2021

# Correlations in Microplastic Abundance Between Water, the Eastern Oyster, Crassostrea virginica, and Their Biodeposits in a Dynamic Florida Estuary

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# CORRELATIONS IN MICROPLASTIC ABUNDANCE BETWEEN WATER, THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, AND THEIR BIODEPOSITS IN A DYNAMIC FLORIDA ESTUARY

by

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B.S. University of Central Florida, 2015

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Spring Term
2021

Major Professor: Linda Walters

# ABSTRACT

Estuaries have been identified as hotspots of microplastic pollution because they are transitional zones where coastal freshwater and oceans converge. Microplastics (MP) are transported through estuaries by a dynamic series of forces such as surface flow and tides, which influence MP abundances and trends. The eastern oyster, *Crassostrea virginica,* is an estuarine bivalve known to ingest MP, resulting in negative impacts on organism physiology. I investigated MP pollution as a threat to *C. virginica* in a dynamic Florida estuary, the Indian River Lagoon (IRL), and determined there are both regional and small-scale spatial and temporal fluctuations in MP abundance. Tributaries were identified sources of MP, while inlets flush them out of the system. The south IRL is a hotspot for MP, where the St Lucie Estuary is the primary tributary. Throughout the IRL, fibers dominated MP and polyethylene terephthalate (PET) was the most abundant polymer type (>50%). Overall, *C. virginica* had a mean of 2.2 MP/individual and lagoon water had 1.5 MP/L. An *in-situ* biodeposition experiment revealed *C. virginica* of all sizes were able to egest environmental MP at a rate of 1 MP per 1 hour through feces, and 1 MP per 2 hours through pseudofeces. Oysters had a mean MP egestion efficiency of 62.1%, and 32.1% of oysters were able to egest all MP from their tissues within 2 hours. Smaller *C. virginica* were more efficient at egesting MP, and egestion efficiency decreased by 0.8% for every 1-g increase in tissue weight. Overall, I provide an argument that MP are ubiquitous in this hydrologically dynamic estuary in both the water and in a keystone, filter-feeding invertebrate. I estimate there are currently ~1.4 trillion microplastics in the Indian River Lagoon.

For my family, and to those whose shoulders I stand upon.

# ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Linda Walters, for mentoring me throughout my years with her, investing her time in me, and giving words of encouragement when needed. I thank her for understanding the value of a Master's student and am grateful for the opportunities I've had. I would also like to acknowledge my committee members, Dr. Kristy Lewis and Dr. Lisa Chambers, for their fostering of my professional development. Thank you to Dr. Melinda Donnelly for always lending a helping hand, even in the most inconvenient times. I would like to acknowledge Dr. Paul Sacks for his help and ancillary research support in times of need.

I am so grateful to my lab mates, Jessie Copertino and Becca Fillyaw, for their constant support and encouragement as friends and colleagues. This experience would not have been the same without you. Thank you, also, to my family for their never-ending support and words of encouragement.

Additionally, this work could not have been conducted without an immense collaborative effort from UCF, Marine Discovery Center, Florida Department of Environmental Protection Indian River Lagoon Aquatic Preserves Division, Florida Oceanographic Society, UF/IFAS Extension, and Smithsonian Marine Station. I would like to acknowledge the funding source for this research, the Indian River Lagoon National Estuary Program, whose patience throughout the COVID-19 pandemic is notable. A thank you, also, to Canaveral National Seashore for allowing this research to take place within the park. This research was conducted under Florida Fish and Wildlife Conservation Commission Division of Marine Fisheries Management Permit #SAL-18-2075-SR.

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# CHAPTER I-INTRODUCTION

Plastic is both a common household material and pervasive pollutant despite its relatively short history (e.g., Sekudewicz et al. 2021, Zhao et al 2018). Synthetic plastic was first created by Leo Baekeland in 1907; mass production of plastic, however, did not begin until the 1950s when a new generation of plastics (PVC-polyvinyl chloride, PS-polystyrene, Nylon, PE-polyethylene, PP-polypropylene, PET-polyethylene terephthalate) made this feasible (Baekeland 1909; Crespy et al. 2008). Global plastic production continued to increase, with an estimated 8300 million metric tons (Mt) produced through 2015; 79% is now in landfills or the environment, 9% has been recycled, and 12% was incinerated (Geyer et al. 2017). In 2010, an estimated 5 to 13 Mt of plastic debris entered Earth's oceans from a myriad of sources, including ship overspill, container wash-off, coastal development, and litter (Bouchet and Friot, 2017, Jambeck et al. 2015).

Once in the marine environment, plastics are subject to solar-, thermal-, mechanical and bio-degradation, which can weaken or fragment the plastic into smaller objects called microplastics (MP) (Arthur et al. 2009, Guo & Wang 2019). The National Oceanic and Atmospheric Administration defines a MP as any plastic object less than or equal to 5 mm in size, but can be further categorized into two subclasses, primary and secondary MP (Arthur et al. 2009). Primary MP are those that are manufactured at a small size, whereas secondary MP form via fragmenting from a larger plastic object (Barnes et al. 2009). Primary MP include microbeads in personal care products and 'nurdles', a raw material formed into small pellets for easy transport that are used to make larger plastic items (Auta et al. 2017; Ellison 2007). Secondary MP include fibers, fragments, foams, and films which vary in shape depending how they are formed (Barnes et al. 2009). Oceanic MP are predominantly textile fibers (35%), fragments

associated with city dust (24%), or pieces of tire (28%), with < 3% nurdles or beads (Bouchet and Friot 2017). Fibers are especially common in estuaries and coastal waters (Li et al. 2018, Yu et al. 2017). For example, Simon-Sanchez et al. (2019) found fibers were the most abundant type (70%) of MP in the Ebro Delta estuary in Spain. Luo et al. (2019) documented similar findings of MP fibers in more than 80% of MP in coastal waters in the Shanghai area, and found MP abundance increased in areas closer to the city.

Plastic ingestion in marine biota has been documented in hundreds of species at varying trophic levels (e.g., Wang et al. 2016; Zhang et al. 2020). Species of particular interest are filter-feeders such as oysters, clams, and mussels (Cho et al. 2021, Li et al. 2018, Ward et al. 2019). The Eastern oyster, *Crassostrea virginica*, is an estuarine species known to ingest MP and face negative impacts on physiology (Eierman 2019). Waite et al. (2018) documented MP in tissues of *C. virginica* from Mosquito Lagoon, the northernmost portion of the Indian River Lagoon (IRL), one of North America's most biodiverse estuaries (SJRWMD & IRLNEP 2007). The IRL National Estuary Program identify now MP as a 'contaminant of concern' in this system (IRL CCMP 2019).

The overall goal of this thesis is to quantify MP pollution in the IRL as a threat to *C. virginica*. The first aim is to quantify MP pollution in surface water and *C. virginica* in the IRL estuary and identify any spatial or temporal factors that are important for MP in this system. Along with this I determine what polymer types comprise MP from the IRL to help elucidate identify possible sources of pollution. The second aim of this thesis is to determine MP egestion efficiency in *C. virginica* through an *in-situ* bivalve filtration tank experiment to further investigate how environmental MP accumulate in *C. virginica.*

# CHAPTER II-QUANTIFYING SPATIAL AND TEMPORAL TRENDS IN MICROPLASTIC POLLUTION IN THE INDIAN RIVER LAGOON

## Introduction

Microplastics are transported through coastal systems by a dynamic series of forces such as rain, wind, freshwater discharge, waves, tides, salinity gradients, surface drift, biofouling, and storm events (Hitchcock 2020, Xia et al. 2020). Identifying what factors influence MP abundances in hydrologically complex coastal landscapes is a defined research gap in the MP field (Zhang 2017).

The largest Florida lagoon, the Indian River Lagoon, spans 40% of Florida's east coast (251 km) from Ponce de Leon Inlet in the north (29.075898° N, 80.917571° W) to Jupiter Inlet in the south (26.944768° N, 80.073952° W). It falls within the boundaries of Volusia, Brevard, Indian River, St. Lucie, Martin, and Palm Beach counties and has 5 major inlets (Ponce de Leon, Sebastian Fort Pierce, St. Lucie, and Jupiter). IRL water (hereafter lagoon water) flows through three interconnected water bodies Mosquito Lagoon, Indian River, and Banana River; has an average depth of 1.2 m; and is brackish with freshwater contributions from the St. Johns River and Okeechobee watersheds (IRL CCMP 2019). Saltwater influx comes from the Atlantic Ocean through inlets, while freshwater input is predominantly from rainfall, discharge, and runoff from nearby land (SJRWMD & IRLNEP 2007). Currents, tides, and circulation patterns are influenced by factors dependent on location from the nearest inlet - areas closer to inlets have a larger tidal influence while stretches between inlets are primarily driven by wind and freshwater input (IRL CCMP 2019). This research is centered on the IRL oysters because there are long-term efforts to restore *C. virginica* populations here, and they are commercially harvested for human consumption.

## Material & Methods

*Sample collection and citizen science*

Lagoon water was collected from the IRL over a 12-month sampling period, between March 2019 and February 2020. Water was collected once per month from 35 sites that extended the length of the IRL. All sites were accessible from shore and on public lands (Figure 1, Table 1). Each month, lagoon water samples from all sites were collected within a 4-day time period to limit temporal variation. At each site, five replicate 1-L surface lagoon water samples were collected using a discrete sampling protocol (Cutroneo et al. 2020; Zhu et al. 2019). Sample bottles were triple-rinsed in in 0.45µm filtered deionized water in the laboratory, and then again with lagoon water upon site arrival to remove any existing contamination. Bottle rinsing occurred at least 10 m away from the sample collection location. Rinsed bottles were partially submerged to collect the top 5 cm of lagoon water and capped while submerged. At each site, abiotic parameters of air and water temperature were recorded using a thermometer (°C), salinity using a refractometer (ppt), and mean wind speed using an anemometer (km/h). Samples were transported back to laboratories for temporary storage and processing at room temperature.

Citizen scientists associated with UCF and three partnering conservation agencies along the IRL assisted with water sample collection and processing. Citizens were recruited through existing volunteer pools of agencies, social media posting, or by word of mouth. Citizen recruits underwent an MP training where they were educated about MP generation and pollution, scientific procedures used to sample MP from surface water, and how to inspect samples once MP are extracted. Once trained, citizen scientists were independently deployed to collect water samples each month at local sites. Lagoon water samples were processed and inspected for MP in the laboratories under direct supervision of conservation agencies.

*Crassostrea virginica* was collected quarterly for 1 year from 12 intertidal reefs in the IRL, 6 in the north, 3 in the central, and 3 in the south IRL (Figure 1). Sampling reefs in the north region were randomly selected using a random number generator (www.random.org), while central and southern reefs were the only sustainable, intertidal reefs accessible in each respective region (E. Dark, per comm.). Sampling distribution is skewed to the north to be more representative of *C. virginica* abundance in the IRL, as there is a downward trend in abundance as latitude decreases (Garvis et al. 2015). At each reef, 30 individual *C. virginica* and five 1-L lagoon water samples were collected. Fifteen large (shell length ≥ 36 mm) and fifteen small (shell length < 36 mm) *C. virginica* were haphazardly collected from each reef, wrapped in aluminum foil, bagged, and placed on ice. Five, 1-L water samples were collected immediately prior to oyster collection using the surface water discrete sampling protocol described above. Oysters from all 12 reefs were collected within a 7-day window to limit temporal variation. Samples were brought to the University of Central Florida Department of Biology laboratory for storage in a -20 °C freezer until processing.

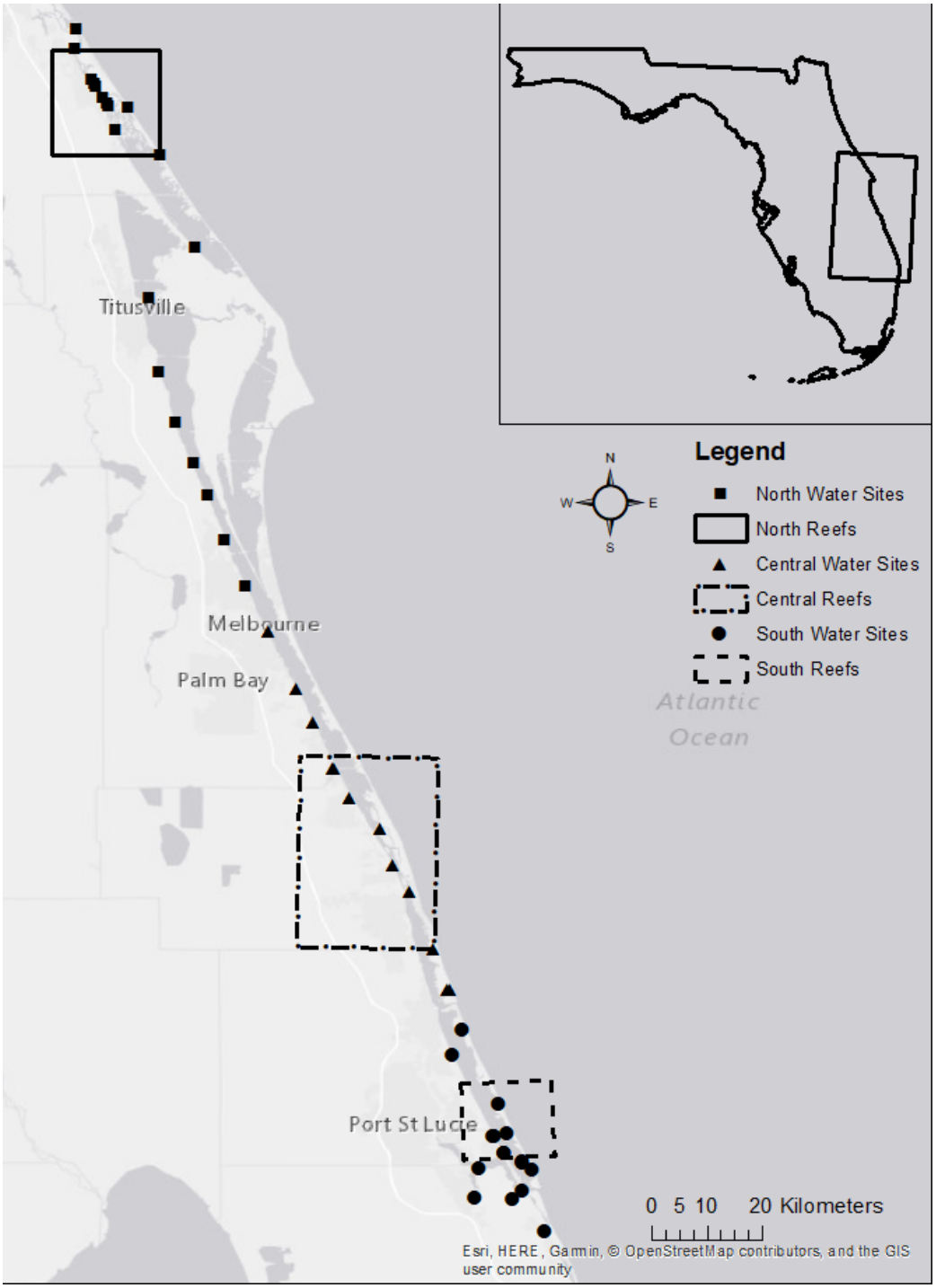


*Figure 1. Indian River Lagoon microplastic water sampling sites (points) and oyster reef areas (boxes).*

*Table 1. Site key for Indian River Lagoon water sampling sites.*

| Site No. | Site Name | Abbreviation | Region | Latitude | Longitude |
|---|---|---|---|---|---|
| *1* | Smyrna Dunes Park | SDP | N | 29.063822 | -80.915744 |
| *2* | Marine Discovery Center | MDC | N | 29.030158 | -80.917641 |
| *3* | River Breeze Park | RBP | N | 28.898601 | -80.85174 |
| *4* | CANA Boat Ramp | CANAB | N | 28.934251 | -80.829475 |
| *5* | CANA Parking Lot #5 | CANA5 | N | 28.857672 | -80.777248 |
| *6* | Haulover Canal | HOC | N | 28.706285 | -80.720657 |
| *7* | Parrish Park | PPK | N | 28.623625 | -80.794767 |
| *8* | Campground | CAMP | N | 28.504 | -80.7801 |
| *9* | Briarwood | BW | N | 28.42123 | -80.75245 |
| *10* | Lee Wenner Boat Ramp | LWBR | N | 28.355086 | -80.722994 |
| *11* | Rockledge | ROCK | N | 28.3014 | -80.7005 |
| *12* | Rotary Park | RPK | N | 28.2295 | -80.6714 |
| *13* | Pineapple | PINE | C | 28.154 | -80.6382 |
| *14* | Front Street | FS | C | 28.079558 | -80.599847 |
| *15* | Malabar | MAL | C | 27.9862 | -80.5532 |
| *16* | Christensen | CHR | C | 27.93112 | -80.526022 |
| *17* | Outriggers | OUT | C | 27.855367 | -80.492992 |
| *18* | Sebastian | SEB | C | 27.80892 | -80.466215 |
| *19* | Environmental Learning Center | ELC | C | 27.758069 | -80.415706 |
| *20* | Vero | VERO | C | 27.654303 | -80.368983 |
| *21* | Round Island | RI | C | 27.561131 | -80.328635 |
| *22* | Wildcat | WC | C | 27.495292 | -80.303114 |
| *23* | Bear Point | BP | S | 27.429391 | -80.281382 |
| *24* | Midway | MID | S | 27.38723 | -80.297868 |
| *25* | Jensen Beach | JEN | S | 27.308302 | -80.22226 |
| *26* | Palm City Bridge | PCB | S | 27.155333 | -80.261 |
| *27* | Riverwalk | RW | S | 27.20225 | -80.253883 |
| *28* | Fish House | FH | S | 27.151083 | -80.199867 |
| *29* | Twin Rivers | TR | S | 27.164933 | -80.18215 |
| *30* | Driftwood | DW | S | 27.255533 | -80.23085 |
| *31* | Jensen Beach Impound | JBI | S | 27.260117 | -80.209233 |
| *32* | River Cove | RC | S | 27.21435 | -80.183983 |
| *33* | House of Refuge | HOR | S | 27.199617 | -80.166283 |
| *34* | Indian Riverside Park | IRP | S | 27.228535 | -80.212716 |
| *35* | Jimmy Graham Boat Ramp | JGBR | S | 27.09958 | -80.145616 |

*Sample processing*

Indian River Lagoon water samples were vacuum-filtered at room temperature using Whatman nitrocellulose membrane filter paper (47mm, 0.45 µm pore size) to extract MP, and

placed in triple-rinsed, sealed, 60 X 15 mm petri dishes. Filters were inspected once dry using a dissecting microscope (Leica EZ4) at 20X - 40X magnification. MP type, color, and size (mm) were recorded following protocol established by the Marine and Environmental Research Institute (2015). To distinguish between natural and synthetic items, potential MP were prodded using forceps to test breakage, and examined for discrete variation in color, shape, and margins (smooth, jagged, fraying) along their lengths.

Individual *C. virginica* were thawed and shell heights (mm) were recorded using calipers. Each oyster was shucked, soft tissue weighed (g) using a balance (Ohaus Scout Pro), and placed in individual, glass, Erlenmeyer flasks (125 mL for small, 250 mL for large oysters). Digestion protocol followed procedures established by Thiele et al. (2019) for optimal extraction of MP from bivalve tissues. A 10% potassium hydroxide (KOH) solution was added to each flask at a ratio of 3:1 volume (mL) to wet tissue weight. Flasks were covered and placed in a shaking incubator at 40 °C at 60 rpm for 24 hours, and then removed and left at room temperature for an additional 24 hours where tissue digestion was completed. A 1.0-M citric acid solution was added to the digested tissue solution until a neutral pH (7.0) was reached to prevent an interaction with filters. The neutralized solution was vacuum-filtered under a fume hood using Whatman glass microfiber filters (90 mm, 1.2 µm pore size) and placed in triple-rinsed petri dishes for later quantification.

*Limiting polymer contamination and degradation*

Procedural MP contamination was controlled for by triple-rinsing all equipment used during digestion and filtration with 0.45 µm filtered deionized (DI) water prior to each use (M. McGuire, pers comm.) Solutions used during digestions were also made with 0.45 µm filtered DI water. Chemical digestion of oysters was conducted in a fume hood to prevent polymer

contamination during the filtration process (Foekema et al. 2013). KOH was preferrable to digest bivalve soft tissue and extract MP particles as small as single-µm in size because it preserves major polymers, including rayon (Thiele et al. 2019).

Aerial contamination was quantified during microscopy by using five filter-control blanks (filters dampened with 0.45 µm filtered, deionized water placed in triple-rinsed petri dishes) (Foekema et al. 2013, Granek et al. 2020). Blanks (exposed filters) were haphazardly placed on the table immediately around the microscopy station at all times during inspection to quantify potential air contamination while samples were exposed (Granek et al. 2020). Blanks were inspected for MP, then normalized to a mean contamination rate per minute.

*Fourier-transform infrared spectroscopy*

To supplement MP identification, polymer composition was determined using attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) at the University of Central Florida Nanoscience Technology Center using a Shimadzu IRSpirit-T instrument. A subset of samples containing MP (10% of each sample for water and *C. virginica*) were randomly selected (www.random.org) and all potential MP larger than 0.5 mm in size were scanned (Gago et al. 2016). MP were scanned in the 600 $cm^{-1}$ to 4000 $cm^{-1}$ range and spectra were matched to the reference library from Shimadzu using differential derivative point matching (ATIR-FTIR Polymer and Polymer Additives Database #: 220-93143-07, 2020). A score, also known as hit quality index (HQI), for each spectrum was calculated to measure percent match using the equation:

$$HQI = \frac{\left(\left(1 - \frac{D}{S}\right)^{1/3} + 1\right)}{2}$$

where *D* is the summation of the primary and reference spectra by calculation of a fitting method, and *S* is the area of the primary derivative curve of the sample spectrum. Spectra were automatically included as a polymer if score match was 700 or higher, ambiguous scores of 600-700 were manually sorted for inclusion in analysis, and scores of 600 or below were excluded (Frias et al. 2016; Zhao et al. 2018). Ambiguously scored spectra were inspected and differentiated by visual peak matching. Polyester is predominantly PET and could not be elucidated as a distinctly different polymer, so polyester signals were classified as PET (Geyer et al. 2019). A subset of signals (10%) on MP identified in control blanks were scanned using ATR-FTIR to determine any overlap between polymers found in the IRL and aerial contamination.

*Statistical methods*

Lagoon water and *C. virginica* MP abundance data were broadly dispersed and had a high presence of zeros, so each were analyzed using negative binomial generalized linear modelling (GLM) for zero-inflated data (R package "pscl"). To quantify spatial and temporal variations in MP abundance in lagoon water, predictor variables tested in models included region, site, and season. Seasons were defined by standard meteorological season (Spring = March - May, Summer = June - August, Fall = September - November, Winter = December - February). To determine what factors may influence MP abundance in water, distance to the nearest tributary (km) and distance to the nearest inlet (km) were tested as predictor variables in models. Distinct regional differences in MP abundance were apparent, so both whole-IRL and independent regional model analyses of lagoon water were incorporated to distinguish trends more precisely. Since the IRL is so expansive, models with predictors of distance to an inlet and tributary were only included within the individual regional analyses.

Control blanks were normalized to a contamination rate per minute ($C_M$) using the formula:

$$C_M = \frac{MP_B}{T_B}$$

where $MP_B$ is the mean number of MP per blank and $T_B$ is the time that blanks were exposed in minutes. Contamination per minute values then were used to calculate a contamination per sample ($C_S$) using the formula:

$$C_S = C_M \times T_E$$

where $T_E$ is the length of time each filter was exposed during inspection. Contamination per sample values were incorporated as a covariate in water models but only included if significant in the model.

To quantify MP abundance and fluctuations in IRL oysters, predictor variables tested in models included region, site, season, and shell height. To determine what factors may influence MP abundance in oysters, distance to the nearest tributary, and distance to the nearest inlet were also tested as predictor variables. Contamination per sample and tissue weight were incorporated in all oyster models but only included in analyses if significant. Model selection using Akaike information criterion (AIC) was used to determine which variables best predict MP abundance in lagoon water and oysters from the IRL. Differences in MP abundance between lagoon water and reef water (water collected from oyster reefs) were analyzed using a zero-inflated negative binomial GLM. Regional differences in MP abundance were apparent so both whole-IRL and independent regional model analyses of *C. virginica* were incorporated. Regional models used the same predictor variables as the whole-IRL models to distinguish trends.

Linear regressions were used to determine differences in air and water temperature between IRL regions, and seasons. Regressions were also used to determine wind speed and

salinity differences between IRL regions, sites, and months. All statistical analyses were performed using R 4.0.3 and RStudio (R Core Team 2019; RStudio Team 2019).

## Results

*Microplastics in lagoon water*

Overall, a total of 3755 MP were observed in 44% of lagoon water samples. Fibers, fragments, films, and foams were found in water, though fibers were dominant and comprised 95.6% of MP. Fragments, foams, and films comprised the remaining 3.9%, 0.3%, and 0.2% of MP, respectively. Plastics ranged in size from 0.1 mm to 30.0 mm and had a mean size (± CI) of 1.9 ± 0.1. Lagoon water density ranged from 0 to 25.0 MP/L and had a mean (± CI) of 1.47 ± 0.09 MP/L (Table 2). Abundance differed between each of the IRL regions. Central sites had the lowest MP abundance, followed by the north, then south regions ($p < 0.001$ for all, Figure 2).

*Table 2. Microplastic abundance per liter of Indian River Lagoon water, overall, and in the north, central, and south regions. Two sets of values are reported, raw MP count (Abundance) and normalized abundance accounting for aerial contamination ($Abundance_N$). Values reported are mean abundance, the 95% confidence interval of the mean, and abundance range.*

| Value | Parameter | IRL | North | Central | South |
|---|---|---|---|---|---|
| Abundance | Mean | 1.47 | 1.54 | 0.57 | 2.1 |
| | C.I. | 0.09 | 0.13 | 0.10 | 0.18 |
| | Range | 0 - 25 | 0 - 17 | 0 - 9 | 0 - 17 |
| $Abundance_N$ | Mean | 1.52 | 1.72 | 0.58 | 2.1 |
| | C.I. | 0.10 | 0.16 | 0.10 | 0.18 |
| | Range | 0 - 24.6 | 0 - 24.6 | 0 - 8.9 | 0 - 16.9 |

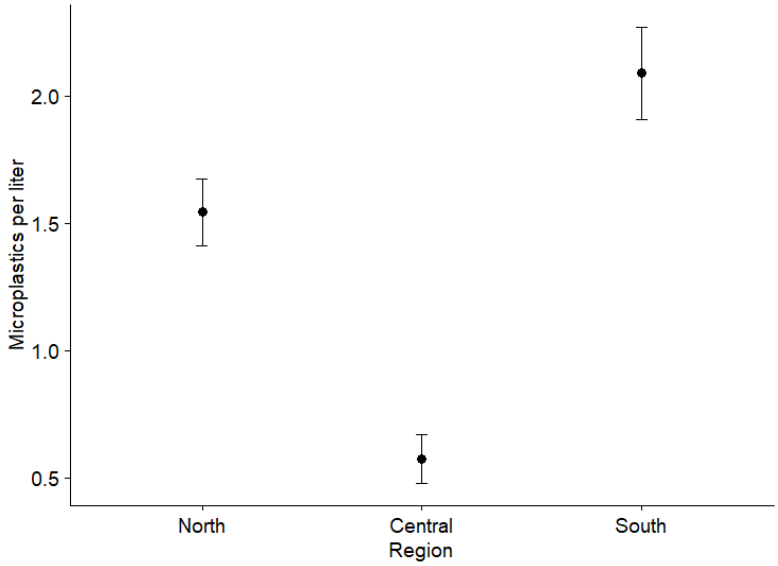


*Figure 2. Microplastic abundance per liter of water from the north, central, and south Indian River Lagoon. Values are mean abundance (point) and 95% confidence interval of the mean. (GLM, $p < 0.05$, North=716, Central=598, South=776)*

The most plausible model to predict MP abundance in IRL water included site and season as predictor variables (AIC = 6283.6; McFadden pseudo$R^2$ = 0.12, Table 3). Site Wildcat was selected as a reference in the model to compare to all others because it is also an oyster sampling site. Spring was selected at the seasonal reference because it was when MP abundance was lowest. In comparison to Wildcat, central sites, Christensen and ELC, and southern site, Jensen, contained less MP. Higher abundances were observed at southern sites Driftwood, Fish House, House of Refuge, PC Bridge, Indian Riverside Park, and Riverwalk compared to Wildcat ($p <$ 0.05 for all). Model results also indicated northern sites Briarwood, River Breeze Park, Haulover Canal, Front St, Parrish Park, Lee Wenner, and Rotary Park, central site, Pineapple, and southern sites Fish House, House of Refuge, Indian Riverside Park, River Cove, Riverwalk, and PC Bridge were significant predictors of MP presence ($p < 0.05$).

Abundance was higher in lagoon water in winter and fall ($p \leq 0.02$) and winter was also a significant predictor of MP presence ($p = 0.005$).

The most plausible predictor of MP abundance in north lagoon water was season, and abundance was lower in winter (AIC = 2422.9; McFadden pseudo$R^2$ = 0.01, $p < 0.001$, Table 3). Spring was used as a reference in the model as abundance was lowest. MP presence was not predicted by a particular season within the north IRL.

In central lagoon water, the most plausible predictors of MP abundance were site and season (AIC = 1131.2, McFadden pseudo$R^2$ = 0.10, Table 3). Wildcat was selected as a site reference, and spring as a season reference, for comparison in the model. Within the central IRL, abundance was lower at sites ELC, Vero, and Outriggers, and during the summer and fall seasons ($p \leq 0.04$ for all). Sites, Front St. and Pineapple, were predictors of MP presence ($p < 0.01$).

A similar trend was observed in the south region where the most plausible predictors of MP abundance in lagoon water were site and season (AIC = 2710.5; McFadden pseudo$R^2$ = 0.12, Table 3). Jensen Beach was used as a reference for comparison in the southern models as it had lower abundance. Southern sites Driftwood, Fish House, House of Refuge, Indian Riverside Park, JB Impound, Jimmy Graham, Midway, PC Bridge, River Cove, Riverwalk, and Twin Rivers had higher MP abundance ($p \leq 0.001$ for all). Abundance was also higher in fall and winter ($p < 0.001$ for both). Neither site, or season were significant predictors of MP presence in south lagoon water.

Microplastic abundance trends also differed between IRL regions with regard to oceanic and freshwater influences. In the north IRL, MP abundance did not vary with distance to a tributary or inlet. However, a different trend was apparent in the central IRL, where MP abundance decreased with increasing distance from a tributary ($p < 0.001$) and increased with increasing distance from an inlet ($p = 0.001$). Abundance decreased by 0.99 MP/L for every 1-

km increase in distance from a tributary and increased by 0.82 MP/L for every 1-km increase in distance from an inlet in the central IRL. In the southern region, no oceanic influence was apparent, however tributary influence was, and MP abundance decreased by 0.88 MP/L for every 1-km increase in distance from a tributary ($p < 0.001$).

Analysis of MP abundance in lagoon water and oyster reef-adjacent water revealed abundance was higher in site water than in reef-adjacent water, by 1.7 MP/L on average ($p = 0.03$). This can likely be attributed to the filter-feeding behavior of *C. virginica*.

*Table 3. Zero-inflated negative binomial GLM models of MP abundance in lagoon water, overall and by region. Values reported are AIC, delta AIC, degrees of freedom, and AIC weight.*

| **Indian River Lagoon Water** | **AIC** | **ΔAIC** | **df** | **AIC weight** |
|---|---|---|---|---|
| Site + season | 6283.6 | 0 | 77 | 1 |
| Site | 6306.6 | 23 | 71 | <0.001 |
| Tributary + region + season | 6569.9 | 286.3 | 15 | <0.001 |
| Tributary + region | 6584.1 | 300.5 | 9 | <0.001 |
| Region + season | 6644.6 | 361 | 13 | <0.001 |
| Region | 6660.2 | 376.6 | 7 | <0.001 |
| Season | 6938.3 | 654.8 | 9 | <0.001 |
| Inlet | 6946.8 | 663.2 | 7 | <0.001 |
| Tributary | 6951.4 | 667.8 | 5 | <0.001 |
| **North Lagoon** | **AIC** | **ΔAIC** | **df** | **AIC weight** |
| Season | 2422.9 | 0 | 9 | 0.8221 |
| Site + season | 2426.0 | 3.1 | 31 | 0.1748 |
| Site | 2434.1 | 11.2 | 25 | 0.0031 |
| **Central Lagoon** | **AIC** | **ΔAIC** | **df** | **AIC weight** |
| Site + season | 1131.2 | 0 | 27 | 0.7207 |
| Site | 1133.1 | 1.9 | 21 | 0.2771 |
| Inlet + season | 1143.6 | 12.4 | 11 | 0.0015 |
| Inlet | 1145.0 | 13.8 | 5 | <0.001 |
| Tributary | 1179.1 | 47,9 | 5 | <0.001 |
| Season | 1209.9 | 78,7 | 9 | <0.001 |
| **South Lagoon** | **AIC** | **ΔAIC** | **df** | **AIC weight** |
| Site + season | 2710.5 | 0 | 33 | 1 |
| Site | 2742.7 | 32.2 | 27 | <0.001 |
| Tributary + season | 2912.9 | 202.5 | 11 | <0.001 |
| Tributary | 2920.5 | 210 | 5 | <0.001 |
| Season | 3003.5 | 293 | 9 | <0.001 |

*Microplastics in oysters*

*Crassostrea virginica* from the IRL contained a total of 3181 MP (*n* =1402). The composition of MP was dominantly fibers (95.0%), while fragments comprised 4.4%, and films and foams comprised less than 1% of MP combined. Seventy percent (*n*=981) of *C. virginica* contained MP in their tissues, and the dominant color of MP was black. Plastics ranged in size from 0.1 mm to 35 mm, with a mean size (± CI) of 2.79 ± 0.10 mm. Oysters had a mean MP abundance (± CI) of 2.26 ± 0.16 MP/individual and density of 2.43 ± 0.52 MP/g tissue weight (Table 4).

Abundance differed between *C. virginica* from IRL regions; northern oysters contained less MP than central and south oysters, but abundance did not differ between the central and south oysters ($p < 0.001$, Figure 3).

*Table 4. Microplastic abundance in Indian River Lagoon oysters, overall and in each region. Four values are reported: raw MP count (Abundance) and density (Density) per oyster, normalized abundance ($Abundance_N$) and density ($Density_N$) per oyster accounting for aerial contamination, Measurements are mean, 95% confidence intervals of the mean, and range. Units for abundance are MP/individual and MP/g tissue weight for density.*

| Value | Parameter | Oysters | North | Central | South |
|---|---|---|---|---|---|
| Abundance | Mean | 2.26 | 1.85 | 2.66 | 2.72 |
| | CI | 0.16 | 0.20 | 0.35 | 0.33 |
| | Range | 0 - 32 | 0 - 32 | 0 - 22 | 0 -20 |
| $Abundance_N$ | Mean | 2.21 | 1.81 | 2.59 | 2.67 |
| | CI | 0.16 | 0.20 | 0.35 | 0.33 |
| | Range | 0 - 31.9 | 0 - 31.9 | 0 - 21.9 | 0 - 19.9 |
| Density | Mean | 2.43 | 2.02 | 2.73 | 2.97 |
| | CI | 0.52 | 0.91 | 0.78 | 0.61 |
| | Range | 0 - 318.6 | 0 - 318.6 | 0 - 78.1 | 0 - 49.9 |
| $Density_N$ | Mean | 2.43 | 2.02 | 2.73 | 2.97 |
| | CI | 0.52 | 0.91 | 0.78 | 0.61 |
| | Range | 0 - 318.6 | 0 - 318.6 | 0 - 78.1 | 0 - 49.9 |

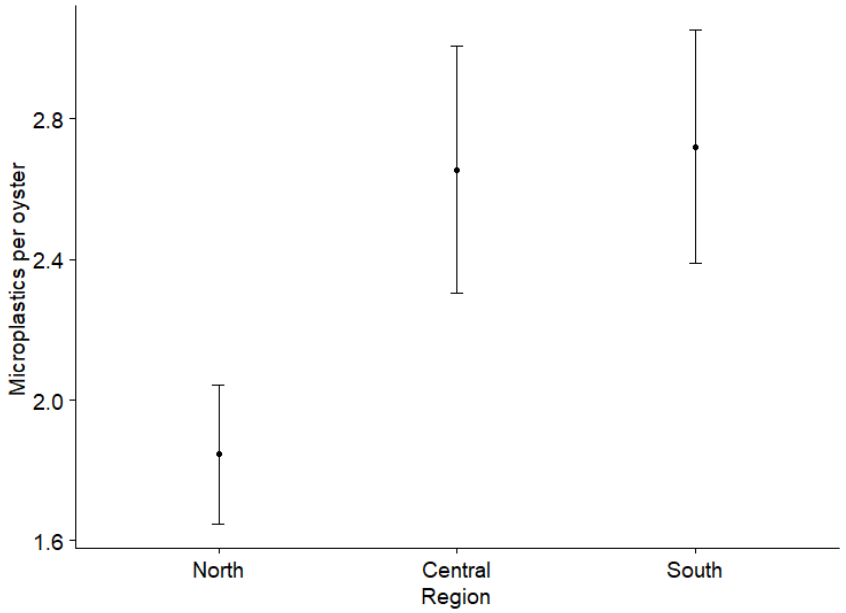


*Figure 3. Microplastic abundance by region in Indian River Lagoon oysters. Values reported are the mean (point) and the 95% confidence interval of the mean (error bar). (GLM, p = 0.01, North=710, Central=345, South=347).*

The most plausible model to predict MP abundance in IRL *C. virginica* included season, site, and shell height as predictor variables (Table 5). Site, Wildcat, and the spring season were selected as a reference in the model to match the IRL water models. Model results indicated MP abundance was lower in oysters sampled in summer, fall, and winter ($p < 0.03$ for all) and were also predictors of MP presence ($p \leq 0.05$). Oysters from northern reefs, MLR1 and MLR3, central reefs, Sebastian and Vero, and southern reefs, Driftwood, Indian Riverside Park, and FOS had more MP ($p \leq 0.03$). The model also indicated MP abundance increased by 1.4 MP/individual for every 1-mm increase in shell height ($p < 0.001$).

*Table 5. Zero-inflated negative binomial GLM models of MP abundance in Indian River Lagoon oysters, overall and by region. Values reported are AIC, delta AIC, degrees of freedom, and AIC weight.*

| **Indian River Lagoon Oysters** | **AIC** | **ΔAIC** | **df** | **AIC weight** |
|---|---|---|---|---|
| Site + season + shell height | 4984.0 | 0.0 | 35 | 1 |
| Season + shell height | 5020.6 | 32.6 | 13 | <0.001 |
| Site + shell height | 5053.1 | 69.1 | 29 | <0.001 |
| Shell height | 5088.2 | 104.2 | 7 | <0.001 |
| Site + season | 5158.6 | 174.6 | 35 | <0.001 |
| Season | 5208.4 | 224.4 | 13 | <0.001 |
| Site | 5248.5 | 264.5 | 29 | <0.001 |
| Tributary | 5277.0 | 293.0 | 9 | <0.001 |
| Region | 5289.1 | 305.1 | 11 | <0.001 |
| **North Lagoon** | **AIC** | **ΔAIC** | **df** | **AIC weight** |
| Site + season | 2376.0 | 0.0 | 23 | 0.63 |
| Site + season + shell height | 2377.1 | 1.0 | 23 | 0.37 |
| Season + shell height | 2404.2 | 28.2 | 13 | <0.001 |
| Season | 2407.2 | 31.2 | 13 | <0.001 |
| Site | 2411.0 | 34.9 | 17 | <0.001 |
| Site + shell height | 2413.5 | 36.5 | 17 | <0.001 |
| Tributary | 2435.6 | 59.6 | 9 | <0.001 |
| Inlet | 2435.6 | 59.6 | 9 | <0.001 |
| Shell height | 2436.5 | 60.5 | 7 | <0.001 |
| **Central Lagoon** | **AIC** | **ΔAIC** | **df** | **AIC weight** |
| Season + shell height | 1203.5 | 0.0 | 13 | 1 |
| Shell height | 1263.2 | 59.7 | 7 | <0.001 |
| Site + season | 1289.7 | 86.2 | 15 | <0.001 |
| Season | 1310.2 | 106.7 | 13 | <0.001 |
| Site | 1364.5 | 161.0 | 9 | <0.001 |
| Tributary | 1371.7 | 168.2 | 9 | <0.001 |
| **South Lagoon** | **AIC** | **ΔAIC** | **df** | **AIC weight** |
| Season + shell height | 1335.8 | 0.0 | 13 | 1 |
| Shell height | 1372.0 | 36.2 | 7 | <0.001 |
| Season | 1408.7 | 72.9 | 13 | <0.001 |

The most plausible predictors of MP abundance in *C. virginica* varied between the IRL regions (Table 5). In the north IRL, site and season were included in the most plausible model to predict MP abundance in *C. virginica* (AIC = 2376.0; McFadden pseudo$R^2$ = 0.11, Table 5). MLR5 was selected as the site of reference for comparison the model as it had lower abundance. Reefs MLR1, MLR3, and MLR6 had higher MP abundance ($p < 0.01$ for all) and abundance was

lower in summer and winter ($p = 0.02$ for both). Summer, fall, and winter were significant predictors of MP presence in oysters from the northern region ($p \leq 0.04$).

A different trend was apparent in the central IRL, as season and shell height were predictors of MP abundance in *C. virginica* from this region (AIC = 1203.5; McFadden pseudo$R^2 = 0.13$, Table 5). Wildcat reef and spring were used as a reference in the model to be consistent with IRL water models. Abundance was higher in *C. virginica* in summer and fall ($p < 0.001$), and summer, fall, and winter oysters were predictors of MP presence ($p \leq 0.04$). The same model indicated MP abundance increased by 1.51 MP/individual for every 1-mm increase in shell height in *C. virginica* from the central IRL.

A similar trend of MP abundance was apparent in the southern IRL, as season and shell height were predictors in the most plausible model for this region (AIC = 1335.8; McFadden pseudo$R^2 = 0.07$, Table 5). Spring was used as a season reference in the model, while reef FOS was used as a site refence as it had lower MP abundance. Model results revealed MP abundance was lower in the summer and fall, and abundance increased by 1.45 MP for every 1-mm increase in shell height ($p \leq 0.002$ for all). No variable was a significant predictor of MP presence.

Microplastic abundance trends in *C. virginica* also differed between IRL regions with regard to oceanic and freshwater influence. In the northern and central IRL, MP abundance in oysters decreased with every 1-km increase in distance from a freshwater tributary, by 0.77 MP/individual and 0.52 MP/individual, respectively ($p \leq 0.01$ for both). However, no oceanic influence on MP abundance was detected any region, and in the southern IRL, there was also no tributary influence.

*Abiotic parameters*

Air and water temperature differed significantly between IRL regions and seasons but did not differ between sampling sites ($p < 0.005$ for all). As expected, temperatures were higher in

summer and spring, and lower in winter. Water temperature was also higher in the southern region. Salinity did not vary between regions or seasons but did vary between water sites ($p <$ 0.05 for all). Wind speed varied between regions and seasons, but not months. Wind speed was slower in the central IRL region ($p = 0.004$), and faster at northern water sampling sites CANA Parking Lot 5, Marine Discovery Center, Haulover Canal, and Parrish Pk ($p <0.01$). Wind speed was slower at northern oyster reef MLR1 ($p = 0.006$) and southern oyster reef FOS ($p < 0.02$).

*Polymer composition and contamination*

A total of 122 signals of suspected MP were obtained using ATR-FTIR spectroscopy, and 78 (64%) were confirmed synthetic polymers. Fibers, fragments, foams, and films were found in both lagoon water and *C. virginica.* Fibers dominated type composition and comprised 95.6% and 95.0% of MP in water and *C. virginica,* respectively (Figure 4). Fragments were second most abundant and comprised 3.9% of water MP and 4.4% of *C. virginica* MP. Films and foams comprised the remaining 1% of MP. Colors varied across the spectrum, but black MP were the most common.

Polyethylene terephthalate (PET) was the most abundant polymer in lagoon water and *C. virginica* in the IRL, and comprised 50%, and 56% of MP, respectively. Polypropylene (PP), polystyrene (PS), and polyamide (PA) were also found in lagoon water in differing proportions (Figure 5). All scanned MP were fibers, except for two clear fragments, one was polyethylene (PE) and the other synthetic wax. There was only one rayon fiber found, which was in an oyster. Miscellaneous polymers included polymer blends in water and *C. virginica*, acrylic adhesive in lagoon water, and polyacrylates in *C. virginica*.

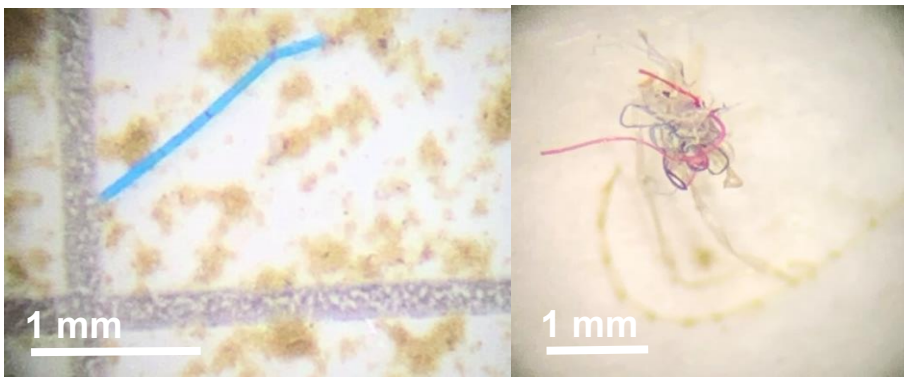


*Figure 4. Light blue microplastic fiber extracted from lagoon water and bundle of synthetic and natural fibers extracted from C. virginica.*

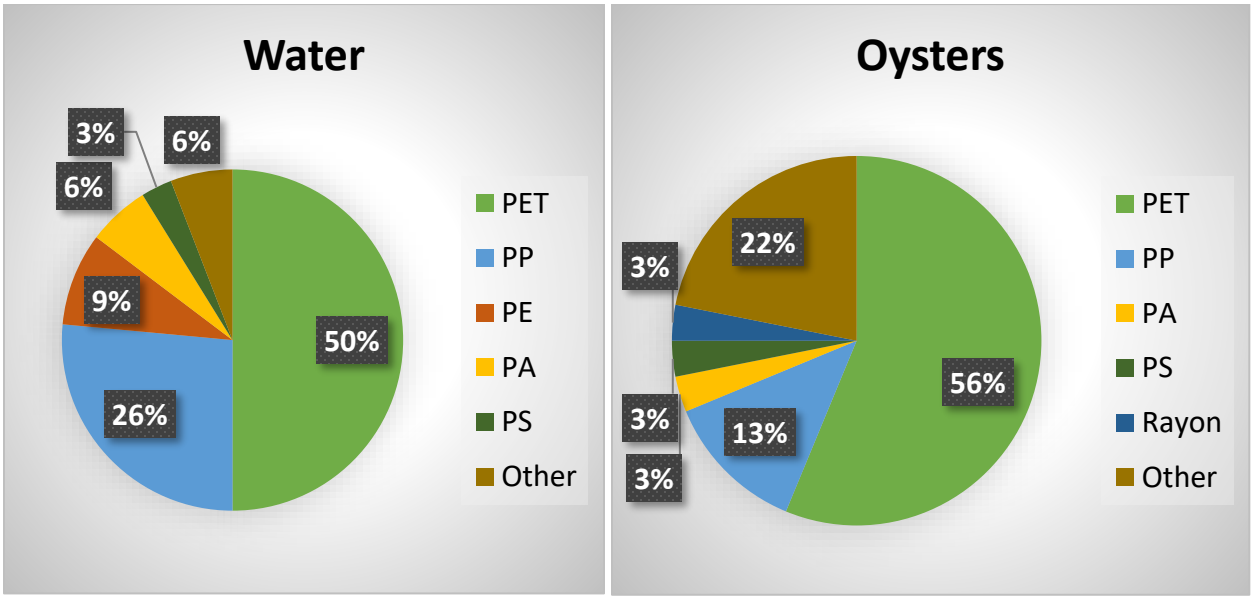


*Figure 5. Synthetic polymers in microplastics from water and oysters from the Indian River Lagoon. Polymers are polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), polyamide (PA), polystyrene (PS), rayon, and miscellaneous polymers (Other).*

Aerial control blanks associated with lagoon water had a mean contamination rate of 0.016 MP/min. The mean time of exposure associated with microscopy for water samples was 8 minutes. Mean aerial contamination rate for *C. virginica* samples was 0.02 MP/min and samples were exposed for a mean time of 5 minutes. There was a 15% overlap (3 PET particles) between polymers found in samples and polymers from aerial control blanks.

# Discussion

Microplastics are a ubiquitous pollutant in the marine environment and potential risk to marine biota, emphasizing the need to understand MP abundance and the factors that influence these patterns in marine systems (Law et al. 2010, Erkes-Medrano et al. 2015, Wang et al 2016). Growing research suggests oceans function as sinks for MP while coastal surface waters are sources (Siegfried et al. 2017, van Franeker and Law 2015, Wooddall et al. 2014). This study quantified MP abundance in surface water and *C. virginica* from the Indian River Lagoon to determine if spatial and temporal factors influence MP abundances within this system. Overall, *C. virginica* had 2.26 MP/individual, or 2.43 MP/g tissue weight, and lagoon water had 1.47 MP/L, on average. Significant variations and trends in MP abundance were detected across seasons, and within spatial extents less than 5 km, indicating both site and season should be incorporated into microplastics research designs. Abundance varied significantly within the IRL although trends differed between regions. The south IRL is an identified hotspot for MP pollution. MP abundance was influenced by oceanic inlets and freshwater tributaries in both systems, but the effects of each differed between the lagoon regions.

MP studies of estuaries and coastal bivalves have reported variable levels of MP abundances in coastal systems (Han et al. 2020, McEachern et al. 2019). Comparisons of MP abundance in the IRL to abundances of other estuaries in the United States are summarized in Table 6. Abundances reported in this study are lower than those previously documented in seawater and *C. virginica* from Mosquito Lagoon, the northernmost portion of the IRL (Waite et al. 2018). This difference in abundance can likely be attributed to the distinctly different MP collection and extraction procedures between the studies as FTIR was not included in earlier studies and large numbers of natural fibers are present in the system (CC, pers. obs.). Also,

polymer composition data was not reported and so could not be compared to validate confirmed polymers using FTIR results. I found MP abundance in IRL water was comparable to a surface water study in the Tampa Bay estuary (FL), but not to earlier studies in Charleston Harbor, SC or Winyah Bay, SC (Gray et al. 2018; McEachern et al. 2019). MP abundance in *C. virginica* from the IRL was also dissimilar to MP abundance in *Crassostrea gigas* from the Oregon coast, but was similar to *C. gigas* in the Salish Sea (WA; Baechler et al. 2020; Martinelli et al. 2020). The MP abundances reported in this study were also comparable to those in *C. gigas* along the French Atlantic coast (1.7 MP/individual; Phuong et al. 2018).

*Table 6. Comparison of microplastic abundance in water and oysters from the Indian River Lagoon and other United States estuaries. Values reported are mean abundance per liter of water, microplastics per individual oyster, and standard error of the mean.*

| **Water** | **Location** | **Abundance** | **Reference** | |
|---|---|---|---|---|
| | Indian River Lagoon, FL | 1.46 ± 0.05 | Present study | |
| | Mosquito Lagoon, FL | 23.1 | Waite et al. 2018 | |
| | Tampa Bay Estuary, FL | 0.94 ± 0.52 | McEachern et al. 2019 | |
| | Charleston Harbor, SC | 6.6 ± 1.3 | Gray et al. 2018 | |
| | Winyah Bay, SC | 30.8 ± 12.1 | Gray et al. 2018 | |
| **Oysters** | **Location** | **Abundance** | **Reference** | **Species** |
| | Indian River Lagoon, FL | 2.26 ± 0.08 | Present study | *Crassostrea virginica* |
| | Mosquito Lagoon, FL | 16.5 | Waite et al. 2018 | *Crassostrea virginica* |
| | Salish Sea, WA | 1.75 | Martinelli et al. 2020 | *Crassostrea gigas* |
| | Oregon Coast | 10.95 ± 0.77 | Baecher et al. 2020 | *Crassostrea gigas* |

*Spatial microplastic fluctuations and influences*

Overall, MP abundance in the IRL differed between the north, central, and south regions. The highest abundances were observed in the south IRL, which indicates it is a hotspot for MP pollution. Within the lagoon water-MP system, each region had distinctly different MP abundances from another, suggesting MP abundance is likely influenced by differing factors in the north, central, and south IRL. This was confirmed by analyses that indicated MP abundance in both lagoon water and *C. virginica* were influenced differently by hydrological factors such as

distance to a tributary or inlet. The IRL is a bar-built lagoon that has limited water exchange through five inlets and is hydrologically complex (IRL CCMP 2019, Rosario-Llantin & Zarillo, 2021, Smith 1993). Four of the five inlets within the lagoon, Ponce de Leon, Sebastian, Ft Pierce, and St Lucie, were included within the defined boundaries of this study. Freshwater contribution to the IRL comes from land runoff and a dynamic matrix of rivers, drainage canals, creeks, and ditches, which are unevenly distributed throughout the lagoon (IRLNEP & SJRWMD 2007).

In the northern IRL there was no inlet influence detected on MP abundance in water or oysters. There was, however, a tributary influence on MP abundance in oysters though not in lagoon water. The primary tributary in the north IRL, the Halifax River, empties into the lagoon in the same location as Ponce de Leon Inlet, which is the only inlet in the north region. The next closest inlet to Ponce de Leon is Sebastian, which is 142 km south, at the boundary of Brevard and Indian River counties. There is a second inlet in the north Banana River (Port Canaveral) that is within the bounds of the northern IRL, however it is regulated using an engineered locking system and only opened for vessel passing, so natural tidal flow is inhibited (IRL CCMP 2019; Saberi and Weaver 2016, Zarillo 2020). As a result, parts of the north IRL are considered microtidal and water residence times (50% renewal time) within the region vary greatly; for example, residence time for the northernmost and southernmost portions of Mosquito Lagoon are 15 days and 172 days, respectively (Rosario-LLantin and Zarillo 2021).

Within the northern IRL, MP abundance in water did not differ between sites, however abundance was different in oysters from different reefs. Abundance in *C. virginica* from the north IRL was higher at reefs MLR1, MLR3, and MLR6. All northern sampling reefs were located within 15 km of Ponce de Leon inlet, and 5 km of each other so spatial influences on MP abundance in *C. virginica* were hard to distinguish. All reefs were located in the central

Mosquito Lagoon region, where water residence time is lowest (15 days; Rosario-LLantin and Zarillo 2021). It is possible that the higher MP abundance at reef MLR1 can be attributed to proximity to the Halifax River, however increased MP abundance was also observed at the southernmost reef in the north region, MLR6, as well as MLR3, so influences on MP abundance observed in these reefs remains unanswered. Investigation into abiotic conditions between sampling reefs in the north IRL were inconclusive as conditions did not differ. It is likely that the variation in MP abundance in the north IRL is not explained by variables captured in this study, and a finer-scale study is needed to distinguish influences. Stormwater outfalls were not incorporated in this study but have been identified as sources of MP pollution in coastal systems and may have an influence on MP abundance (Mak et al. 2020, Wang et al. 2020).

In the central IRL, a similar trend of freshwater influence on MP abundance was apparent in both water and *C. virginica*, as abundance decreased with increasing distance from a tributary suggesting that the tributaries are sources of MP. Freshwater tributary presence in the central lagoon is higher than that in the north lagoon; primary tributaries within the boundaries of the central IRL are the Sebastian and Eau Gallie Rivers, Turkey and Crane Creeks, and various manmade canals (e.g. Vero Main, Vero North, Vero South, Taylor; IRL CCMP 2019, IRLNEP & SJRWMD 2007). The Sebastian River is the second largest tributary to the IRL (Bergman and Donnangelo 2007). Despite the increase in tributary presence, water from the central IRL contained the lowest MP abundances, which may be attributed to the inlet influence flushing MP out of the region. There are two inlets within the boundaries of the central IRL, Sebastian and Ft Pierce, that contribute to lower water residence times and increased tidal flushing in the region (Kim 2003). In oysters from the central IRL, however, there was no inlet influence which can likely be attributed to the proximity of two of the three reefs (Sebastian and Vero) to freshwater

tributaries. Reefs Sebastian and Vero are located where two tributaries (Sebastian River and Vero Main Canal) empty into the lagoon, and the third reef in this region (Wildcat) is located further south, 3 km away from Ft Pierce inlet. Although reef Wildcat is near the Ft Pierce inlet, it is located on the western side of the central lagoon, where there is a lessened tidal influence compared to the eastern shore (Kim 2003). In central IRL water, abundance was lower at site Outriggers, which is located at the mouth of the Sebastian River. While at first glance this may seem contradictory, Sebastian River discharge data (USGS Surface Water Data for the Nation) indicated the river had a mean annual discharge rate of ~100 $m^3$ $s^{-1}$ throughout this study, and Law et al (2010) found MP accumulate in areas with water velocities slower than 2 cm $s^{-1}$, suggesting MP in this area are flushed out by the increased water velocity of the Sebastian River. Abundance in central lagoon water was also lower at adjacent sites ELC and Vero, however investigation into the hydrological and abiotic influences at this site did not provide insight to explain the variation observed, and further investigation into stormwater outfalls is likely needed to help distinguish this. Conversely, *C. virginica* sampled from the central IRL revealed there was no difference in MP abundance between reefs, but MP abundance increased with increasing oyster shell height. This suggests the increased mixing and tidal circulation in this region promotes the flushing of MP out of the central IRL but in the process delivers MP from tributaries to areas further away with slower surface water currents, resulting in similar abundances. Abundance in oysters in the central IRL differed between oysters of varying size, unlike north IRL oysters, and those with longer shells had more MP.

Southern IRL MP abundance was highest in both *C. virginica* and lagoon water. Following the trend of tributary contribution in the central region, MP abundance in southern lagoon water decreased with increasing distance from a tributary, however abundance in *C.*

*virginica* did not. There is one primary tributary in the south IRL, the St Lucie Estuary (SLE), which is also the largest tributary to the IRL as a whole and connects the lagoon to Lake Okeechobee through the C44 canal (IRL CCMP 2019, Ji et al. 2007). The south IRL deviated from other regions in that MP abundance was not influenced by distance to an inlet, in either lagoon water or *C. virginica*. There is one inlet within the southern IRL boundary defined in this study, the St Lucie inlet, which has constricted water flow into the area, and as a result there is less tidal influence in this area, and in the SLE (Ji et al. 2007). This suggests the St Lucie inlet is not flushing MP out of the southern IRL at rates fast enough to accommodate deposition from the SLE. In southern lagoon water, MP abundance was higher at all southern sites except Bear Point. This site is the northernmost site in the southern IRL, adjacent to the Ft Pierce Inlet, which is an identified exit point for MP in the IRL which likely attributes to the lower abundance observed. Abundance in *C. virginica* in the southern IRL did not differ between sampling reefs, matching the trend observed in oysters from the central region, but MP abundance did increase with increasing shell height. The lack of variation in abundance in southern reefs can likely be attributed to their location, as they are all north of the SLE and St. Lucie Inlet where water circulation patterns are different than in the SLE/Inlet area (Ji et al. 2007, Kim 2003).

When assessing MP abundance at the whole-IRL scale, MP abundance was highest in lagoon water from Driftwood, Fish House, House of Refuge, PC Bridge, Indian Riverside Park, and Riverwalk, all of which are in the southern IRL, a hotspot for MP. Three of these sites (Fish House, PC Bridge, and Riverwalk) were in the SLE, further providing evidence it is delivering MP pollution to the IRL. Abundance was lower at central sites, Christensen and ELC, which can be attributed to the increased inlet flushing in the region, and southern site, Jensen Beach. Jensen Beach is located directly in the middle of the southern IRL region and is the site furthest from the

two nearest tributaries in the area (SLE and Taylor canal), suggesting this site may receive less MP deposition from these two tributaries resulting in lower abundances. In the IRL-*C. virginica* system, MP abundance was higher at northern reefs MLR1 and MLR3, central reefs, Sebastian and Vero, and all southern reefs (Driftwood, Indian Riverside Park, and FOS). The increase in abundance at these reefs, except for MLR3, is attributed to freshwater MP deposition from nearby tributaries. Abundance also increased with increasing shell height, indicating larger oysters have more MP, on average. It is probable that the increase in abundance at MLR3 could not be explained by the variables captured in this study.

*Temporal microplastic fluctuations and influences*

Temporal trends in MP abundance in IRL water and *C. virginica* varied between each of the IRL regions. In the northern IRL, MP abundance was higher in lagoon water in winter, but lower in *C. virginica* in summer and winter. Investigation into these trends revealed no correlation to an increase in tributary discharge, rainfall, or wind in the area. As previously mentioned, it is possible that there are other seasonal variables that influence MP abundance, such as stormwater outfall flushing, that are not encompassed in this study. Another potential explanation is a limitation of the study design, in that abiotic parameters are recorded at the time of sample collections, and so are only representative of one point in time, on one day of each collection, and not of an entire season. Continuous, public, abiotic data was not available at the small scales needed to help distinguish trends in MP abundances in this study. In the central IRL, temporal trends in MP abundance in water and *C. virginica* were identical, with lower abundances in summer and fall. However, investigation into the abiotic parameters in these seasons revealed no correlation a change in discharge, rainfall, wind, or temperature at these times. Interestingly, summer and fall are seasons when east Florida has increased rainfall, or a 'wet season' (Lascody 2002) which indicates the rain could be diluting the IRL resulting in

lower MP abundance. Oysters from the southern IRL also had lower abundances in summer and fall, though southern lagoon water MP abundance was higher in fall and winter. Ultimately, further investigation into the effects of rainfall, and other seasonal influences on MP abundance in the lagoon such as outfalls is needed.

Unexplained temporal variation in MP abundance was likely impacted by extreme events that happened during the study period. Hurricane Dorian, which paralleled Florida's east coast between September 1-3, 2019, may have impacted MP abundance in the lagoon. Hitchcock (2020) found MP abundance levels in the Cooks River estuary (AUS) were 40-fold during a storm event. Around the time of Hurricane Dorian, discharge out of the Sebastian River and SLE around this time increased to ~1000 $ft^3$ $s^{-1}$ (Figure 6, Figure 7). There were also prolonged high-water levels associated with a new lunar cycle and slowing of the Atlantic current in November 2019, which may also have influenced MP abundance (Figure 8).

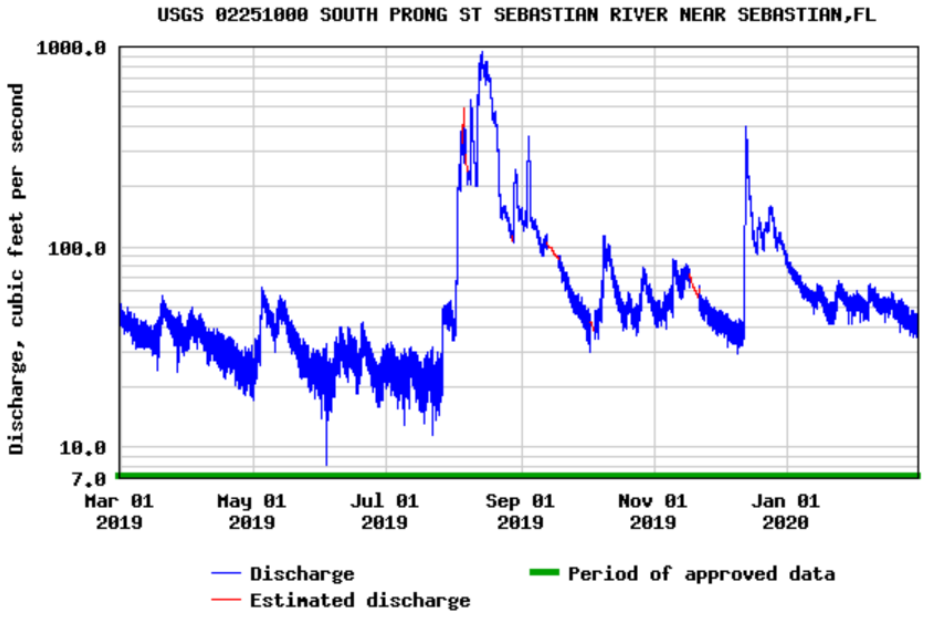


*Figure 6. Discharge, in cubic feet per second, from the St. Sebastian River from March 2019 to February 2020.*

*Source: USGS Surface Water Data for the Nation*

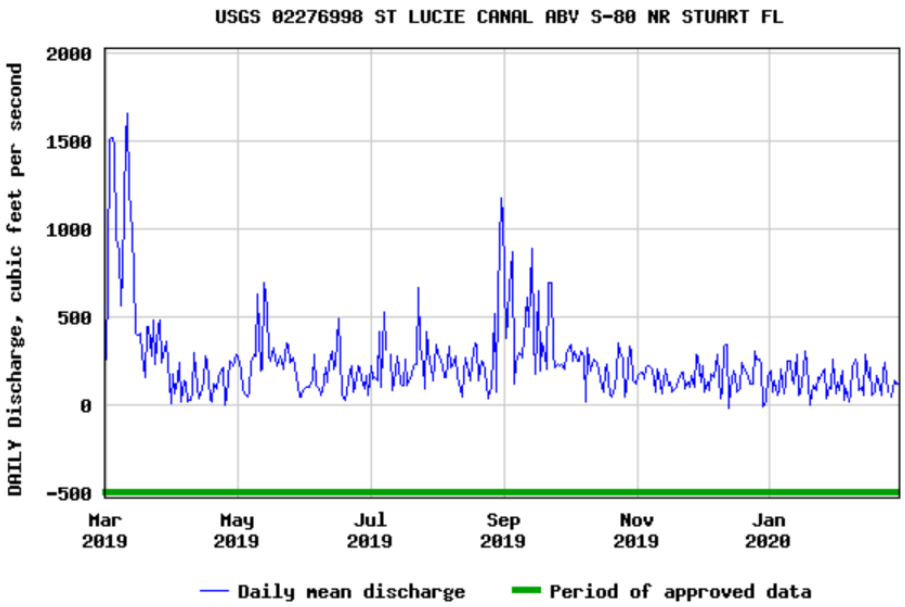


*Figure 7. Discharge, in cubic feet per second, from the St. Lucie Estuary from March 2019 to February 2020.*

*Source: USGS Surface Water Data for the Nation*

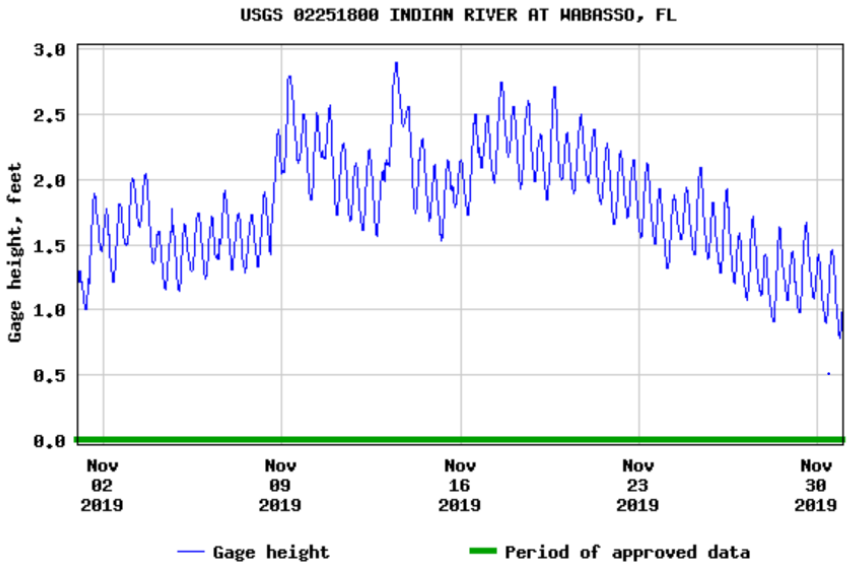


*Figure 8. Gage heigh, in feet, in the central Indian River Lagoon in November 2019 illustrating extreme high-water levels associated with the lunar cycle.*

*Source: USGS Surface Water Data for the Nation*

*Polymer composition*

Polyethylene terephthalate (PET) was the dominant polymer found in both oysters and water. PET is prominent in the single use plastic industry, particularly plastic water bottles (Beck 2005, Delle Chiai et al. 2020, Moore 2008). Polyester, also known as PET, is the most produced synthetic textile material in the world and is common in clothing (Jaffe et al. 2020). Since 95% of MP in the IRL were fibers, it is probable MP originate from wastewater treatment plants or septic systems. Bouchet and Friot (2017) estimate 35% of plastics in oceans are from synthetic textiles associated with laundry.

Of the 44 misidentified non-polymers, 39% were natural textile fibers including as wool, cotton, and silk fibroin (Koh et al. 2015). An additional 30% were cellulose derivatives (e.g. microcrystalline, microfibrillated cellulose), and 10% were ramie fiber. These are fibers engineered to be resistant to breakage (e.g. ramie, silk fibroin, cellulose derivatives) suggesting that there may be a weakness in identification procedures where resistance to breakage may be too heavily relied upon as a characteristic to classify a particle as a MP.

*Conclusions and citizen science*

Over the period of this study, 84 citizens participated hands-on with water sampling, processing, and inspecting. A total of 48 MP trainings were held, and citizen scientists contributed over 1600 hours of their time to Indian River Lagoon MP research.

In the IRL, MP abundance was variable, both spatially and temporally, which can be attributed to the unique hydrology in each region. A total of 6,936 MP were found in IRL water plus oysters, 95% of which were fibers. The southern IRL is a hotspot for microplastic pollution where the primary tributary is the also the largest tributary to the IRL, the St. Lucie Estuary. Overall, freshwater tributaries in the IRL were the suggested sources of MP pollution, while the Sebastian and Ft. Pierce inlets flushed MP out of this system. Here I provide an argument that

MP abundance is highly variable within hydrologically dynamic estuarine systems like the Indian River Lagoon, and small scale spatial and temporal context should be considered in future microplastics study designs. Using a mean abundance of 1.5 MP/L and lagoon volume of 953,000,000 $cm^3$ (Smith 1992), I estimate there are ~ 1.4 trillion MP in the Indian River Lagoon.

# CHAPTER III: IN-SITU MICROPLASTIC EGESTION EFFICIENCY OF THE EASTERN OYSTER, CRASSOSTREA VIRGINICA

## Introduction

Microplastics are a pervasive environmental pollutant that accumulate in subtropical regions, especially estuaries, posing a risk to marine biota (Law et al. 2010, McEachern et al. 2019, Sankoda and Yamada 2021, Zhang et al. 2020). Oysters have been documented to ingest microplastics in laboratory settings and the environment (Chapter 2, this document, Waite et al. 2018). To survive, oysters in the genus *Crassostrea* filter-feed by extracting particulate matter from the water, encasing it in mucous, and rejecting or digesting the material. If rejection is the chosen pathway, the material passes along the mantle and is excreted as pseudofeces: If digestion is elected, the material is brought into the mouth opening, nutrients removed, and the remainder excreted as feces (Gaspar 2018; Newell 1983).

Laboratory studies of microplastic (MP) ingestion in *Crassostrea gigas* documented the disruption of reproductive processes, including reduced oocyte production and size and delayed larval metamorphosis (Sussarellu et al. 2016). In a separate study of juvenile *C. virginica* exposed to polyethylene terephthalate (PET), Eierman et al. (2019) documented significantly decreased survival and growth, and a skewed sex differentiation favoring females. Ward et al. (2019) rejected using *C. virginica* as bioindicators of MP pollution after documenting selective ingestion and egestion of virgin MP beads and fibers <1 mm in a laboratory setting. In the Indian River Lagoon, MP in water and *C. virginica* had a mean size of 1.9 mm and 2.8 mm, respectively (Chapter 2, this document). Ingestion and egestion abilities of MP by *C. virginica* likely differs with varying sized particles; selective ingestion of particles by *C. virginica* is influenced by size, shape, and physiochemical properties such as static charge and films on the

particle surface (Rosa et al. 2013). Additionally, MP have high-surface area to volume ratios and may accumulate large quantities of harmful compounds called persistent organic pollutants (POPs) from the environment, which alter MP surface properties (Guo and Wang 2019, Tueten et al. 2007). Fibers have especially high aspect ratios and can accumulate POPs 6 times more than the surrounding water. Ninety-five percent of MP in the IRL were fibers (Chapter 2, this document, Mato et al. 2001). POPs such as dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) adsorb to MP because they are hydrophobic (Bakir et al. 2012, Tueten et al. 2007). Biofilms on MP have been identified vectors of transport of POPs and heavy metals (e.g. chromium, lead, zinc) through the food chain (Zhang et al. 2020). A limitation of MP ingestion and egestion research is that studies use virgin MP so rates and results reported do not account for degraded MP in the environment which have different surface properties (Eiermann et al. 2019, Ward et al. 2019, Zhang et al. 2020). As a result, ingestion and egestion rates in *C. virginica* likely differs in laboratory and environmental settings.

## Material & Methods

*Sample collection*

To investigate the ability of *C. virginica* to ingest and excrete MP, *in-situ* biodeposition experiments were conducted at Smithsonian Marine Station (SMS) in Fort Pierce, Florida in the Indian River Lagoon (IRL). Oysters were collected from 12 intertidal reefs in the north, central, and south IRL, less than 7 days prior to running the trials in July 2019 (Figure 9). At each reef, 6 large (shell length ≥ 36 mm) and 6 small (shell length < 36 mm) oysters were hand-collected and scrubbed of any fouling organisms. Individuals were hung from SMS dock into the water in wire cages until used.

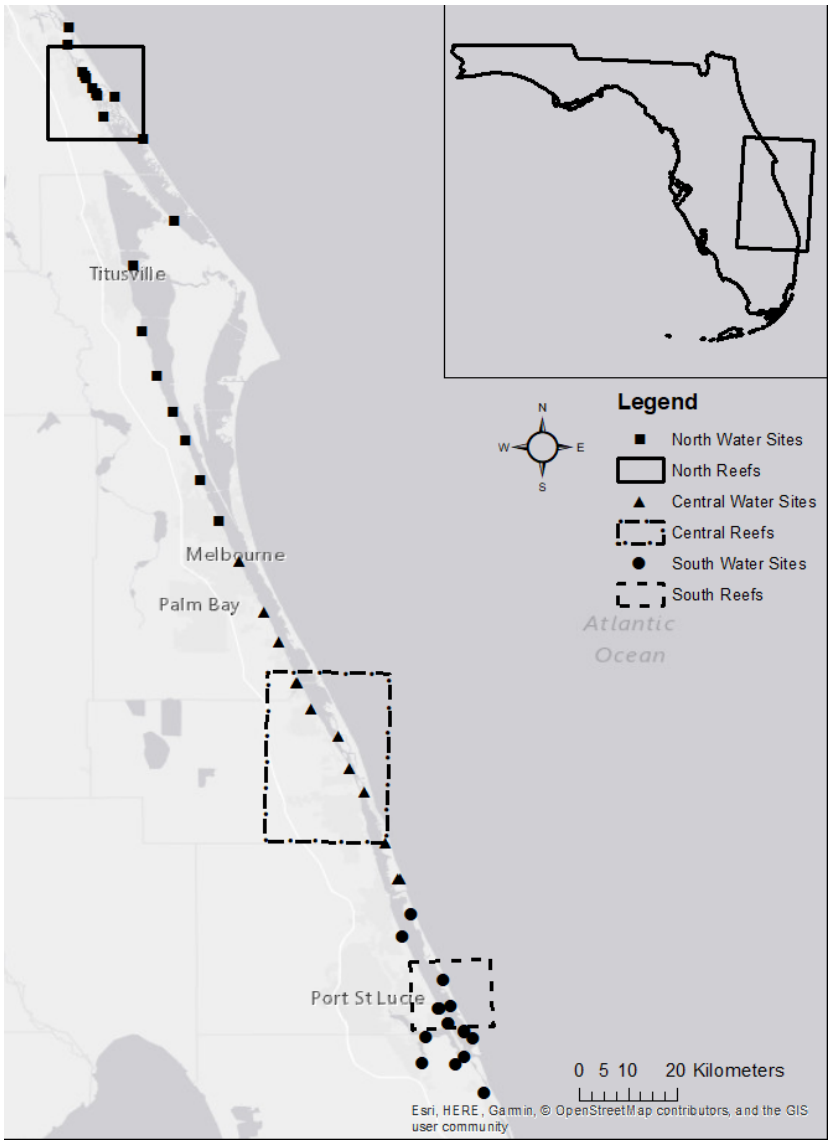


*Figure 9. Indian River Lagoon oyster collection locations (boxes).*

*Biodeposition experiments*

Experimental trials were conducted on the SMS dock using two flow-through filter-feeding biodeposition tanks (Galimany et al. 2011, 2017, 2018). Each device pulled lagoon water through a PVC intake hose into a 20-L PVC reservoir tank, which then flowed into 10 individual PVC chambers (45 x 180 x 60 mm) (Figure 10). The flow rate of water into each chamber was maintained at 12 L $hr^{-1}$, to mimic local filter-feeding conditions: Flow rates were checked every

15 minutes throughout each trial (Galimany et al., 2018). Eight trials were conducted over a 4-day period. Each trial included a different IRL region and oyster size class. Abiotic conditions of air and water temperature (°C), salinity (ppt), and mean windspeed (km/h) were recorded once per trial using a refractometer and anemometer, respectively. Before each trial began, four *C. virginica* were haphazardly selected to calculate gut transit time for oysters from that group (GTT = time between algal exposure, *Nannochloropsis spp,* and bright green feces production in 300 mL of ambient lagoon water). After GTT was calculated, *C. virginica* (*n*=18) were haphazardly placed in separate chambers in one of the two replicate devices to acclimate for GTT before starting each trial. In the tenth chamber of each device, an empty, articulated oyster shell was placed as a negative control (Galimany et al. 2011, 2017, 2018). Immediately prior to the start of data collection, chambers were cleaned of any feces and pseudofeces produced while *C. virginica* acclimated using a glass pipette. Once chambers were cleaned, feces and pseudofeces excreted from each individual were separately collected with glass pipettes every 5 minutes for 2 hours. In preliminary trials, it was determined that these collections did not alter filtering behaviors of *C. virginica.* Feces and pseudofeces for each oyster and control were placed in separate glass scintillation vials (20 mL) that had been triple-rinsed with 0.45 µm filtered deionized water. Upon the conclusion of each trial, *C. virginica* were individually wrapped in aluminum foil, bagged, and stored in a -20 °C freezer for later processing.

*Figure 10. Flow-through filter-feeding bivalve filtration tank used in biodeposition trials.*

*Sample processing in the laboratory*

To collect MP that were not egested by each oyster in their feces or pseudofeces, individual *C. virginica* were thawed and shell heights (mm) were recorded using calipers. Each oyster was then shucked, weighed (g) using a balance (Ohaus Scout Pro), and placed in individual, glass Erlenmeyer flasks (125 mL for small, 250 mL for large oysters). The digestion protocol followed procedures established by Thiele et al. (2019) for optimal extraction of MP from bivalve tissues. A 10% potassium hydroxide (KOH) solution was added to flasks at a ratio of 3:1 volume (mL) to wet tissue weight. Flasks were covered and placed in a shaking incubator at 40 ℃ at 60 rpm for 24 hours, and then removed and left at room temperature for an additional 24 hours where tissue digestion was completed. A 1.0-M citric acid solution was added to the digested tissue solution until a neutral pH (7.0) was reached to prevent an interaction with filters. The neutralized solution was vacuum-filtered under a fume hood using Whatman glass microfiber filters (90 mm, 1.2 µm pore size) and placed in sealed, triple-rinsed, petri dishes for later quantification.

*Limiting polymer contamination and degradation*

Procedural MP contamination was controlled by triple-rinsing all equipment used during digestion and filtration with 0.45 µm filtered deionized (DI) water prior to each use (Arthur et al. 2009). Solutions used during digestions were made with 0.45 µm filtered DI water. Chemical digestion of oysters was conducted in a fume hood to prevent aerial polymer contamination during the filtration process. KOH was used to digest bivalve soft tissue and extract MP particles single-µm in size and larger because it preserves retention rates of major polymers, including rayon (Thiele et al. 2019).

Aerial contamination was quantified during microscopy by using five filter-control blanks (filters dampened with 0.45 µm filtered, deionized water placed in triple-rinsed petri dishes) (e.g., Foekema et al. 2013, Granek et al. 2020). Exposed blank filters were haphazardly placed on the table around the immediate microscopy station at all times during inspection to quantify potential air contamination while samples were uncovered for examination (e.g. Granek et al. 2020). After sample microscopy was completed, blanks were similarly inspected for MP, and this contamination was used to calculate a mean contamination rate per minute that was incorporated into all analyses.

*Fourier-transform infrared spectroscopy*

To supplement MP identification, polymer composition was determined using Attenuated Total Reflection Fourier-transform infrared spectroscopy (ATR-FTIR) using a Shimadzu IRSpirit-T instrument at the UCF Nanoscience Technology Center. A subset of biodeposits and *C. virginica* tissue samples (10% of each) containing MP were randomly selected for analysis using a random number generator (www.random.org), and all potential MP larger than 0.5 mm on each sample were tested (Gago et al. 2016). MP were scanned in the 600 $cm^{-1}$ to 4000 $cm^{-1}$ range and spectra were matched to the reference library from Shimadzu using differential

derivative point matching (ATIR-FTIR Polymer and Polymer Additives Database #: 220-93143-07, 2020). A score, also known as hit quality index (HQI), for each spectrum is calculated to measure percent match using the equation:

$$HQI = \frac{\left(\left(1 - \frac{D}{S}\right)^{1/3} + 1\right)}{2}$$

where *D* is the summation of the primary and reference spectra by calculation of a fitting method, and *S* is the area of the primary derivative curve of the sample spectrum. Spectra were automatically included as a polymer if the score match was 700 or higher, ambiguous scores of 600-700 were manually sorted for inclusion in analysis, and scores of 600 or below were excluded (Frias et al. 2016; Zhao et al. 2018). Polyester is predominantly PET and could not be elucidated as a distinctly different polymer, so polyester signals were classified as PET (Geyer et al. 2019). A subset of signals (10%) on MP identified in control blanks were scanned using ATR-FTIR to determine any overlap in polymers found in samples and controls.

*Statistical methods*

All statistical analyses were performed using R 4.0.3 and RStudio (R Core Team 2019; RStudio Team 2019). The biodeposit count data was zero-inflated and thus analyzed using zero-inflated negative binomial generalized linear models (GLM). To determine influences on MP abundance in biodeposits, region of origin of *C. virginica* (north, central, south), biodeposit type (feces and pseudofeces), shell height (mm), and tissue weight (g) were tested as predictor variables in models. Contamination per sample and biodeposit weight were tested as covariates in every biodeposit model but only included in analyses if significant in the model. A Kruskal-Wallis rank sum test was applied to determine differences in MP density between biodeposit types. To determine MP size difference between feces and pseudofeces, a gamma GLM was

applied, after a Shapiro-Wilk test for normality revealed log transforming the data did not meet linear regression assumptions.

Controls blanks were normalized to a contamination rate per minute ($C_M$) using the formula (McGuire, M; pers. comm.):

$$C_M = \frac{MP_B}{T_B}$$

where $MP_B$ is the mean number of MP per blank and $T_B$ is the time that blanks were exposed, in minutes. Contamination per minute values then were used to calculate a contamination rate per sample ($C_S$) using the formula:

$$C_S = C_M \times T_E$$

where $T_E$ is the length of time each filter was exposed during inspection.

MP abundance in biodeposits and *C. virginica* tissue were used to calculate MP egestion efficiency (*EE*) using the following formula:

$$EE = \frac{MP_{EG}(MP_F + MP_P)}{MP_{ING}(MP_{CV} + MP_{EG})} \times 100$$

where $MP_{EG}$ is the sum of MP abundance in feces ($MP_F$) and pseudofeces ($MP_P$), and $MP_{ING}$ is the sum of MP abundance in *C. virginica* tissue ($MP_{CV}$) and biodeposits ($MP_{EG}$). Contamination per sample was subtracted from $MP_F$, $MP_P$, and $MP_{CV}$ values prior to efficiency calculations. To determine what factors influenced MP egestion efficiency in *C. virginica,* binomial GLMs were applied. Tested predictor variables included tissue weight, shell length, and *C. virginica* region of origin. AIC model selection was used to determine what variables best predicted MP egestion efficiency.

# Results

*Microplastics in feces and pseudofeces*

Overall, 331 MP were present in 67% of *C. virginica* biodeposits (feces plus pseudofeces). Biodeposits contained fibers, fragments, and films, though fibers were dominant (88.3% of MP found). Most of the remaining MP were fragments (11.2%) and one film was found in feces. MP color was variable, with black the most abundant color followed by clear. Plastics ranged in size from 0 to 6.00 mm in feces, and 0 to 20.00 mm in pseudofeces (Table 7). MP had a mean size (± CI) of 1.46 ± 0.16 mm and 1.73 ± 0.32 mm in feces and pseudofeces, respectively. A gamma GLM indicated there was no difference in MP size between biodeposit types ($p$ = 0.09, Feces= 169, Pseudofeces=162).

Over a 2-h period, *C. virginica* excreted a mean (± CI) of 1.45 ± 0.24 MP through feces and 1.22 ± 0.23 MP through pseudofeces. A zero-inflated negative binomial GLM indicated there was no difference in MP abundance between biodeposit types (Figure 11; $p$= 0.42, F=140, P=140). MP abundance in feces ranged from 0 to 6 MP, while pseudofeces abundance ranged from 0 to 7 MP. Feces contained a mean density (± CI) of 0.33 ± 0.15 MP/mg biodeposit weight, while pseudofeces had 0.28 ± 0.15 MP/mg biodeposit weight. A Kruskal-Wallis rank sum test indicated MP density did not differ between biodeposit types ($p$ = 0.81, $x^2$ = 0.06, F = 140, P=140). MP abundance could not be determined by *C. virginica* shell height ($p$ = 0.90) or tissue weight ($p$ = 0.24). However, a significant difference in MP abundance was detected in biodeposits of oysters from the different IRL regions. Biodeposits of central oysters contained significantly less MP than northern biodeposits, and southern biodeposit abundance did not differ from north biodeposits ($p$ < 0.005, North=140, Central=68, South=72).

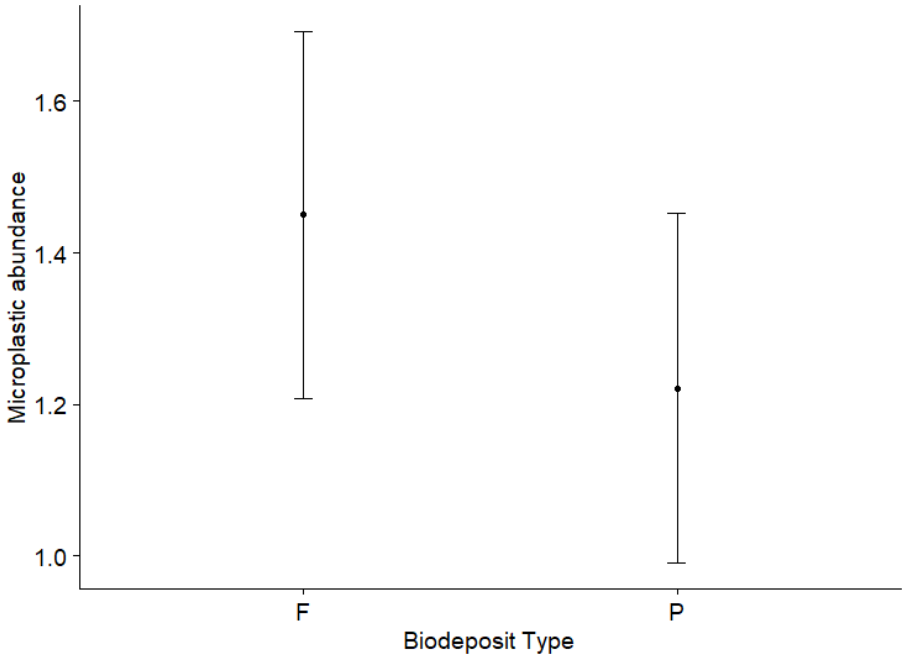


*Figure 11. Microplastics excreted per 2 hours in oyster feces (F) and pseudofeces (P). Values reported are mean (point) and the 95% confidence interval of the mean (error bar). (GLM: p=0.42, F=140, P=140).*

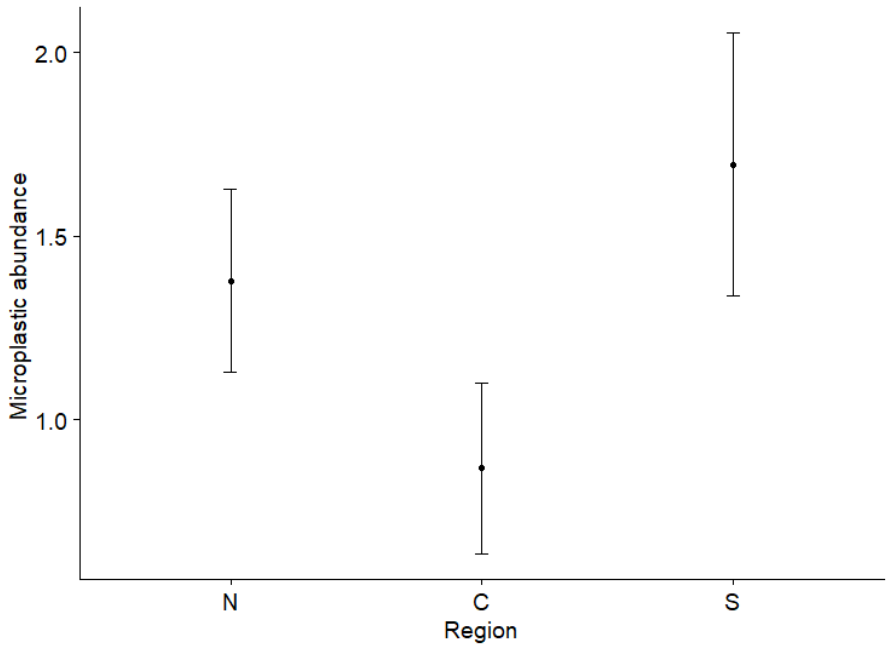


*Figure 12. Microplastics excreted per 2 hours in biodeposits of oysters from the north, central, and south Indian River Lagoon. Values reported are the mean (point) and 95% confidence interval of the mean (error bar). (GLM: p = 0.004, North=140, Central=68, South=72).*

*Table 7. Microplastics found in C. virginica and biodeposits. Values reported are means ± S.E. and ranges for size (mm), raw abundance (Abundance), normalized abundance which accounts for aerial contamination, and density. Biodeposit abundances are reported as microplastics excreted per 2-hours and C. virginica abundances are reported as microplastics per individual.*

| Sample Type | Length (mm) | Length Range | Abundance | Range | $Abundance_N$ | $Range_N$ | Density |
|---|---|---|---|---|---|---|---|
| Biodeposits | 1.60 ± 0.17 | 0.5 - 20 | 1.33 ± 0.17 | 0 - 7 | 1.31 ± 0.17 | 0 - 6.8 | 0.30 ± 0.11 / mg |
| *Feces* | 1.46 ± 0.16 | 0.5 - 6 | 1.45 ± 0.24 | 0 - 7 | 1.43 ± 0.24 | 0 - 6.8 | 0.33 ± 0.15 / mg |
| *Pseudofeces* | 1.73 ± 0.32 | 0.1 - 20 | 1.22 ± 0.23 | 0 - 6 | 1.20 ± 0.23 | 0 - 5.8 | 0.28 ± 0.15 / mg |
| Oysters | 2.48 ± 0.37 | 0.1 - 30 | 1.79 ± 0.60 | 0 - 29 | 1.74 ± 0.59 | 0 - 28.8 | 0.42 ± 0.11 / g |
| *Small* | 1.55 ± 0.47 | 0.2 - 12 | 0.89 ± 0.31 | 0 - 7 | 0.88 ± 0.31 | 0 - 6.9 | 0.33 ± 0.20 / g |
| *Large* | 2.79 ± 0.46 | 0.1 - 30 | 2.69 ± 1.14 | 0 - 29 | 2.61 ± 1.11 | 0 - 28.8 | 0.51 ± 0.21 / g |

*Abiotic conditions during biodeposition trials*

Across the four-day trial period, mean air and water temperature (± CI) was 29.7 ± 1.6 and 28.0 ± 0.4 °C, respectively. Mean wind speed ranged from 3.7 km/h to 19.0 km/h and had a mean (± CI) of 10.7 ± 4.0 km/h. Salinity varied between 30 and 35 ppt, and mean salinity was 33 ppt. A thunderstorm passed during the final trial, bringing sporadic rainfall for a period of 45 minutes.

*Egestion efficiency*

Oysters used in trials ranged in size from 9.5 mm to 94.0 mm and had a mean shell height (± CI) of 26.7 ± 1.39 mm (range: 9.5-36.0 mm) in small oysters, and 57.4 ± 3.16 mm (range: 40.4-94.0 mm) in large oysters. Six oysters were of harvestable size (> 76 mm), and all came from the north or central IRL. Mass of *C. virginica* tissue ranged from 0.1 g to 15.3 g and had a mean (±CI) of 0.8 ± 0.13 g in small oysters, and 4.9 ± 0.71 g in large oysters. A total of 231 MP were found in oyster tissues. All were fibers (94.0%) or fragments that ranged in size

from 0.1 to 30.0 mm. MP in oyster tissue were significantly larger than those in biodeposits, by 1.0 mm on average (Figure 13; GLM: $p < 0.001$, Feces=169, Pseudofeces=162, Tissue=252).

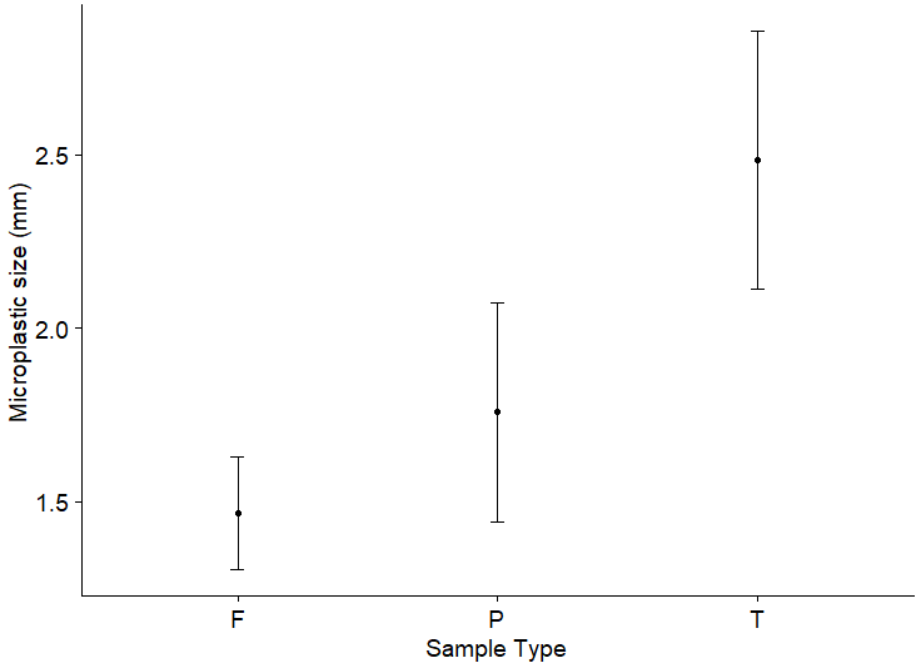


*Figure 13. Microplastic size in feces (F), pseudofeces (P), and oyster tissue (T). Values reported are the mean (point) and 95% confidence interval of the mean (error bar). (GLM: p < 0.01, F=169, P=162, T=252).*

Oysters had a mean MP egestion efficiency of 62.1%, and 32.1% of oysters were able to egest all MP from their tissues within 2 hours. There was no difference in egestion efficiency between oysters from the different IRL regions (GLM: $p$ =0.47, North=70, Central=34, South=36). MP egestion efficiency decreased significantly with increasing oyster shell height (GLM: $p$ = 0.03, $n$=140) and tissue mass (GLM: $p$=0.02, $n$=140). Oyster tissue mass was most plausible at predicting MP egestion efficiency in *C. virginica* and efficiency decreased by 0.8% for every 1-gram increase in tissue weight ($p$=0.02, $n$=140, McFadden pseudo$R^2$= 0.05, Figure 14, Table 8).

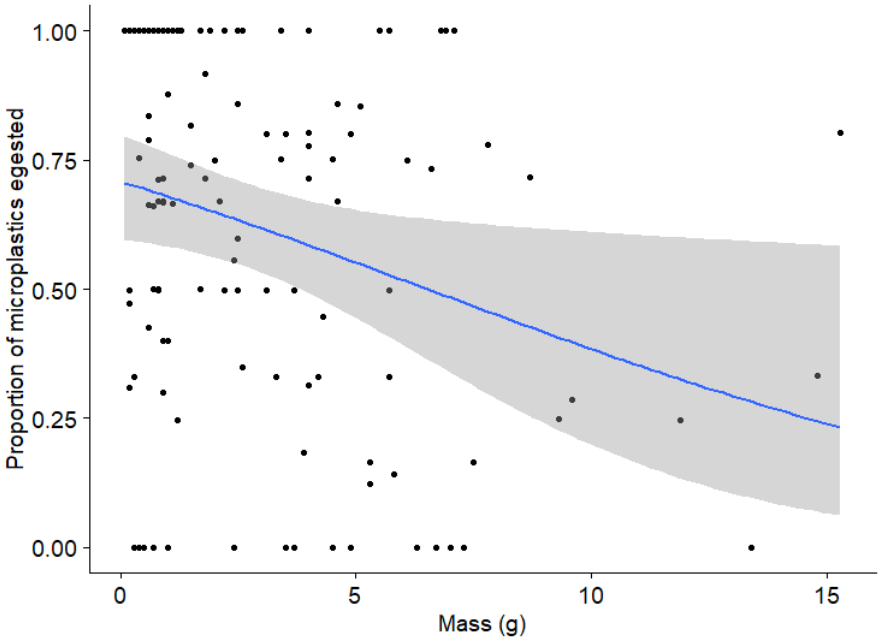


*Figure 14. Binomial regression of microplastic egestion efficiency in* C. virginica *with 95% confidence interval of the model (gray shading). (p=0.03, n=140, McFadden pseudo$R^2$=0.05).*

*Table 8. Binomial regressions of MP egestion efficiency in* C. virginica*. Values reported are AIC, delta AIC, degrees of freedom, and AIC weight.*

| Variable | AIC | ΔAIC | df | AIC weight |
|---|---|---|---|---|
| Mass | 171.9 | 0 | 2 | 0.6938 |
| Shell height | 175.2 | 3.3 | 2 | 0.1326 |

*Polymer composition and contamination*

A total of 43 suspected MP from biodeposits and *C. virginica* tissues were scanned using ATR-FTIR spectroscopy. Of the suspected MP scanned, 70% were confirmed synthetic polymers. Fibers and fragments were present in biodeposits and oyster tissue, and fibers comprised 88.5% and 94.0% in each sample medium, respectively. The remaining MP were fragments, except for one film in feces. Black was the most abundant color of MP found in both sample media. Polyethylene terephthalate (PET) was the most abundant polymer in biodeposits and oyster tissue, and comprised 50% and 58% of confirmed MP, respectively. The next polymer of highest abundance was polypropylene (PP, 10%). Other synthetic polymers found include

polystyrene (PS), polyethylene (PE), polyamide (PA), polyethylene vinyl acetate (PEVA), polybutylene terephthalate (PBT), and polymer blends (Figure 16). All MP scanned were fibers, except for a clear PS fragment in an oyster and a clear PVC fragment in pseudofeces. Of the 30% of misidentified fibers, most were natural textile fibers (46%), including wool, cotton, and Bemberg™. The rest were cellulose (n=6) with the exception of one ramie fiber.

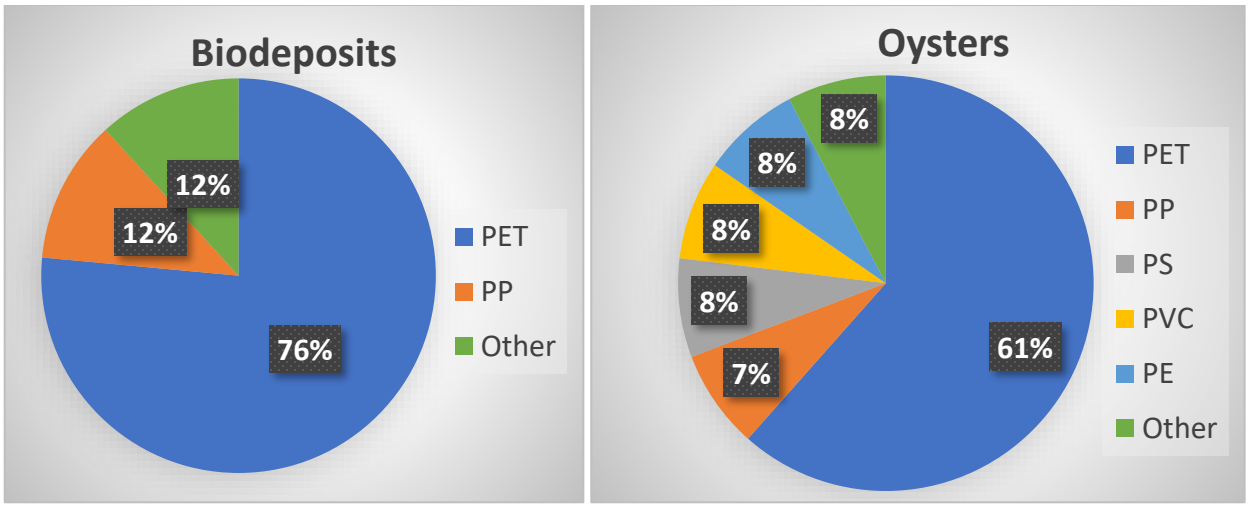


*Figure 15. Synthetic polymers in microplastics found in feces, pseudofeces, and in oyster tissues from biodeposition trials. Polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyethylene (PE), and miscellaneous polymers or poly-blends (Other).*

Aerial control blanks associated with *C. virginica* had a mean contamination rate of 0.016 MP/min and filters were exposed for a mean of 3 minutes. Mean aerial contamination rate for biodeposit samples was 0.02 MP/min and mean filter exposure time was 5 minutes. There was a 14.2% overlap (3 PET particles) between polymer types found in samples and polymers from aerial control blanks.

## Discussion

Estuarine filter-feeders like *Crassostrea virginica* are prone to ingest MP and may experience negative impacts on individual reproduction, growth, and survival (Eierman et al. 2019, Sussarellu et al. 2016). In this study an observational, *in-situ* biodeposition apparatus was

used to determine the efficiency for *C. virginica* to egest environmental MP. We found both large and small oysters (< 36 mm) are capable of egesting MP through both feces and pseudofeces, suggesting *C. virginica* are not just rejecting MP but digesting them. On average, *C. virginica* were able to egest 1 MP per 1 hour through feces, and 1 MP per 2 hours through pseudofeces. MP egestion efficiency was best predicted by *C. virginica* tissue mass, and efficiency decreased by 0.8 % for every 1-gram increase in mass. Oysters had a mean MP egestion efficiency of 62.1%, however only 32.1% were able to expel all MP ingested after 2 hours, and the larger an oyster was the lower it's MP egestion efficiency. This study helps to understand how MP accumulate in wild *C. virginica* populations.

The egestion rates observed in this study apply to MP fragments less than 4 mm, and fibers 0.5 mm to 20 mm in size. Fibers comprised 88.5% of MP egested, and 11 of these (3.3%) were macroplastics (> 5 mm). Abundance in biodeposits did not differ between feces and pseudofeces, suggesting *C. virginica* do not differentially egest MP and have an equal chance of being digested or rejected as pseudofeces. These findings contradict results of selective egestion of MP fibers <1 mm in length by *C. virginica* in laboratory studies (Ward et al. 2019). Oysters differentially egest MP based on size, shape, and particle surface properties (Teuten et al. 2007) which likely attributes to the difference in results. This was *in-situ,* observational study, so we investigated *C. virginica* egestion instead of ingestion, as MP came from the environment either before or during a trial.

This study was conducted in July when IRL water temperature (mean = 28.1 °C) and salinity (mean = 33 ppt) are ideal for optimum filtration capacity in *C. virginica* (Grizzle et al. 2008). Oysters regulate filtration rates to adapt to stress from changes in water temperature, salinity, pollution (Hutchinson and Hawkins 1992, Jones et al 2019, Lannig et al. 2006), so MP

egestion rates are expected to vary in different abiotic conditions. There are significant fluctuations in MP abundance in IRL water between seasons (Chapter 2, this document), so egestion rates may also vary based on MP abundance and availability in the water. Ambient MP abundance in IRL water near Smithsonian Marine Station (3 km distance away) where trials were conducted was 0.1 MP/L on average (Chapter 2, this document). Biodeposits excreted by oysters from the central lagoon contained less MP than biodeposits of northern and southern oysters. This variation is unexplained by the variables captured in this study. Oyster shell length or tissue mass did not vary between individuals from different regions, and all acclimated for more than 24 hours in ambient site water prior to trials. Further research is needed understand the possible interactive effects of abiotic parameters (e.g. salinity, temperature, MP abundance, water quality) on MP ingestion and egestion in *C. virginica* to elucidate this.

Overall, oysters had a mean MP egestion efficiency of 62.5%, however egestion efficiency in *C. virginica* that were of harvestable size in Florida (76+ mm, $n$= 6), was much lower at 26.1%. MP found in *C. virginica* tissue were 1.0 mm larger, on average, than MP in biodeposits suggesting oysters egest smaller MP, while larger micro- and macroplastics remain in tissues. The mouth opening of *C. virginica* is 100 to 150 µm in width but will stretch to ingest particles much larger in size (Galtsoff 1964, Peharda et al. 2012), so an upper size limit of MP that can be ingested is difficult to determine. Fibers were the dominant MP type (88.3%) found in biodeposits and *C. virginica*, which are elongated and thin. Bundles of entangled fibers were extracted from oysters from the Indian River Lagoon (Figure 4, Chapter 2, this document). It is possible that larger MP fibers (e.g., > 1 mm) can become twisted while moving through the digestive tract and get trapped in tissues, however further research is needed to confirm this.

Since *C. virginica* preferentially ingest and egest particles based on surface properties, understanding the ability oysters to egest environmental MP contributes to our comprehension of MP accumulation in this species. Here I provide evidence that *C. virginica* do not differentially egest or reject environmental MP > 1mm in an *in-situ* setting, and larger oysters, especially ones of harvestable size, are much less efficient at egesting MP. While ingestion studies typically focus on MP less than 1mm in size, or nanoplastics (Eiermann et al. 2019, Gardon et al. 2018, Gaspar et al. 2019, Green et al. 2017, Green 2016, Sussarellu et al. 2016, Ward et al. 2019), I found *C. virginica* were able to egest MP fibers up to 20 mm in size (fiber: largest dimension). The implications of these research findings are of relevance to the resource managers for *C. virginica* populations in Florida, and other United States estuaries, as it further contributes to our understanding of MP accumulation in wild populations of filter feeders.

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