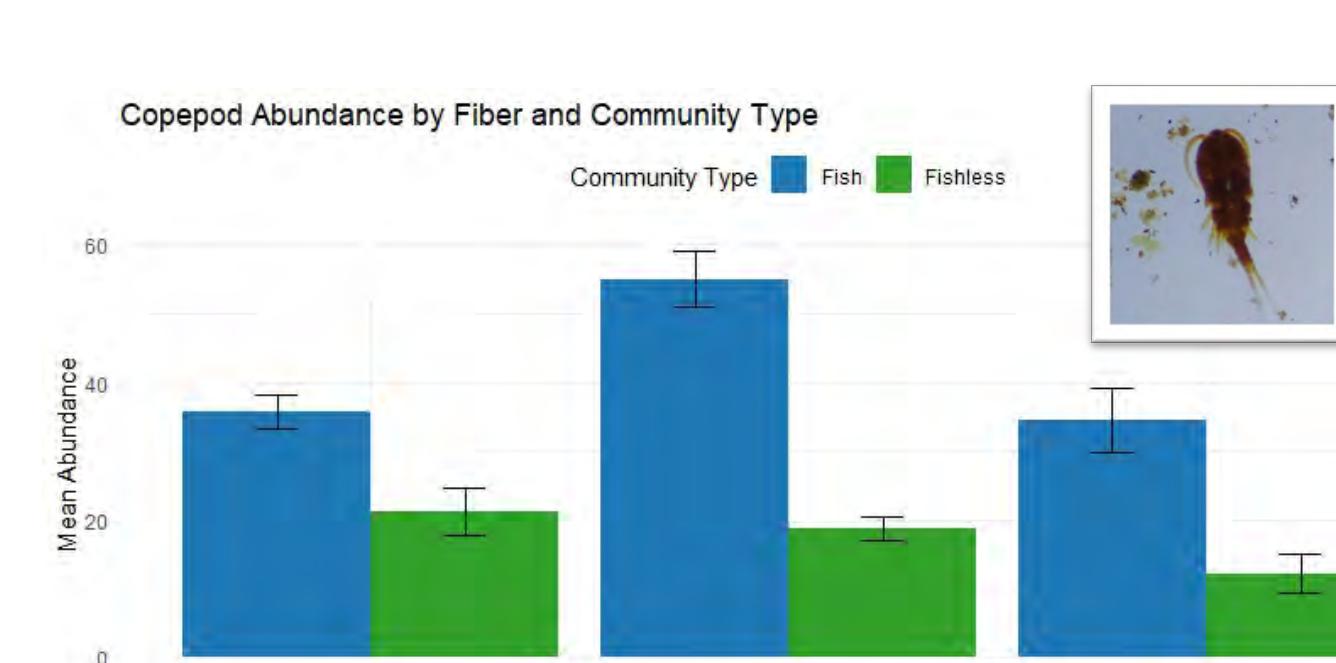


Figure 7: Mean copepod abundance across fiber treatments and community types. Error bars indicate standard error. Both fiber type ( $F_{2,18} = 9.35$ ,  $p = 0.0028$ ) and community type ( $F_{1,18} = 53.33$ ,  $p < 0.001$ ) significantly affected abundance. Cotton increased the abundance of copepods in fish systems, while polyester reduced counts overall. Inset: Copepod image.

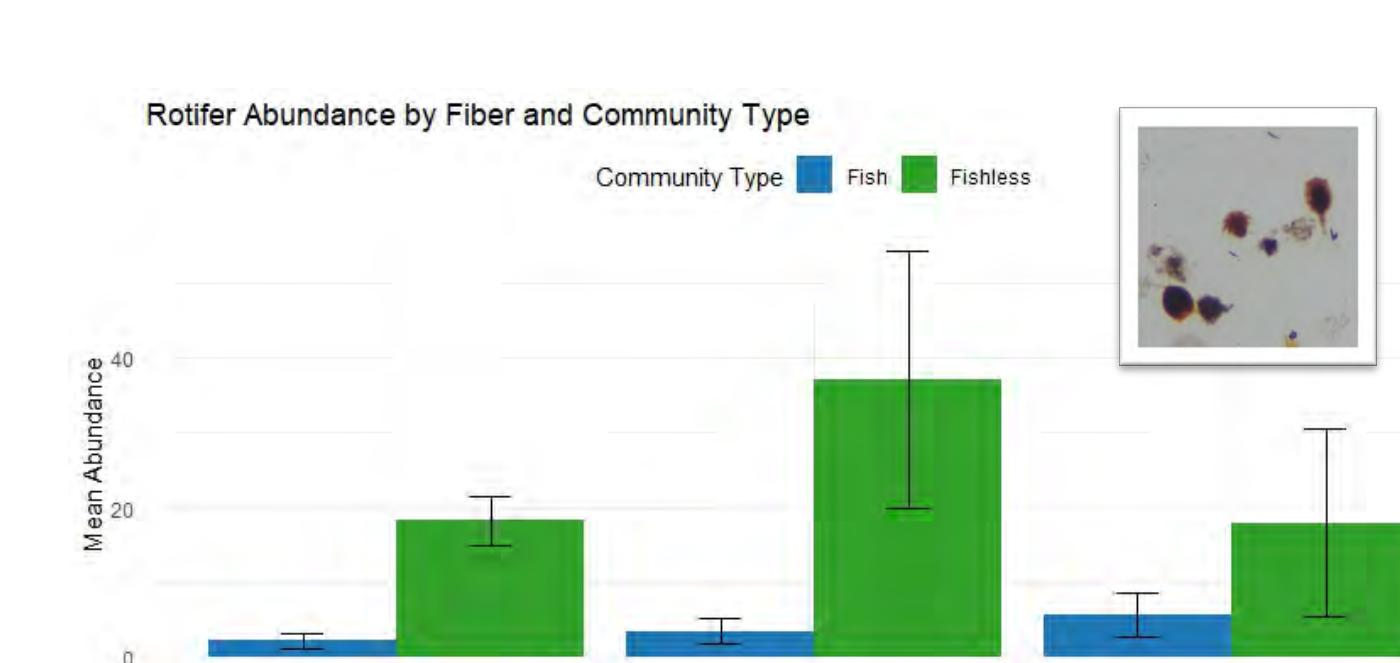
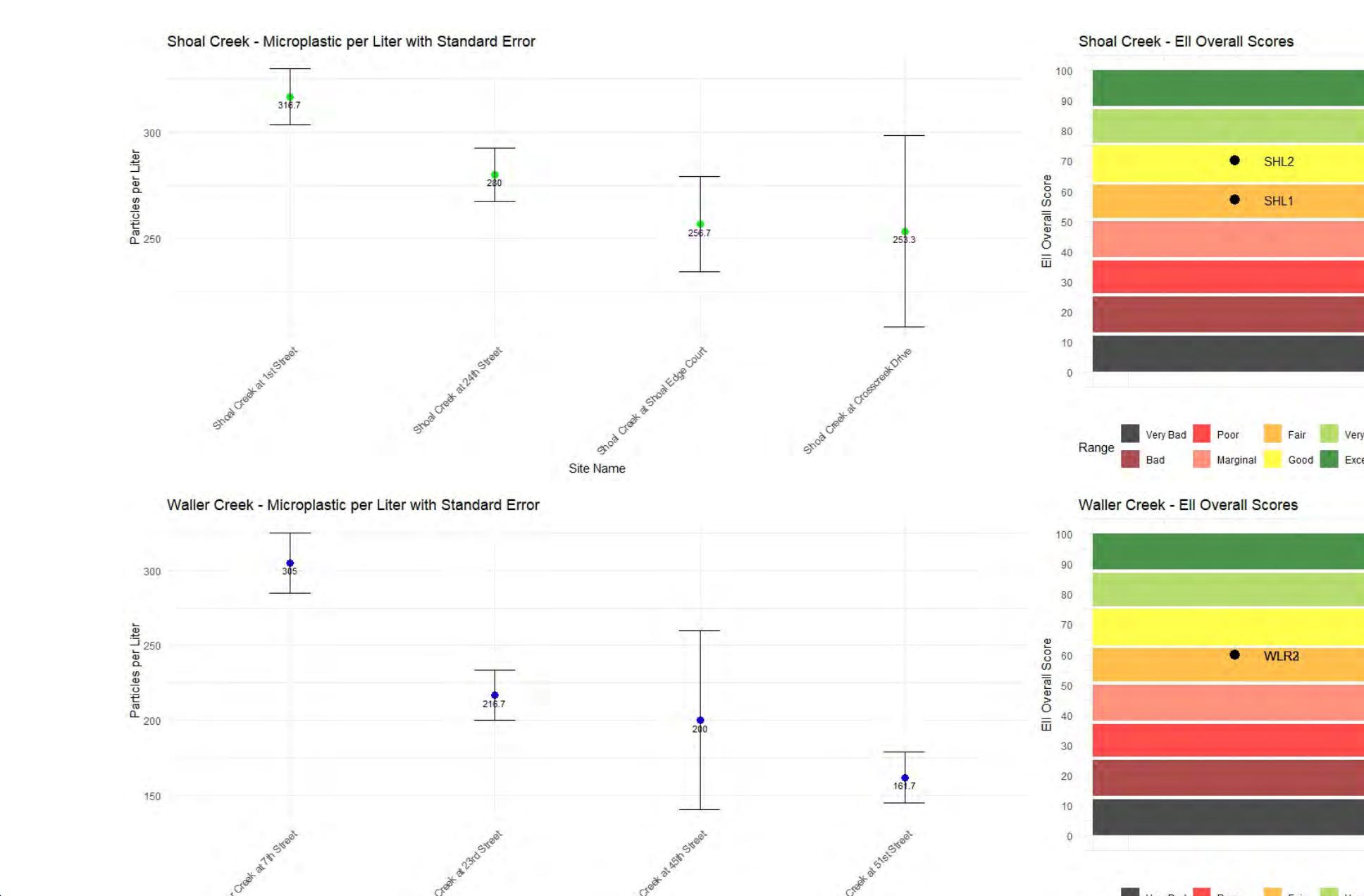


Figure 8: Mean rotifer abundance across fiber treatments and community types. Error bars show standard error. Rotifers thrived in fishless ecosystems, with the highest variability in cotton treatments. Fiber type had no significant effect; community type was the main driver. Inset: Rotifer image.

### Microplastic Survey

Microplastics were found at all sites, with fibers consistently more abundant than fragments (Figure 12). Both creeks showed increasing concentrations of microplastics downstream. Contrary to expectations, Shoal Creek, despite active conservation efforts, recorded higher microplastic levels than Waller Creek. The highest average microplastic concentrations of both streams were observed before entering Lady Bird Lake.

Black and white fibers dominated in both creeks, indicating common urban sources like textiles or construction runoff. Microplastic distribution patterns generally aligned with the streams' EII scores, reinforcing the connection between urbanization and pollutant load.



Figures 10 & 11: Microplastic concentrations and Environmental Integrity Index (EII) scores for Shoal Creek and Waller Creek. (Left Panels): Microplastic concentrations (particles/L) with standard error across four sites. Shoal Creek peaked at 1st St. (316.5 particles/L), while Waller Creek peaked at 7th St. (305 particles/L). (Right Panels): EII scores indicate "Fair" to "Good" conditions in Shoal Creek (SHL1, SHL2) and "Fair" for Waller Creek (WLR3).

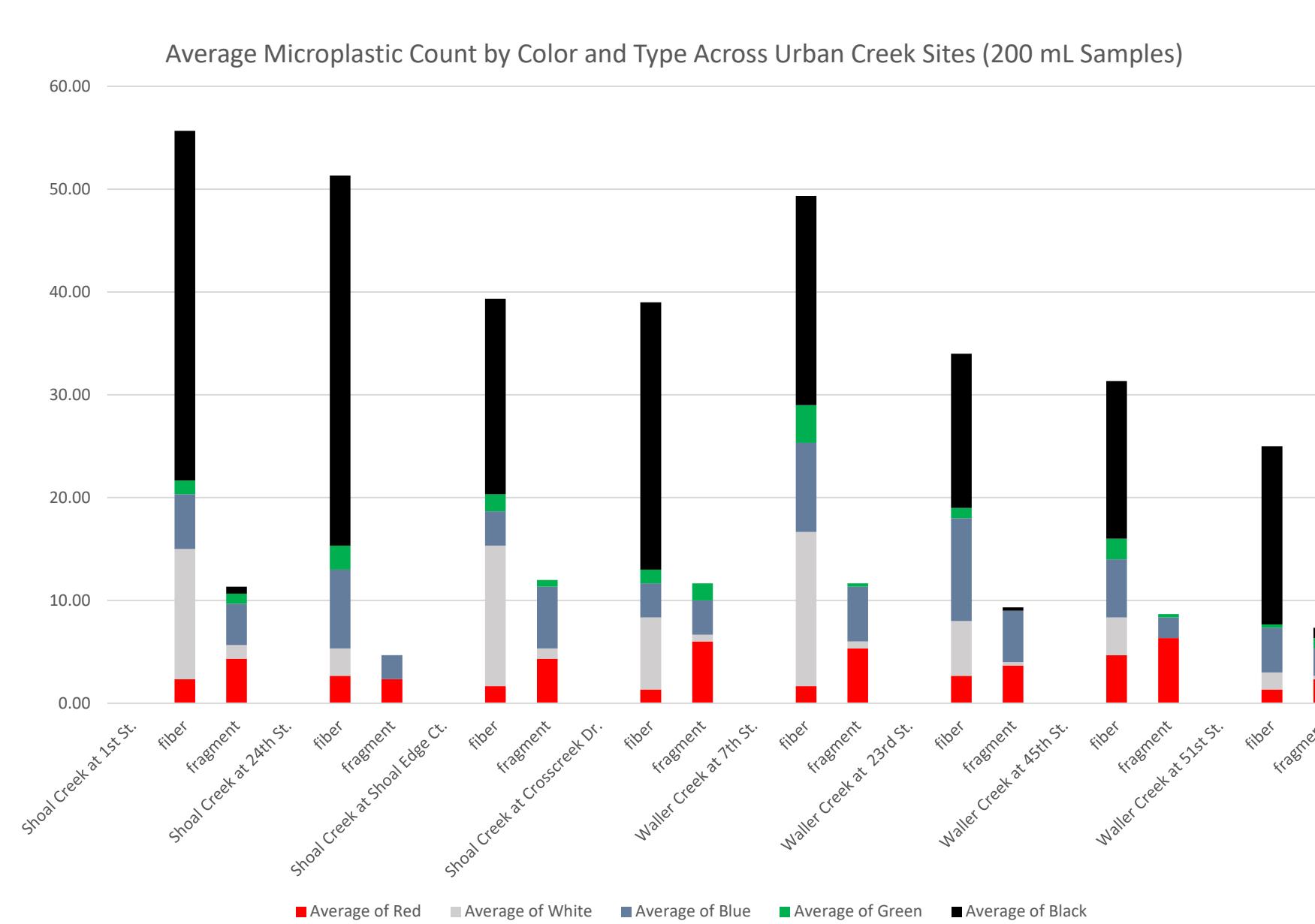


Figure 12: Stacked bar graph showing average microplastic counts per 200 mL water sample across Shoal Creek and Waller Creek sites, separated by fiber and fragment types. Colors represent microplastic types (Red, White, Blue, Green, Black), with black fibers consistently most abundant. Data were averaged from replicates filtered through 0.45  $\mu\text{m}$  filters.

## Discussion

Microplastic impacts are context-dependent, shaped by ecosystem dynamics and pollutant types. The microcosm experiment demonstrated that copepods responded to fiber type, suggesting that microplastic exposure may influence certain taxa. However, broader community patterns remained stable, indicating that short-term exposure at tested concentrations may not cause immediate shifts in species composition.

Field data showed the expected downstream accumulation of microplastics. Population density mapping suggests a link between urbanization and microplastic concentrations (Figure 4). While urban density may contribute to these trends, further investigation is needed to determine how factors like stormwater management, infrastructure, and localized pollution sources interact to influence microplastic distribution.

Future studies should integrate urban planning perspectives to assess how population density, waste management, and city infrastructure impact microplastic pollution. Additionally, long-term monitoring is needed to evaluate potential ecological consequences, particularly for taxa sensitive to fiber exposure.



Figure 13: Image of Shoal Creek sampling site with a callout showing a magnified view of a Bosmina with a blue microfiber on a membrane filter.

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