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Microplastics, Oysters, and the Indian River Lagoon – Final Report to IRL NEP, March 2021

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Microplastics, Oysters, and the Indian River Lagoon – Final Report

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Smithsonian Marine Station, Ft. Pierce; Environmental Learning Center, Vero Beach; St. Lucie County's Oxbow Eco-Center, Port St. Lucie; Friends of the Spoil Islands

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UCF Project Account: 24-03-7065

EXECUTIVE SUMMARY

UCF and partners initiated microplastics (MP) sampling in the Indian River Lagoon in March 2019, with monthly sampling of water samples at 35 locations, and quarterly sampling of oysters through February 2020. Additionally, the ability of the eastern oyster *Crassostrea virginica* to excrete microplastics was examined.

Forty-four percent of water samples contained MP and there was an overall mean (± S.E.) of 1.47 (± 0.05) MP per liter of lagoon water (range: 0 – 25 MP). Fibers were the dominant MP type of MP (95.6%) and no nurdles or microbeads were collected. The dominant color of MP in water was clear followed by light blue, black and dark/royal blue. The mean length of MP was 1.94 (± 0.13) mm with a range in length from 0.1 to 30 mm. Water from the southern regions of the lagoon contained significantly higher abundances of MP and these MP were smaller in length. A significant difference in MP abundance across sampling months was also detected. Samples collected during April, May, June, October, December and February samples had significantly more MP than all other months.

Oyster sampling took place in May, August, and November 2019, and February 2020. Overall, 70% of 1440 oysters contained MP. A total of 3181 MP was found in oysters. Lagoon-wide, the mean abundance of MP in oysters was 2.26 (± 0.08), and mean density per gram tissue weight was 2.43 (± 0.26) MP. The mean length of MP was 2.79 (± 0.06) mm. Fibers dominated collections (95.0%) and black, clear and dark blue were the dominant colors. Smaller oysters (< 35 mm) contained higher densities of MP per gram tissue weight than adults and contained larger MP. Oysters from the southern IRL had more MP than other regions, but there was no difference in MP length between locations.

Experimental trials to examine MP in oyster biodeposits (feces + pseudofeces) were conducted from 15 July through 18 July 2019. MP were present in both feces and pseudofeces from both small and adult oysters. Overall, 67% of oysters produced biodeposits that contained MP. MP ranged in length from 0.05 - 20 mm in pseudofeces and 0.05 - 6 mm in feces. Mean length of MP in pseudofeces was 1.73 (± 0.16), and 1.46 (± 0.08) mm in feces. Fibers accounted for 88.3% of MPs found in biodeposits, and black MPs were the most abundant color. MPs in biodeposits from small oysters were significantly larger than those in adult biodeposits by an average of 1.07 mm. Oysters had a mean MP egestion efficiency of 62.1%, and 32% of oysters were able to egest all MP from their tissues during the 2-h trials. There was no egestion efficiency difference among IRL regions. MP egestion efficiency decreased significantly with increasing oyster shell height and tissue mass; AIC model selection revealed tissue mass was best at predicting MP egestion efficiency in *C. virginica*. Egestion efficiency decreased by 0.8% for every 1-gram increase in tissue weight. Overall, results indicated that oysters excreted at a rate of approximately 1 MP per 1 hour through feces, and 1 MP per 2 hours through pseudofeces.

INTRODUCTION

Plastic pollution in our oceans reached 30 million tons a year in 2013, up from 0.5 million tons annually in the 1960s (Beaman et al. 2016). MP are defined as plastic pieces less than 5 mm in size and can either be manufactured at this small size (primary) or fragment from larger plastic objects (secondary). Primary MP originate from industrial raw materials sometimes referred to as "nurdles" which are melted and used to create larger plastic items. Alternatively, primary microplastics were produced for use in personal care products, including toothpastes and facial cleansers. Secondary MP were degraded through various physical, chemical, and biological processes. The most commonly found microplastics are fibers and fragments, with beads, foams, and films found occasionally (e.g., Boucher and Friot 2017).

MP infiltrate estuaries and subtidal regions and have been documented in more than 180 marine species (Wang et al. 2016). Filter-feeding organisms are especially vulnerable to MP ingestion due to their feeding mechanisms. In the laboratory, ingestion was found to negatively impact reproductive processes in bivalves (Sussarellu et al. 2016). Dr. Walters and colleagues conducted a study of the eastern oyster, *Crassostrea virginica*, and the Atlantic mud crab, *Panopeus herbstii,* in Mosquito Lagoon and recorded an average of 4.2 MP in crab tissues/individual (n=90), and 16.5 MP in adult oyster tissues/individual (n=90) (Waite et al. 2018). Fibers were the most common type of MP found in samples collected from Mosquito Lagoon (70%).

These Mosquito Lagoon results prompted questions about the temporal and spatial extent of MP throughout the Indian River Lagoon (IRL) from Ponce de Leon Inlet to St. Lucie Estuary. Were the results of Waite et al. (2018) collected from central Mosquito Lagoon at a single point in time in the spring of 2017 representative of the entire system? Moreover, microplastics research has greatly evolved in recent years to include contamination protocols that were not reported in Waite et al. (2018). Likewise, polymer identification was not available to Waite's team. Both enhanced contamination protocols and polymer identification via Fourier-transform Infrared Spectroscopy (FTIR) to identify polymer composition of MPs were incorporated in our project to better understand this pollutant in the IRL.

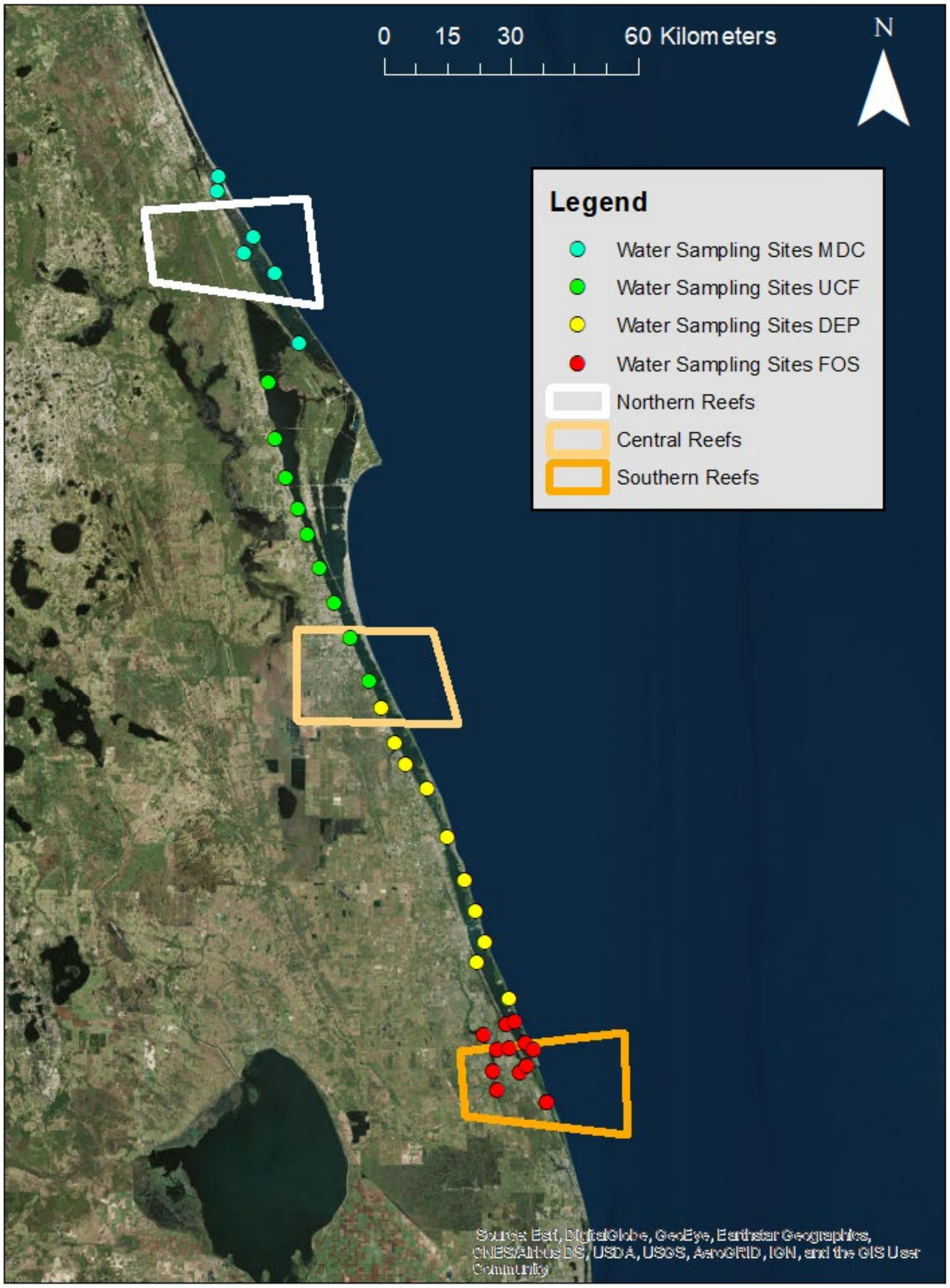
I. OBJECTIVES

The following objectives were completed for this project:

(1) Conduct coordinated monthly water sampling at 35 locations throughout the IRL for 12 consecutive months.
(2) Conduct coordinated quarterly oyster sampling of 12 oyster reefs in the IRL (6 in the north region, 3 in the central, and 3 in the south) for one year.
(3) Conduct feces/pseudofeces experimental microplastic excretion trials on adult and juvenile oysters.
(4) Include public engagement of a minimum of 300 hours and 60 individuals through citizen-science efforts.
(5) Provide a final report of our understanding of MP abundance and diversity in IRL water and oysters.

II. SCOPE

Monthly water samples were collected for 35 sites for the March 2019-February 2020 (Figure 2.1, Table 1). Quarterly oyster samples were collected from 12 reefs with 6 from northern IRL system (Mosquito Lagoon), 3 from central IRL reefs, and 3 from southern IRL reefs (Figure 2.1, Table 1).



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 2.1. Water and oyster sampling locations.

Sample Collections	Scheduled	Completed
Water Sampling		
March	3/2/19 - 3/5/19	3/2/19 - 3/5/19
April	4/6/19 - 4/9/19	4/6/19 - 4/9/19
May	5/4/19 - 5/7/19	5/4/19 - 5/7/19
June	6/1/19 - 6/4/19	6/1/19 - 6/4/19
July	7/6/19 - 7/9/19	7/6/19 - 7/9/19
August	8/3/19 - 8/6/19	8/3/19 - 8/6/19
September	9/7/19 - 9/10/19	9/7/19-9/10/19
October	10/5/19 - 10/8/19	10/5/19-10/8/19
November	11/2/19 - 11/5/19	11/1/19 - 11/5/19
December	12/7/19 - 12/10/19	12/7/19 - 12/10/19
January	1/4/20 - 1/7/20	1/4/20 - 1/7/20
February	2/1/20 - 2/4/20	2/1/20 - 2/4/20
Oyster Sampling		
1st Quarter	May-19	5/22/19, 5/26/19
2nd Quarter	Aug-19	8/20/19, 8/21/19
3rd Quarter	Nov-19	11/14/19, 11/21/19
4th Quarter	Feb-20	2/18/20, 2/29/20
Feces/Pseudofeces Sampling		
Feces/Pseudofeces	July 2019	7/15/19-7/18/19

Table 1. Project Sampling Dates.

III. METHODS AND RESULTS OF IRL-WIDE WATER SAMPLING

3.1 Water Sampling Methods: Water samples were collected once per month within a four-day period, starting with the first Saturday of each month, concluding on the following Tuesday. At each of the 35 locations, five 1-L samples were collected from water surfaces. Bottles were triple-rinsed prior to collection with 0.45 micron filtered deionized water in the laboratory, followed by triple-rinsing with lagoon water upon arrival at site. Bottle rinsing occurred at least 10 m away from the collection location. Rinsed bottles were submerged to collect the top 5 cm of lagoon water and capped while submerged. Bottles were taken to processing sites and vacuum filtered using a 0.45 μm nitrocellulose member filter paper. Filters were then inspected for MPs using a dissecting microscope at up to 40X magnification. MP type, color, and length were recorded. Control blanks (n = 5) were utilized during all inspections to quantify aerial MP contamination.

3.2 Water Sampling Results: Water samples were collected for all 35 sites for 12 months ending in February 2020. Due to COVID delays, processing of water samples was completed in November 2020. Forty-four percent of water samples contained MP. The most abundant type of MP, fibers, comprised 95.6% of all plastics found (Fig. 3.2.1). Fragments, foams, and films comprising the remaining 3.9%, 0.3%, and 0.2% of MP, respectively. No microbeads or nurdles were found in any water samples. The most abundant color of MP found in water samples was clear, followed by light blue, black, and dark/royal blue (Figure 3.2.2). The mean length of plastics found in water samples was 1.94 (± 0.13) mm. Plastics ranged in length from 0.1 mm to 30 mm. Water from southern sites contained significantly smaller MPs than those of the central and north regions (ANOVA, p=8.57e-14; Fig. 3.2.3).

A total of 3755 MPs were found, with a mean of 1.47 (± 0.05) plastics per liter of lagoon water. There was a minimum of 0 MPs per liter and maximum of 25 MP. There was a significant difference of MP abundance in water from different regions of the IRL (GLM, $p < 0.05$, n=2180). Figure 3.2.4 illustrates southern IRL sites had the highest abundance of MPs, followed by the north and central regions. A significant difference was also detected in MP abundance across sampling months (GLM, $p < 0.05$, n=1728; Fig 3.2.5). Water samples from April, May, June, October, December, and February contained significantly higher abundances of MP.

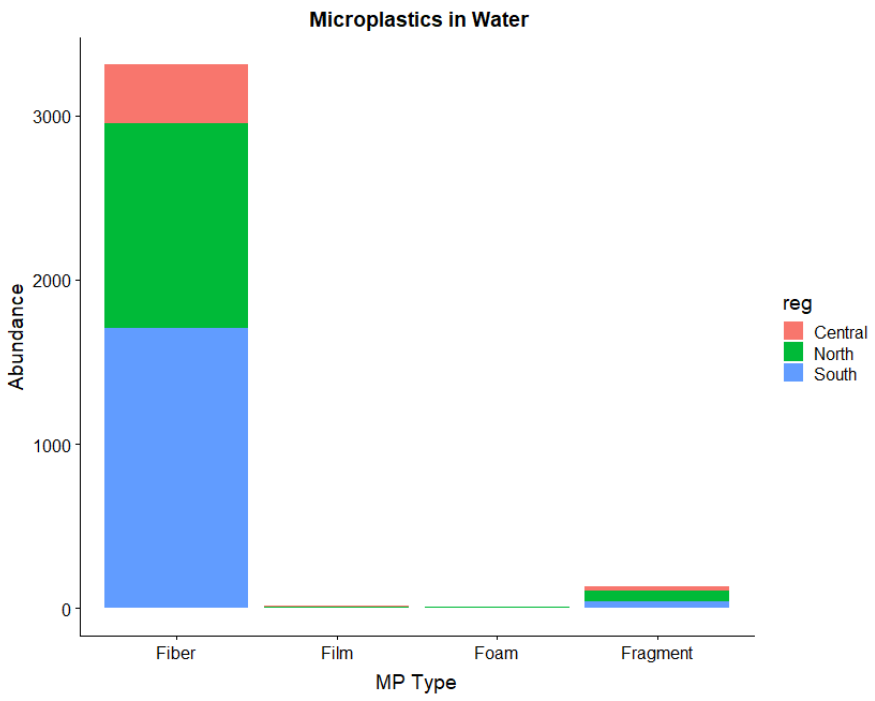


Figure 3.2.1 The total count of MPs analyzed in water samples by type.

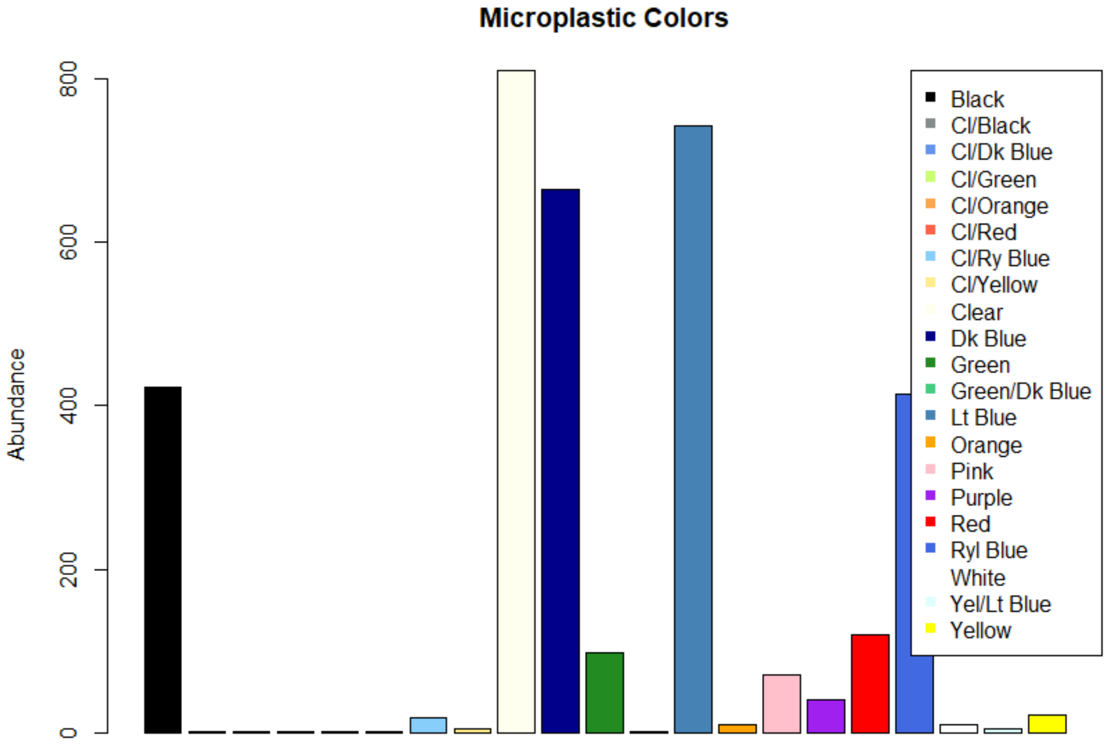


Figure 3.2.2 The total count of MPs analyzed in water samples by color.

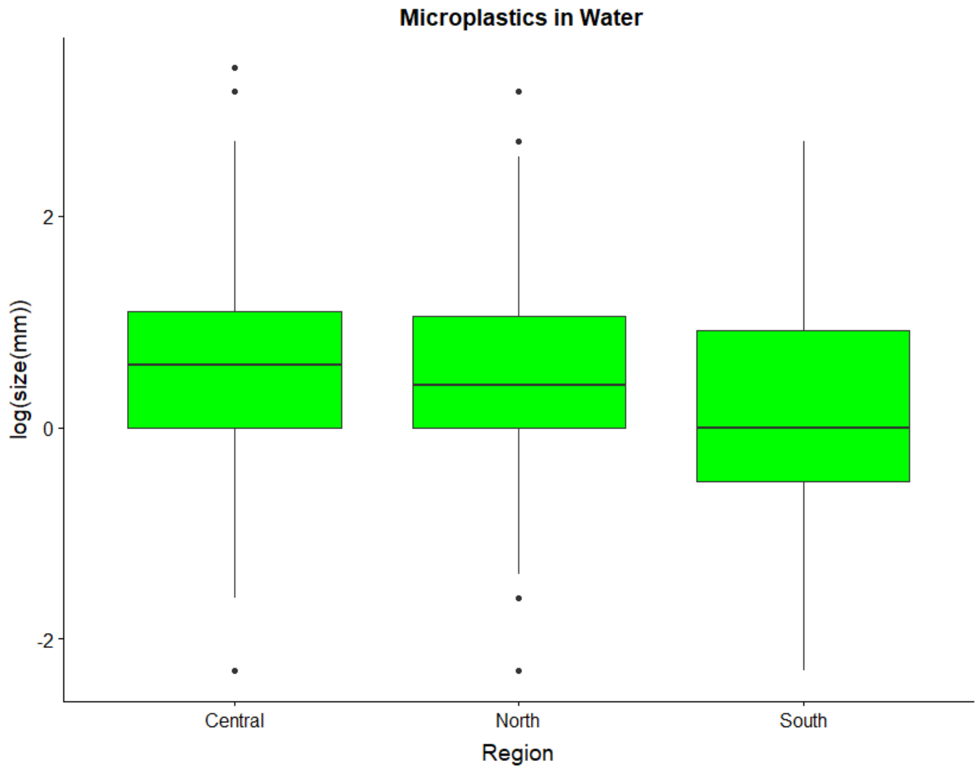


Figure 3.2.3 MP length in water samples by region (ANOVA, p=8.57e-14, n=3462).

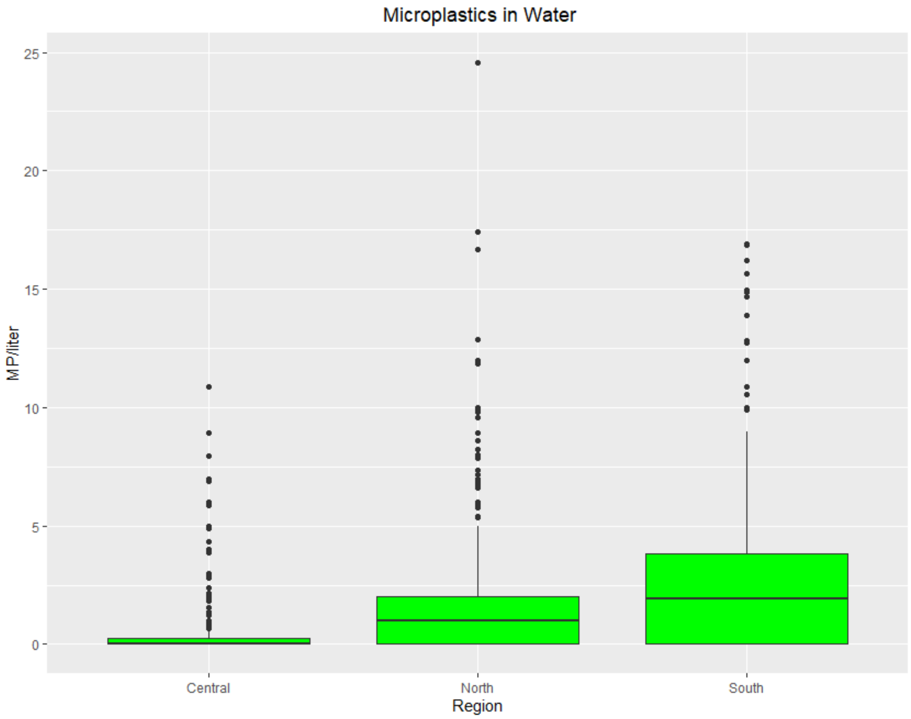


Figure 3.2.4 MP density per liter in samples from different IRL regions (GLM, $p < 0.05$, n=2180).

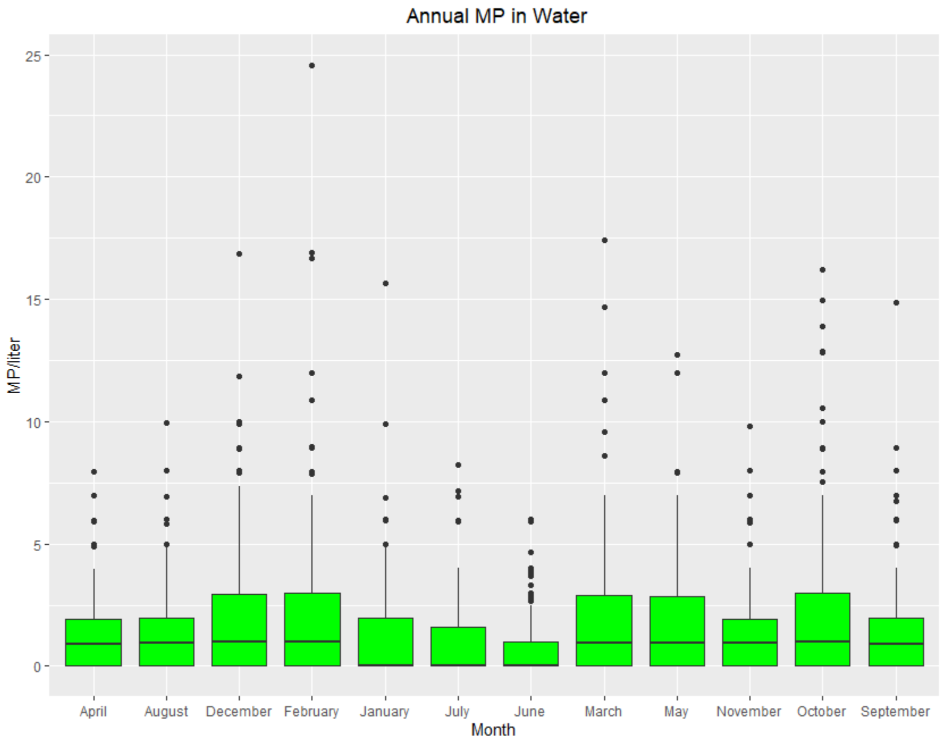


Figure 3.2.5 MP density in IRL water across sampling months (GLM, $p < 0.05$, n=2180).

IV. METHODS AND RESULTS OF OYSTER SAMPLING

4.1 Oyster Sampling Methods: Oyster samples were collected quarterly, within a seven-day period, from 12 reefs selected to encompass the entire IRL (Florida Fish and Wildlife Conservation Commission Division of Marine Fisheries Management Permit #SAL-18-2075-SR). Oysters were collected from 6 reefs in Mosquito Lagoon, 3 reefs from the central IRL, and 3 reefs from the southern IRL. This distribution represented the greater abundance of intertidal oyster reefs in Mosquito Lagoon. 15 large (> 35 mm) and 15 small (≤ 35 mm) oysters were collected from each reef. Collected oysters were carefully wrapped in aluminum foil and stored in a -20° C freezer until processing. Oysters were chemically digested using a 10% potassium hydroxide solution with 3 mL KOH per gram tissue weight (Thiele et al. 2019). Individuals in solution were placed in a shaking incubator at 45° C and 65 rpm for 24 hours, and then cooled at room temperature for another 24 hours. Following digestion, the solution was vacuum-filtered and inspected for MPs as described in 3.1.

4.2 Oyster Results: First, second, third, and fourth quarter oysters were collected from the 3 southern and 3 central reefs on 22 May 2019, 20 August 2019, 14 November 2019, and 18 February 2020, respectively. Samples were collected from the 6 northern reefs on 26 May 2019, 21 August 2019, 21 November 2019, and 29 February 2020. Research progress was not possible between 19 March and 17 June 2020 due to UCF laboratory closures associated with COVID-19. Progress resumed under phased reopening in June 2020. Oyster digestion and processing were completed in December 2020.

A total of 3181 plastics were found in 1440 oysters (70% of total), with a mean of 2.26 (± 0.08) MP per individual oyster. Mean length of MP was 2.79 (± 0.06) mm. When broken down by age class, there was a mean length of 2.82 (± 0.06) mm and 2.75 (± 0.08) mm in large and small oysters, respectively. The most abundant type of plastic in oysters was fibers, comprising 95.0% of all plastics found (Fig. 4.2.1). Fragments comprised 4.4% of our results, while films and foams comprised less than 1% of MP combined. Black and clear MPs were the most abundant, followed by dark blue (Fig. 4.2.2).

There was a mean of 2.43 (± 0.26) MPs per gram tissue oyster weight with a minimum of 0 MP/g tissue weight and maximum of 318.6 MP/g tissue weight. A zero-inflated negative binomial GLM indicated a significant difference in MP abundance per tissue weight in oysters from different regions of the Indian River Lagoon (Fig. 4.2.3; $p < 0.05$; n=1406). Southern oysters had the highest abundances of MPs, followed by central and northern oysters.
When broken down by age class, there was a mean density of 4.11 (± 0.52) and 0.75 (± 0.04) MP/g tissue weight in small and larger oysters, respectively (Fig. 4.2.4). A zero-inflated negative binomial GLM analysis indicated there was a significant difference in MP abundance per gram tissue weight with more in small oysters ($p < 0.05$, n = 1406).

A single factor ANOVA analysis indicated there was not a significant difference in MP length in oysters from different regions of the Indian River Lagoon (Fig. 4.2.5; $p = 0.16$, n=3181). There was, however, a significant difference in length of MPs between the age classes (Fig. 4.2.6; $p = 0.02$, n=3181) with smaller oysters containing larger MP in their tissues.

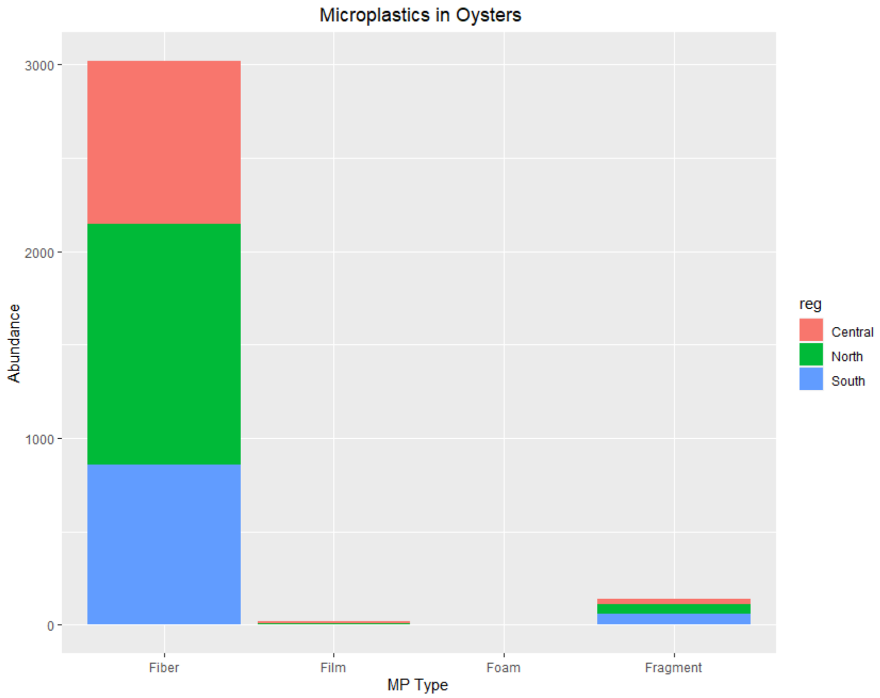


Figure 4.2.1. Total number of MPs found in oysters by type (n = 3181).

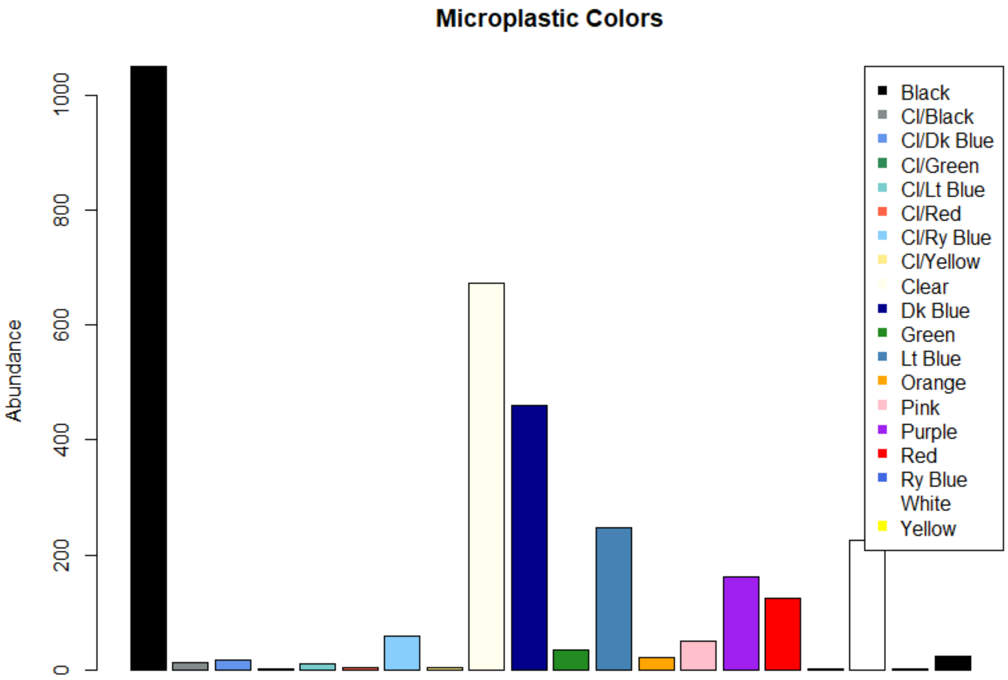


Figure 4.2.2. Total count of MPs analyzed in oysters by color (n = 3181).

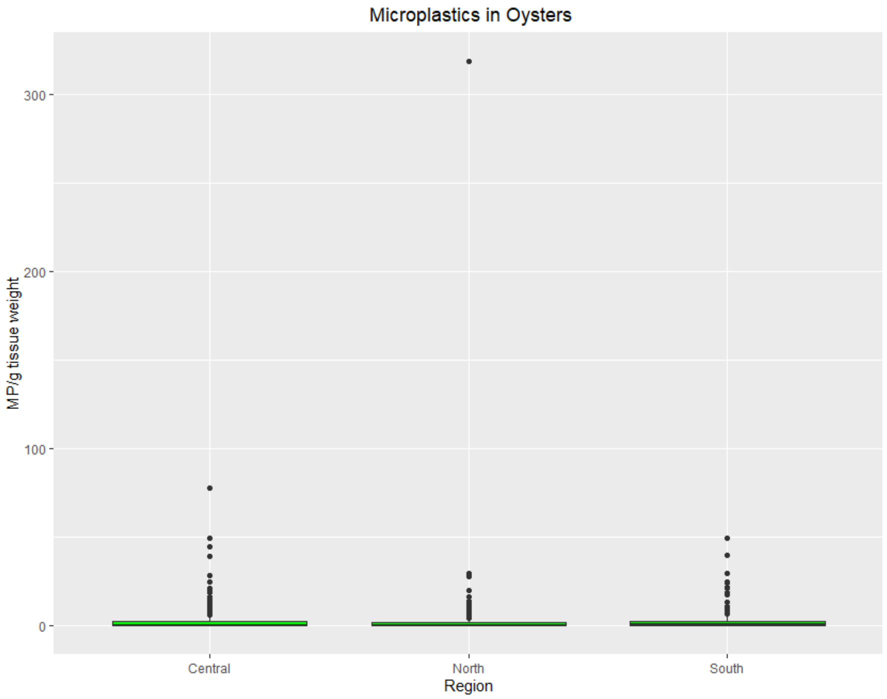


Figure 4.2.3. MP density in eastern oysters from the Indian River Lagoon (GLM, $p < 0.05$; n = 1406).

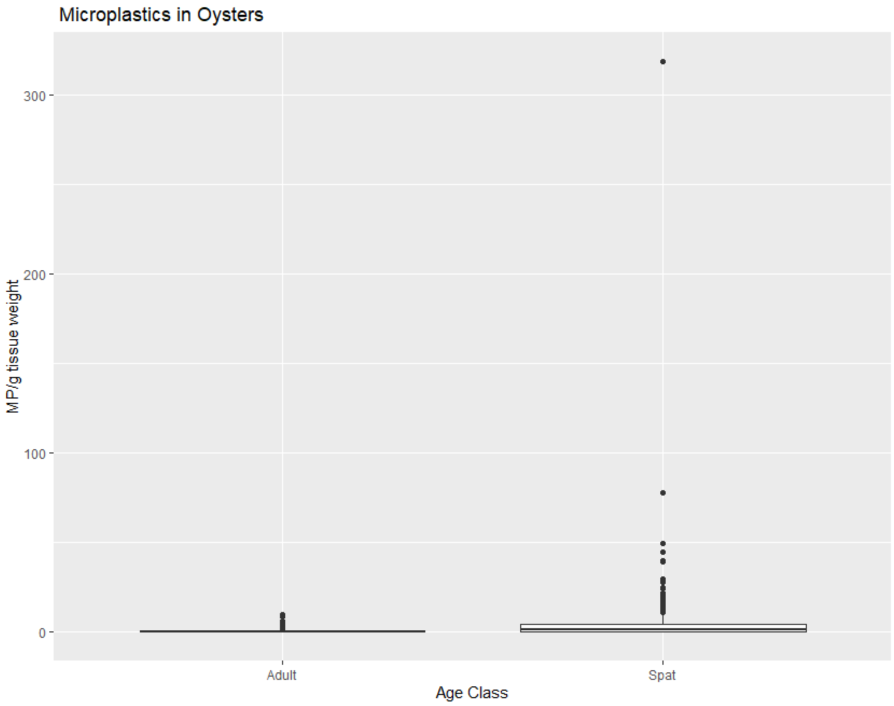


Figure 4.2.4. MP density in adult and small (< 35 mm) oysters (GLM; $p < 0.05$, n = 1406).

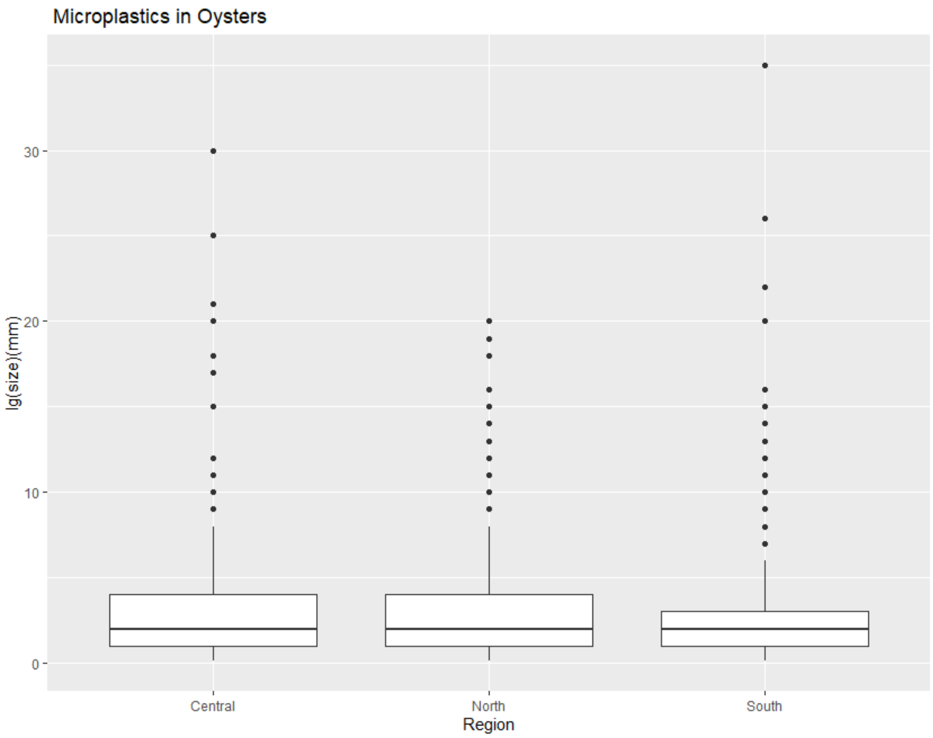


Figure 4.2.5. MP length in eastern oysters from the Indian River Lagoon (ANOVA; p = 0.16; n = 3181).

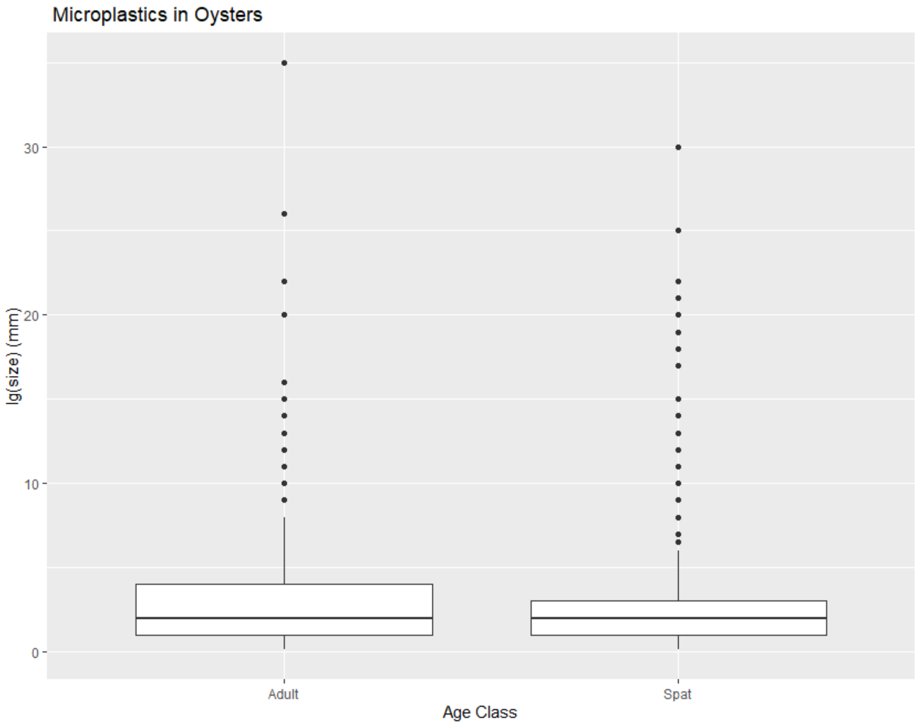


Figure 4.2.6. MP length in small (< 35 mm) and adult oysters from the Indian River Lagoon (ANOVA, p = 0.02; n = 3181).

V. METHODS AND RESULTS OF FECES/PSEUDOFECES SAMPLING

5.1 Feces/Pseudofeces Methods: 18 adult and 18 juvenile oysters were collected from each of 12 study reefs to determine the abundance and diversity of MP in oyster feces and pseudofeces. The bivalve filtration tank at Smithsonian Marine Institute in Fort Pierce, FL was placed at the end of the Smithsonian dock to enable us to directly use IRL water throughout these filtration trials. MPs were extracted from feces/pseudofeces and from oyster tissues using methods described above.

5.2 Feces/Pseudofeces Results: Biodeposit trials took place from 15 July through 18 July 2019. Processing and analysis of excrement samples was completed in December 2020. Overall, a total of 331 MPs were found in 67% of *C. virginica* biodeposits. MP were present in both feces and pseudofeces, ranging in length from 0.05 - 20 mm in pseudofeces and 0.05 - 6 mm in feces. Mean length of MP in pseudofeces was 1.73 (± 0.16), and 1.46 (± 0.08) mm in feces. Fibers, fragments, and films comprised all plastics found, with fibers accounting for 88.3% of MPs found in biodeposits (Fig. 5.2.1). Black MPs were the most abundant color followed by clear and light blue (Fig. 5.2.2). MPs in biodeposits from small oysters were significantly larger than those in adult biodeposits by an average of 1.07 mm (Fig 5.2.3; ANOVA, $p < 0.05$; n=341).

A zero-inflated negative binomial GLM indicated there was a significant difference in MP abundance by excrement type (Fig. 5.2.4; $p < 0.01$, n=288). Oyster feces had a higher abundance of MPs in comparison to pseudofeces. The mean number of MPs excreted per oyster was 1.38 (± 0.12) in feces and 1.11 (± 0.11) in pseudofeces over the 2-hour trials. Both age classes (adult and smaller oysters) were capable of excreting MPs, and adults excreted significantly more MPs, on average, than smaller oysters (Fig. 5.2.5; GLM, $p < 0.01$; n=288). Feces contained a mean density of 0.31 (± 0.08) MP per mg biodeposit weight. Pseudofeces had 0.28 (± 0.07) MP/mg biodeposit weight. A Kruskal-Wallis rank sum test indicated no difference in density of MP between biodeposit types ($p = 0.81$). There was a significant difference in MP abundance in excrement from oysters from different regions of the IRL (Fig. 5.2.6; GLM, $p < 0.01$; n=288) with oysters from the central IRL containing significantly less MP in biodeposits than northern oyster biodeposits ($p < 0.005$).

Oysters had a mean MP egestion efficiency of 62.1%, and 32% of oysters were able to egest all MP from their tissues. There was no egestion efficiency difference among IRL regions (GLM: $p > 0.05$). MP egestion efficiency decreased significantly with increasing oyster shell height (GLM: $P = 0.03$) and tissue mass (GLM: $p = 0.02$). AIC model selection revealed tissue mass was best at predicting MP egestion efficiency in *C. virginica*; efficiency decreased by 0.8% from every 1-gram increase in tissue weight. Overall, results indicated that oysters excreted at a rate of approximately 1 MP per 1 hour through feces, and 1 MP per 2 hours through pseudofeces.

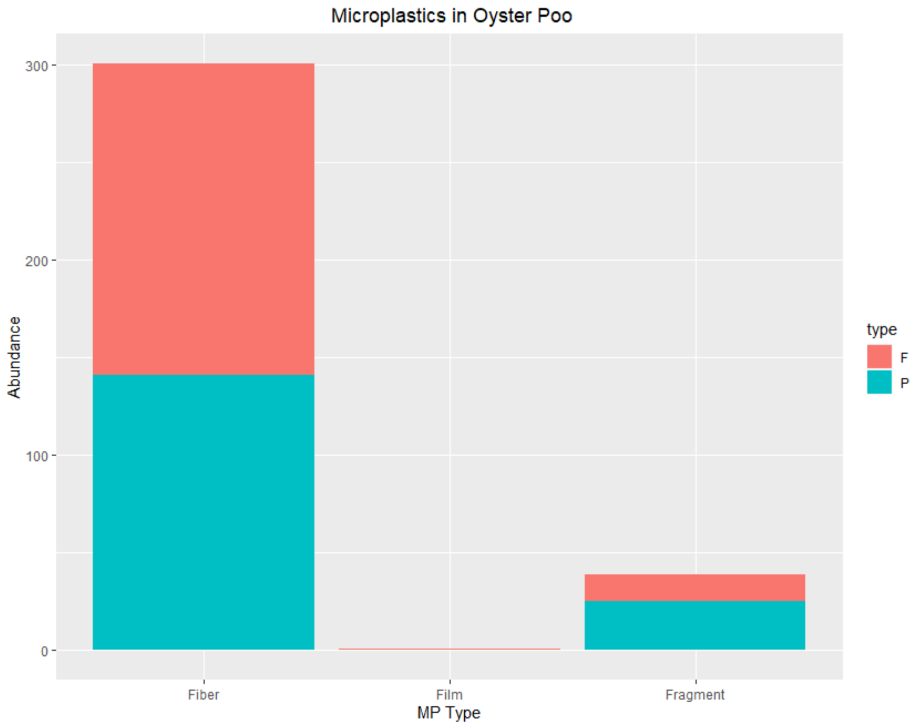


Figure. 5.2.1. MPs found in oyster biodeposits by type. F= feces and P = pseudofeces.

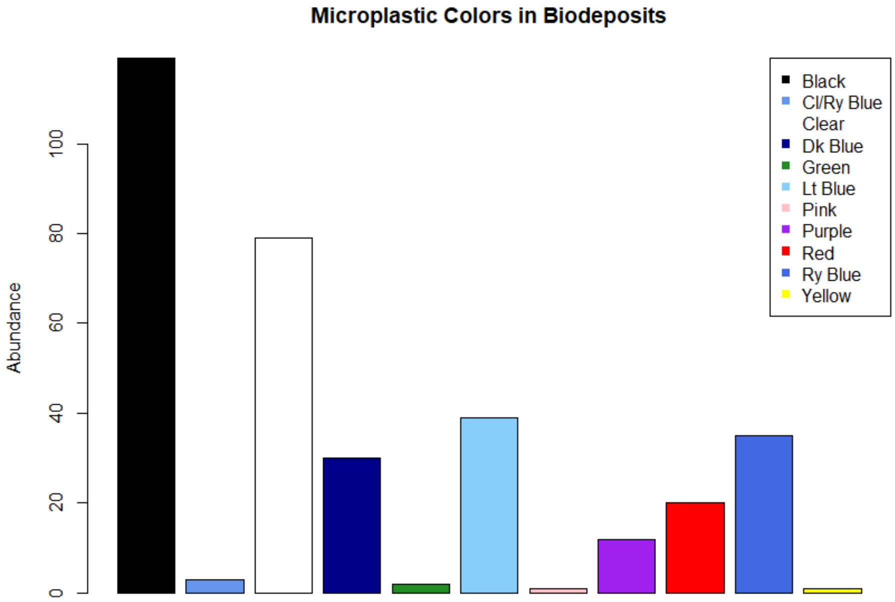


Figure 5.2.2. MPs by color in oyster biodeposits (n = 341).

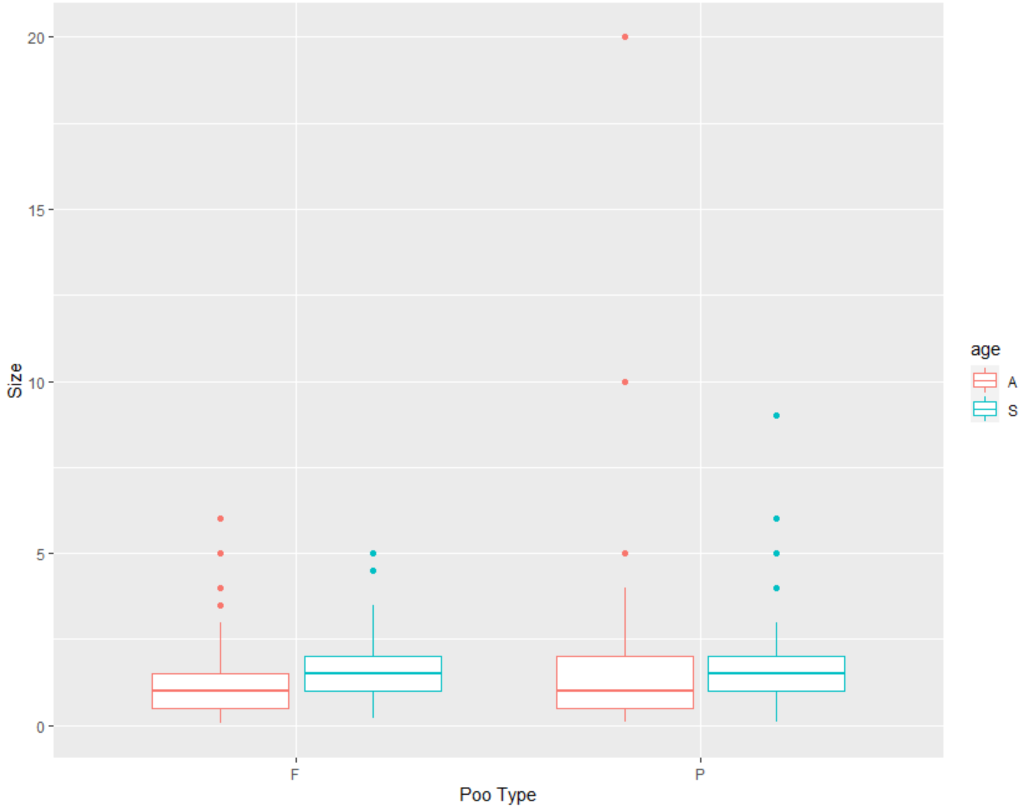


Figure. 5.2.3. MP length by age class and biodeposit type (ANOVA, $p < 0.01$; $n = 341$).

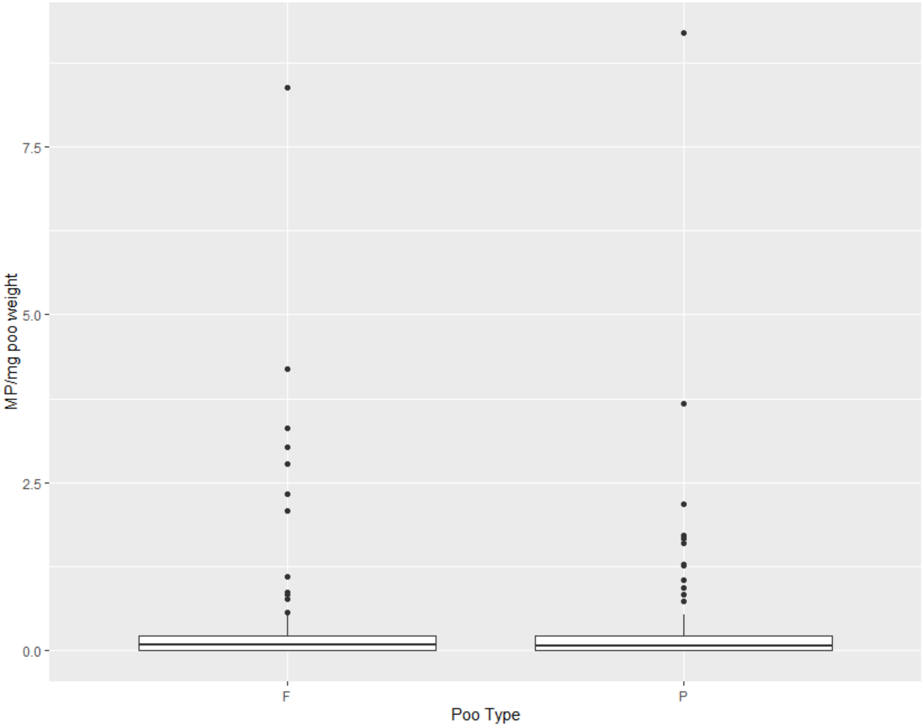


Fig 5.2.4. MP density per mg excrement weight by type after a 2-hour trial (GLM, $p < 0.01$; $n = 288$). F = feces and P = pseudofeces.

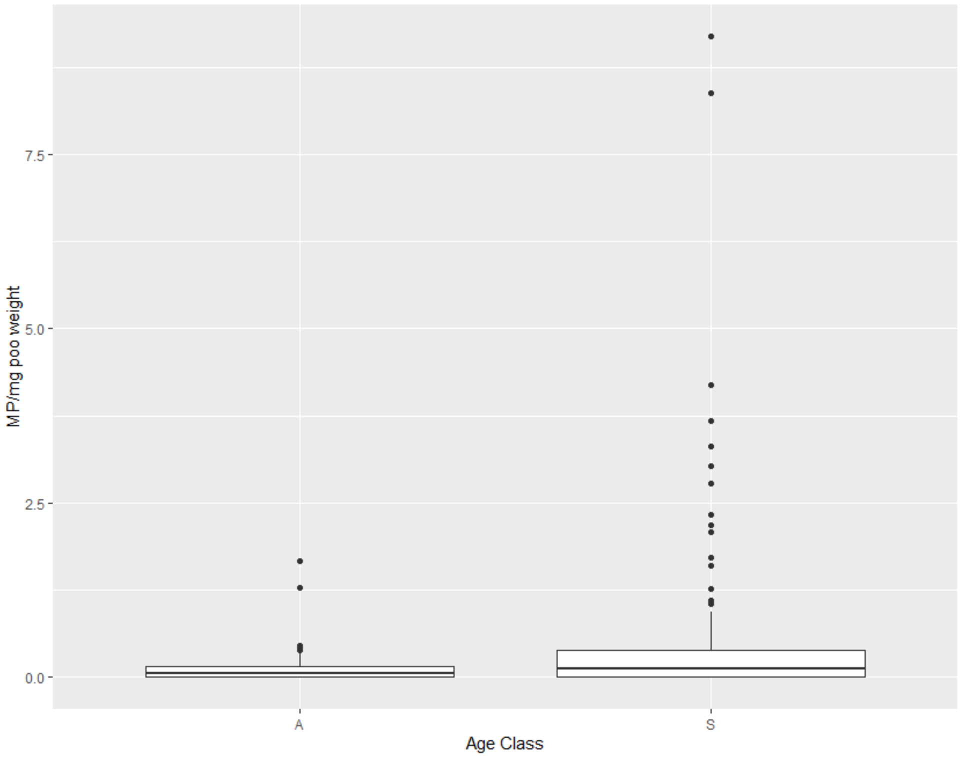


Fig 5.2.5. MPs found in adult (A) and small oysters (S) biodeposits after 2-hour trial (GLM, $p < 0.01$; $n = 288$).

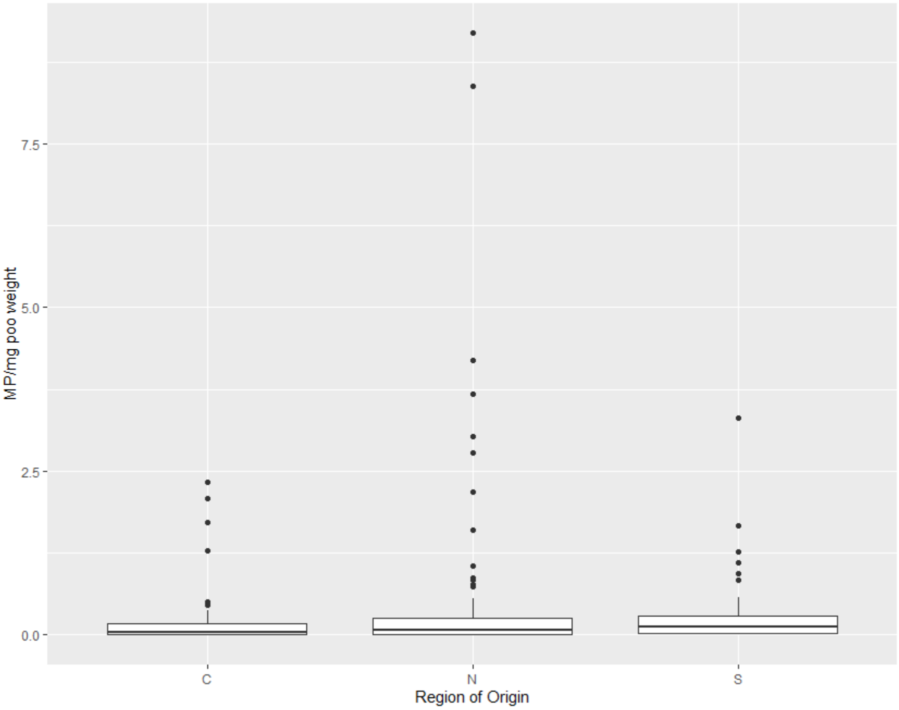


Fig 5.2.6. MPs found in oyster biodeposits from the north (N), central (C), and south (S) IRL after 2-hour trial (GLM, $p < 0.01$; $n = 288$).

VI. COMMUNITY ENGAGEMENT

6.1 Volunteer Recruitment: Community members were recruited via advertisements posted on social media by partnering organizations, as well as by contacting our existing volunteer pools. Citizens were trained using MP identification materials, provided by the Florida Microplastic Awareness Project and the Marine Environmental Research Institute, and workshops instructed by project partners.

6.2 Community Engagement Results: A total of 84 citizen scientists dedicated 1604.9 hours of their time to MP training, sampling, and analysis. Collectively, 48 workshops were held to train our citizen scientists in MP research. An additional 14 educational workshops were held to educate community members on MP issues (Table 2).

Agency	Workshops	Volunteers	Hours
MDC	12	24	427.8
UCF	16	18	429.3
DEP	11	29	505.2
FOS	9	13	242.6
Total	**48**	**84**	**1604.9**

Table 2. Community engagement data through December 2020. Key: MDC = Marine Discovery Center; UCF = University of Central Florida; DEP = Florida Department of Environmental Protection, IRL Aquatic Preserves; FOS = Florida Oceanographic Society.

VII. ADDITIONAL MP ANALYSIS

7.1 FTIR Identification Methods: We supplemented MP data collection using Fourier-transform Infrared Spectroscopy (FTIR). FTIR analysis is used to identify polymer composition of MPs. Plastics from a subset (~10%) of samples were extracted from filters for analysis. UCF Chemistry Department permitted us to use their equipment for these analyses.

7.2 FTIR Results: FTIR has been completed on the selected subset of all sample types and the blank controls used when inspecting all filters. PET (polyethylene terephthalate) was the most abundant polymer found in monthly water samples, oysters, and their biodeposits (Figure 7.2.1). PP (polypropylene) was the dominant polymer found in water samples collected from oyster reefs.

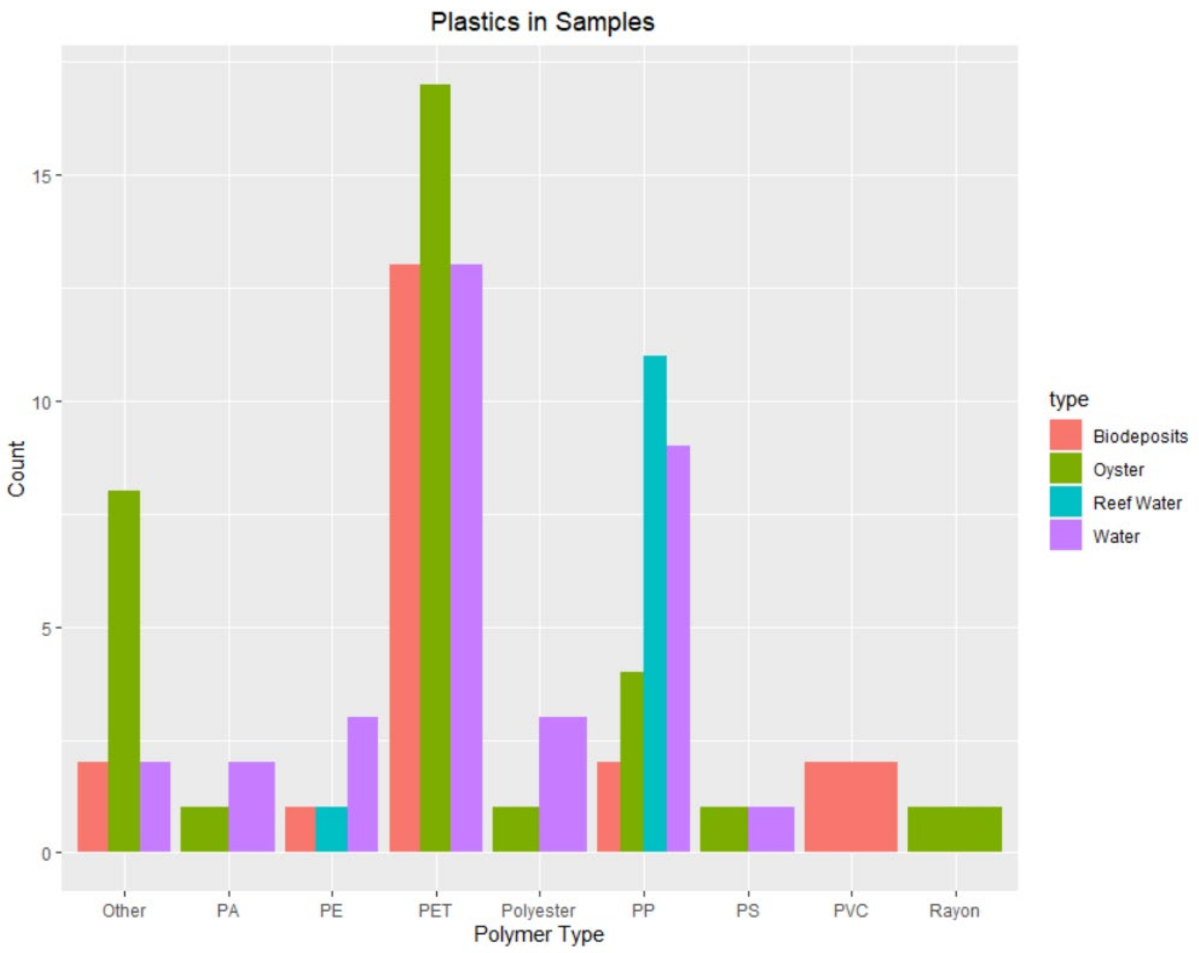


Figure 7.2.1. Plastics found in samples broken down by polymer type. Key: PA-polyamide, PE-polyethylene, PET-polyethylene terephthalate, PP-polypropylene, PS-polystyrene, PVC-polyvinyl chloride.

SUMMARY DATA:

We found the eastern oyster *Crassostrea virginica* had an average of 2.3 MP per individual and 1.5 MP per liter of IRL water. Using this MP density in water and the reported volume of lagoon water (Smith 1992), we estimate there currently are 1.4 trillion microplastics in the Indian River Lagoon.

Our results describe a chronic microplastic contamination problem in both IRL waters and oysters. We suggest future research on MP include these goals: 1) determining important sources of IRL microplastics pollution, 2) understanding potential toxicity of plastics in the IRL, especially in oyster tissues and in the tissues of other economically important species, 3) begin understanding contamination at the nanoplastic scale (1 nano particle = 1/10,000 of a mm), and 4) developing methods to limit future contamination and reduce current pollutant loads. The first two goals are already underway with a focus on IRL stormwater outfalls and microplastic microbiome characterizations.

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