2017

Investigating the Quantity and Types of Microplastics in the Organic Tissue of Oysters and Crabs in the Indian River Lagoon

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Investigating the Quantity and Types of Microplastics in the Organic Tissue of Oysters and Crabs in the Indian River Lagoon

by

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A thesis submitted in partial fulfillment of the requirements
for the Honors in the Major Program in Biology
in the College of Sciences
and in the Burnett Honors College
at the University of Central Florida
Orlando, Florida

Spring Term, 2017

Thesis Chair: Linda J. Walters, PhD

ABSTRACT

Microplastics are widespread and abundant. Few studies have examined the diversity and abundance of microplastics in wild organisms. This study determined the microplastic quantity and types in the organic tissues of the eastern oyster *Crassostrea virginica* and Atlantic mud crab *Panopeus herbstii* from the Indian River Lagoon (IRL). This study also investigated whether location affected the microplastic abundance and variety. Organisms were collected from three sites across Mosquito Lagoon in the northern IRL. Oysters were frozen after collection. Crabs were placed in containers for 5 days before freezing. The soft organic tissue was chemically digested using hydrogen peroxide, filtered, and examined for microplastics. Water samples collected from each study site had an average of 23.1 microplastic pieces per liter and fibers were the most common type. There was a significant interaction for microplastic type and site for both oysters and crabs ($p<0.001$). Crabs had an overall average of 22.7 pieces per crab. More microplastics were found in the crab tank water than in tissues. This suggested microplastics were trapped in the gills and later expelled. Oysters were found to have an overall average of 16.5 microplastic pieces per oyster. In general, microplastic fibers dominated in oyster and crab tissue. Possible sources of fibers include boat ropes, synthetic clothing, and fishing equipment. The high abundance of microplastics in water and animal tissues suggested that microplastics are widespread in the IRL. This research provides a better understanding of microplastics found in the IRL and how their abundance and diversity differ between sites.

DEDICATIONS

For my mother, thank you for supporting me and teaching me the value of education. For my family, thank you for your encouragement and confidence in me. For all the educators in my life, thank you for providing me the skills to succeed and inspiring my passion for science.

ACKNOWLEDGEMENTS

I would like to thank the CEE Lab members and Mathew Waite for field and lab assistance; Paul Sacks for assistance in the field and site map illustrations; Dr. Melinda Donnelly for help with data analysis; Julia Walton for help with laboratory equipment; Anna Forsman from the Core Genomics lab for assistance with vacuum filtration; I would like to thank UCF Department of Biology, Office of Undergraduate Research, NSF grant 1617374, and the College of Sciences for funding this project. Thank you Dr. Lisa Chambers for serving on my committee and providing me valuable feedback. A special thanks to my thesis chair Dr. Linda Walters for all her support, insight, and encouragement.

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CHAPTER 1: INTRODUCTION

Estuaries

Estuaries are semi-enclosed, highly productive coastal ecosystems (Costanza *et al.* 2014). They provide nursery grounds, breeding habitats, and essential nutrient cycling (Costanza *et al.* 2014). Many species of fishes, birds, and crustaceans rely on estuaries for habitat and food (Vermeiren *et al.* 2016). Due to their proximity to land, these environments experience temporal and spatial fluctuations in physical and chemical parameters (Kimmerer 2002). These include large fluctuations in salinity, temperature, and sediment due to inflow of freshwater from rivers and streams (Kimmerer 2002). In addition to these natural stressors, estuarine species are exposed to anthropogenic stressors (Araujo *et al.* 2017). Pollution, overfishing, and habitat loss due to coastal development are a few of those anthropogenic stressors (Seitz *et al.* 2014). Pollution can originate from agricultural and industrial activities as well as urban sewage (Araujo *et al.* 2017). Increased nutrient input from pollution can lead to eutrophication, fish kills, and harmful algal blooms (Seitz *et al.* 2014).

Oysters

Oysters are keystone species and ecosystem engineers that can be found in intertidal areas of estuaries (Drexler *et al.* 2014). They form reef structures that provide habitat for many ecologically and economically important species including decapods, fish, and other bivalves (Drexler *et al.* 2014). Oysters perform many ecologically and economically important functions including water filtration and shoreline stabilization (Drexler *et al.* 2014). Additionally, oysters are an economically important shellfish that is

harvested for human consumption. Overharvesting and harmful algal blooms events can have detrimental effects on oyster reefs (Drexler *et al.* 2014).

The eastern oyster, *Crassostrea virginica*, is a native mollusk species in Florida and along the Atlantic seaboard from Gulf of Mexico to the Canadian Maritime Provinces (Eastern Oyster Biological Review Team 2007). It is found in intertidal coastal and estuarine ecosystems. The average shell length of the eastern oyster ranges from 100 - 115 mm in two years (Buroker 1983). One of the most important ecosystem services the eastern oyster provides is water filtration (Drexler *et al.* 2014). These organisms are sessile suspension feeders that remove organic and inorganic particles from the water column at a rate of approximately 0.12 m^3 g^{-1} dry weight per day or about 50 gallons per day (Newell 1988). The removal of these particles affects water quality and nutrient cycling. Particles are first pumped through the gills of the oyster and then to the labial palps (Ehrich and Harris 2015). There, desirable particles are transferred to the mouth and digestive tract, while undesirable or excess particles are excreted as pseudofeces (Ehrich and Harris 2015). If small pieces of plastic are perceived as desirable, they are transferred to the digestive tract for consumption.

Crabs

The Atlantic mud crab, *Panopeus herbstii,* is found along the Atlantic Ocean from South America to New England (Weber and Epifano 1996). This crab species is found in intertidal or subtidal oyster reefs or salt marshes (Whitefleet-Smith and Harding 2014). It is one of the most common mud crab species in Atlantic estuaries (Weber and Epifano 1996) with an average carapace width of 3-4 cm (Kaplan 1988). Decapods,

such as *P. herbstii*, breathe by running water over their gills to absorb dissolved oxygen from the water.

The Atlantic mud crab is carnivorous and consumes mainly mollusks including oysters (Whitefleet-Smith and Harding 2014) as well as other crustaceans, annelid worms, and snails (Silliman *et al.* 2004). Fish, birds, and other larger crustaceans such as the blue crab, *Callinectes sapidus,* prey on *P. herbstii* (Grabowski 2004). The abundance of *P. herbstii* has been found to limit bivalve population abundance in some areas (Weber and Epifano 1996). Conversely, the Atlantic mud crab relies on oyster reefs for habitat and food (Silliman *et al.* 2004). Thus, Atlantic mud crab abundances can be negatively impacted by overharvesting of oysters and other shellfish (Whetstone and Eversole 1981).

Indian River Lagoon

The Indian River Lagoon system (IRL) is located on the east coast of Florida and extends for 251 km (Lapointe *et al.* 2015). It is a shallow and narrow coastal ecosystem, but one of the most species diverse estuaries in North America (Lapointe *et al.* 2015). Mosquito Lagoon and Banana River are located in the northern portion of the Indian River Lagoon system (Lapointe *et al.* 2015). Freshwater inputs for the IRL ecosystem include rainfall, surface water runoff, groundwater and sewage discharge, and inflow from canals (Lapointe *et al.* 2015). Rapid urbanization has greatly threatened the Indian River Lagoon system in the last few decades (Lapointe *et al.* 2015). This region has seen an increase in human population from a population of about 250,000 in 1960 to about 1.7 million today (Lapointe *et al.* 2015). Where it previously was dominated by

forests and natural areas, the lagoon's watershed is now dominated by urban settlement (39%) and agriculture (24%) (Lapointe *et al.* 2015).

Partially due to urbanization, the lagoon has experienced high pollution rates and eutrophication (Lapointe *et al.* 2015). This pollution has led to several harmful algal blooms in the past (Kang *et al.* 2015). These blooms caused large fish kills, marine disease, "dead zones", and biodiversity loss (Lapointe *et al.* 2015). In recent years, the Indian River Lagoon has experienced brown tide blooms (Kang *et al.* 2015). With an increase in nutrients, especially nitrogen, there has been a call for natural techniques to decrease nutrients and increase water quality. Oysters are an environmentally safe and natural way to increase water quality and assist in denitrification (Kellogg *et al.* 2013). For this reason, Dr. Linda Walters and her collaborators have made efforts to preserve and restore nature eastern oyster reefs in the Mosquito Lagoon, (Walters 2014).

Microplastics

Plastic debris in the ocean has increased drastically within the last couple decades (Avio *et al.* 2016; Beaman *et al.* 2016). Mass production of plastics began in the 1940's with about 1.7 million tons of plastic produced and has increased to about 311 million tons in 2014 (Beaman *et al.* 2016). Due to the low cost of manufacturing and its versatile uses, plastics are common (Beaman *et al.* 2016). It is estimated that about 60 to 80 percent of marine debris is plastic (Beaman *et al.* 2016). Microplastics, defined as plastic pieces < 5mm, are a growing concern as they become increasingly widespread and abundant (Li *et al.* 2015). Microplastics may originate from industrial raw materials in the form of plastic pellets called "nurdles" which are melted and used

by manufacturers to create larger plastic products (Ellison 2007). Other microplastics are created by the breakdown of larger plastic pieces through processes such as wave action and sand grinding (Barnes *et al.* 2009). The mechanical action break down of plastics is further exacerbated by photodegredation, thermal degredation, or biodegredation (Kowalski *et al.* 2016; Vermeiren *et al.* 2016). Plastics, compared to other materials, breakdown more slowly due to their chemical composition and added chemicals called “additives” (Vermeiren *et al.* 2016).

Microplastics may enter marine environments through coastal systems via rivers, wastewater and runoff (Avio *et al.,* 2016). Estuaries are sinks for pollutants where the anoxic conditions and other physical characteristics lead to the slower break down of plastics and longer residence times (Vermeiren *et al.* 2016). The three most common microplastic types are fibers, beads and fragments of irregular shape (Chubarenko *et al.* 2016). Of those types, fibers are the most common microplastic type found in estuaries and subtidal regions (Chubarenko *et al.* 2016). Because estuaries serve as habitat and nurseries for commercially important fish and provide many essential ecosystem services, plastic pollution can also affect human health and livelihoods (Vermeiren *et al.* 2016).

Most plastics contain polymer additives which can leach when traveling through marine systems and when exposed to the digestive tracts of marine organisms (Kowalski *et al.* 2016). In addition, the properties of plastics allow for adsorption of persistent organic pollutants (Wang *et al.* 2016), and concentration of toxins and heavy metals (Avio *et al.* 2016; Kowalski *et al.* 2016). Microplastics have also been found to

house biofilms which can carry harmful algal bloom species and pathogenic microbes (Keswani *et al*. 2016). The biofilms created can also serve as substrate for native and pathogenic communities (Vermeiren *et al.* 2016).

Ingestion of plastics may cause health or other serious issues in marine animals. Microplastic ingestion has been recorded in more than 180 animal species (Wang *et al.* 2016). In several species, plastics have been shown to create blockages in the digestive system, cause abrasion of organs, lead to lower feeding and growth rates, and result in reproductive failure (Vermeiren *et al.* 2016). Filter feeders are readily exposed to microplastics as they draw in water to feed (Green 2016). Some filter feeders, such as oysters, are also a food source for humans (Drexler *et al.* 2014). When fed manufactured microplastics, there was a negative effect on the benthic assemblage and species richness of European flat oysters (Green 2016). Pacific oysters exposed to polystyrene microspheres in the laboratory were found to have a decreased reproductive ability, and decreased survival and development in their larvae (Sussarellu *et al.* 2015). Although some studies found that microplastics were ingested and later egested from the digestive tract, the blue mussel (*Mytilus edulis*) absorbed microplastics into the digestive tract lining and were translocated to other tissues (Wang *et al.* 2016). Other studies have found that mussels show physiological, histological and inflammatory responses to ingestion of microplastics (Von Moos *et al.* 2012).

Shore crabs also take up microplastics via inspiration into the gills and ingestion into the gut (Watts *et al.* 2014). Some microplastics in crab gills were expelled, while others became lodged in the tissue (Watts *et al.* 2014). Oxygen consumption and ion

exchange in these crabs were negatively affected after only acute exposure to manufactured microplastics (Watts *et al.* 2016). Movement of microplastics through the food web (Vermeiren *et al.* 2016) and bioaccumulation of plastics is possible (Ma *et al.* 2016).

Although studies have begun to evaluate the effect of microplastic ingestion on organisms in a laboratory setting using manufactured microplastics, few studies have examined the types and abundance of microplastics present in wild organisms. A study by Li *et al.* (2015) examined microplastics in commercial bivalves in China using a hydrogen peroxide treatment to extract microplastics. The most abundant form of microplastic overall was fibers, however, pellets were the most abundant in one of the eight species (Li *et al.* 2015). This suggests that location and the environment may influence the kinds of microplastics ingested by oysters.

Despite this study, little is known about the abundances or types of microplastics in wild organism's tissues in the southeastern United States or the IRL. This has not been investigated in the eastern oyster, *Crassostrea virginica*, and the Atlantic mud crab, *Panopeus herbstii*, in the northern Indian River Lagoon (Mosquito Lagoon). This study aimed to determine: (1) the quantity and diversity of microplastics in water samples and the organic tissue of *C. virginica* and *P. herbstii;* (2) if location within the lagoon affected the types and amount of microplastics found in the organic tissues; and (3) which species had a higher concentration of microplastics**.**

CHAPTER 2: METHODS

Site selection

Three sites in the northern portion of the Indian River Lagoon system (Mosquito Lagoon) within the Canaveral National Seashore were used as collection sites for oysters and crabs (**Figure 1**). The Mosquito Lagoon has an average water depth of about 1 m and a salinity range of 20 to 35 ppt (Hall *et al.* 2001). Water temperatures in the winter between December and February ranged from approximately 15 to 23°C (Hall *et al.* 2001). Water temperature in the summer between June and August ranged from approximately 27 to 31°C (Hall *et al.* 2001). Temperatures in fall and spring are transitional periods and fluctuates daily by about 2-3°C (Hall *et al.* 2001). The three study sites were natural intertidal oyster reefs and were chosen to reflect distance from shore laterally across the lagoon. This was done to determine if location within the lagoon influenced the types and abundances of microplastics observed. All sites chosen were accessible only by boat. Water samples, oysters, and crabs were collected from each site.

Figure 1 Map of study sites within Mosquito Lagoon, in the northern Indian River Lagoon, Florida.

Oyster, Crab, and Water Sample Collections

Thirty individuals of *C. virginica* and thirty *P. herbstii* were haphazardly collected from each site and placed in labeled buckets. Live oysters and crabs were collected from November 2016 to January 2017. Oysters and crabs were transported to the UCF Biology Field Research Center within 5 hours of collection. Portable bubblers (Hush Bubbles^{TM}) were used to transfer alive crabs in 5 gallon buckets. Five replicate water samples from each site were collected in 1 L bottles. Using NOAA procedures (Masura *et al*. 2015), each container was rinsed three times with lagoon water before collecting the sample. Containers were filled and capped underwater. A few minutes elapsed between collections to allow for suspended particles from the previous collection to settle. Those water samples were filtered for microplastics to determine the abundance and diversity of microplastics in the water at each site. Additionally, four one-gallon

containers of lagoon water were collected and filtered to use as the tank water for the crab experiments.

Quality Control

To avoid microplastic contamination throughout the experiment, all equipment and glassware was rinsed three times with filtered de-ionized (DI) water each use. DI water was filtered through a 0.45 µm nitrocellulose membrane filter paper (47 mm) using a vacuum filtration apparatus and stored in rinsed squirt bottles. All filtration in this project used the same pore size and diameter filter paper. This is the most effective filter paper size for capturing most microplastics.

Chemical Digestion and Filtration

1) Preliminary Trials

To test the effectiveness of the hydrogen peroxide digestive technique, preliminary trials were conducted. Oyster tissue was placed in Erlenmeyer flasks with added pieces of nylon fiber and pieces of polypropylene fiber. Fibers were cut from plastic rope and ranged from 0.3 cm to 1.5 cm in length. The oyster tissue and plastic was then digested and filtered using the technique described below. Filter paper was examined for the added fibers and the percent recovery was calculated for both nylon and polypropylene fibers.

2) Oysters

Once transported to the UCF Biology Field Building, oysters were placed in labeled ziplock bags and retained in a freezer for a minimum of 24 hours. After 24 hours, oysters were thawed, measured using calipers, and shucked. The soft organic

tissue was weighed (in grams) to two decimal places using a portable balance (Scout Pro) and placed into a labeled 500mL Erlenmeyer flask. Following the chemical digestion techniques of Li *et al.* (2015) and NOAA (Masura *et al.* 2015), 30% hydrogen peroxide (H_2O_2) was added to each flask at a 40:1 ratio of 200 mL of H_2O_2 for every 5 grams of organic tissue. The solution was placed in a shaking incubator (311DS Labnet™ Environmental Shaking Incubator) for 24 hours at 65°C and 80 rpm. Then, the solution was stored at room temperature for 24-48 hours. The solution was filtered using a glass vacuum filtration apparatus (**Figure 2**), and the filter paper was examined for microplastics using dissecting microscope at 40X magnification. One filter paper was used per oyster. The type and the amount of microplastic pieces was recorded. After examination, filter papers were placed in individual petri dishes, secured with tape, and stored.

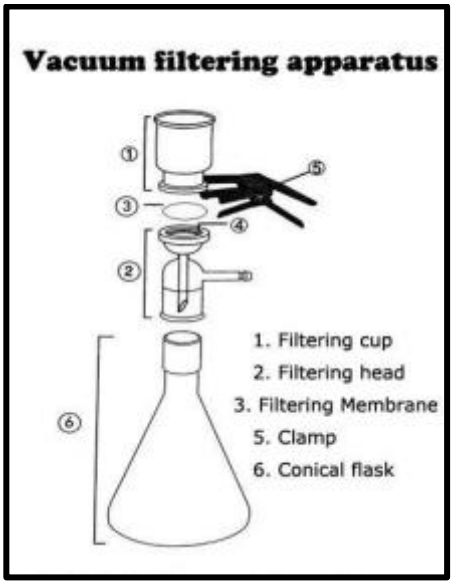

Figure 2 Vacuum filtration apparatus design with filtering cup, filtering head, filter, clamp, and Erlenmeyer flask (© AliExpress).

3) Crabs

In order to determine if crabs expel microplastics from their gills as previous studies suggest (Watts *et al.* 2014), crabs were placed in individual, covered containers (4.5 inch diameter, 1.5 inch height) upon transport to the UCF Biology Field Building. The lagoon water taken during collections was filtered before adding it to the small containers to ensure no microplastics were present. For each container, 200 mL of filtered lagoon water ("tank water") was added. Oxygen was supplied via bubblers and air-stones. Crabs were not fed in the lab. Crabs resided in the containers for 5 days and were then placed in the freezer in individual labeled ziplock bags. Tank water was filtered and examined for microplastics using methods described above for oysters and water samples. Crabs were later thawed and measured using calipers. The digestive tract and gills were dissected and weighed. The dissected organic tissue was placed in separate labeled 125 mL Erlenmeyer flasks. The crab tissue was then chemically digested, filtered, and examined using methods described above for oysters. The data collected from the filtered digested organic tissue was referred to as "tissue". The type and amount of microplastics was recorded. This allowed for comparison of microplastics between those expelled and those retained in the gills and the digestive tract.

Data Analyses

A two-way, full factorial ANOVA statistical analysis (Site x PlasticType) was used to compare the number and type of microplastics between sites for water samples. A two-way ANCOVA full factorial statistical analysis (Site x PlasticType) was used to compare the number and type of microplastics between for oysters. The two

independent variables were site and sample. Site was divided into the three collection locations. A three-way ANCOVA full factorial statistical analysis (Site x PlasticType x Origin) was used to compare the number and type of microplastics found between sites for crabs and between tank water and organic tissue. Origin refers to the filtered tank water (“tank”) which crabs resided in for 5 days before freezing or the filtered digested organic tissue of the crabs (“tissue”). Mass of organisms in grams was used as a covariate in ANCOVA analyses. Plastic type refers to one of the three types of microplastics: fiber, bead, or fragment.

CHAPTER 3: RESULTS

Water Samples

Five replicates of 1 liter water samples were collected from each site and examined for microplastics. Water samples were collected during low tide immediately below the surface of the water. The type and number of microplastics were recorded for each sample. The mean total number of microplastic pieces and types per L were determined (**Figure 3**). Site 1 had a mean of 33.9 microplastic pieces per liter, Site 2 had a mean of 15.6 microplastic pieces per liter, and Site 3 had a mean of 21.6 microplastics per liter. A two-way ANOVA (Site x PlasticType) found a significant Site:Type interaction ($p= 0.03457$; **Table 1**). Site 1 had the most microplastic pieces overall and Site 2 had the least amount of microplastic pieces. Fibers were the most common type of microplastic found in the water samples at all locations ($p= 3.34e-9$; **Table 1**). Beads were the least common types of microplastics and were not found in Site 3.

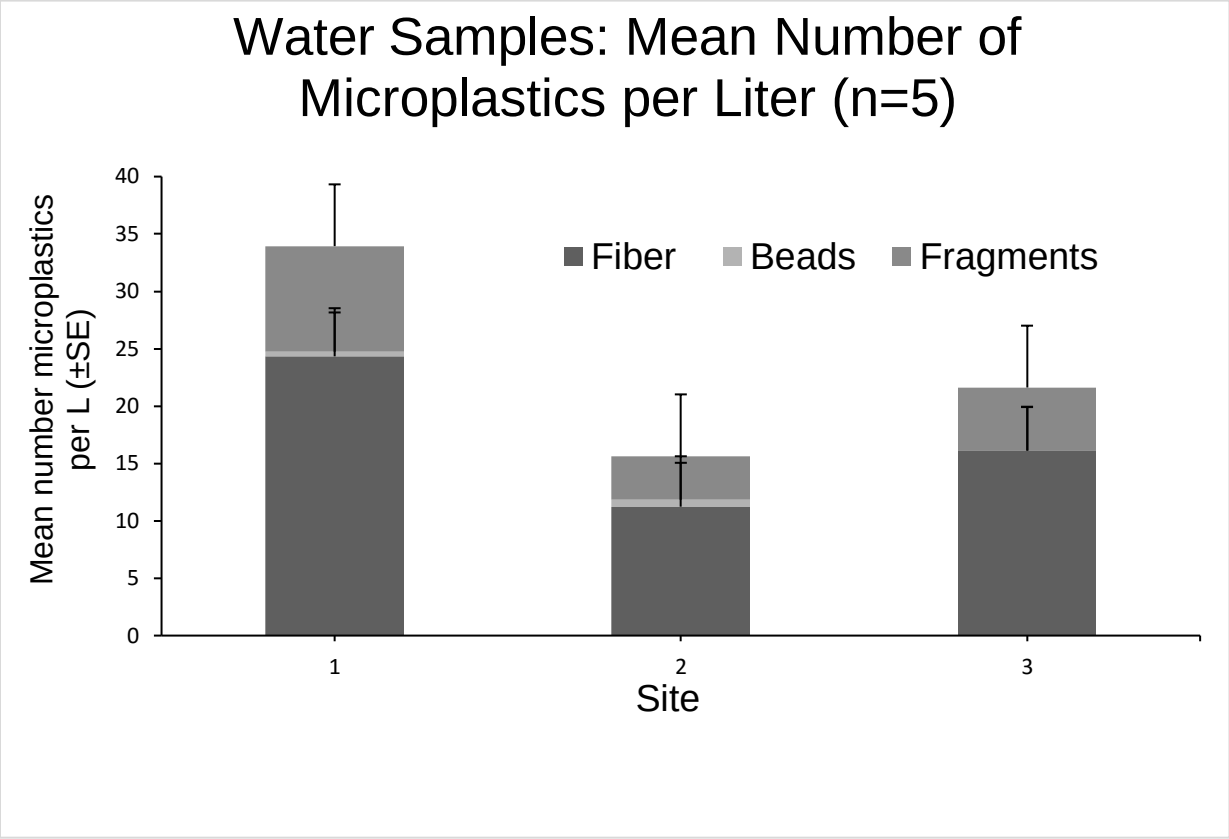

Figure 3 At each site, five 1-liter water samples were collected. The mean number ± standard error of microplastic pieces per 1 L water sample at each site was calculated.

Table 1: Two-way ANOVA results analyzing site and plastic type for water samples.

	Degrees of freedom	Mean of Squares	F value	p value
site	2	166.7	7.195	0.00235
type	2	816.2	35.230	3.34e-09
site:type	4	67.6	2.916	0.03457

Preliminary Trials

Plastic nylon and polypropylene fibers were added to oyster tissue and digested using the same techniques used for the organic tissues of oysters and crabs from all

three sites. The filter paper was examined for the added plastic fibers and recorded. There was a 90.7 percent recovery of nylon fibers and 91.8 percent recovery of polypropylene fibers. Using the data collected in these preliminary trials, a correction factor for fibers was calculated to be about 4%. In all data shown in this manuscript, a 4% correction factor was added to the fiber data only.

Oysters

The weight of soft organic tissue and mean shell length for each individual oyster for all three sites were measured. Mean shell lengths of Site 3 had the largest value while Site 1 and Site 2 had similar mean shell lengths (**Table 2**). Similarly, Site 3 had the largest value for the mean weight of soft organic tissue. For both mean organic tissue weight and shell length, Site 2 had the lowest values. Of a total of ninety *C. virginica* collected between all three sites, the mean weight of organic tissue digested was 5.2 grams and the mean shell length was 63.37 mm. Analysis of oyster data found a statistical variation in the mass of organic tissue of all oyster samples (**Table 3**).

Table 2. Mean soft organic tissue weights and mean shell lengths for oysters at each site were measured (n=30). Soft tissue refers to the organic tissue that was digested and filtered for microplastics.

Site	Mean weight of soft tissue (g)*	Mean shell length (mm)*
1	5.16 ± 1.71	58.1 ± 6.6
2	4.53 ± 1.22	58.0 ± 10.2
3	5.95 ± 2.70	73.7 ± 24.8
Total	5.21 ± 2.04	63.3 ± 17.4

*Mean ± standard deviation

After digestion of the soft organic tissue using hydrogen peroxide, the type and amount of microplastic pieces in each oyster was recorded. Mean number of microplastic pieces were calculated for each microplastic type (**Figure 4**). A two-way ANCOVA found there to be PlasticType effect where fibers were the dominant microplastic type ($p < 2e-16$; **Table 3**) at all three sites. Beads were the least common type of microplastic type. Consistent with the water samples, beads were not found at Site 3. Of those fibers, about 74% were dark blue. Of the fragments found, about 88% were clear. A site effect was also observed ($p = 3.1e-12$; **Table 3**). Site 3 had the least mean amount of microplastic pieces overall and Site 1 had the largest mean amount of microplastics per oyster. Although Site 1 had the highest mean of microplastic pieces per oyster, Site 2 had the highest mean of fibers per oyster. There was a significant

Site:PlasticType interaction (p< 2e-16; **Table 3**), meaning the abundance of microplastic piece variations cannot be explain by site or plastic type effects alone.

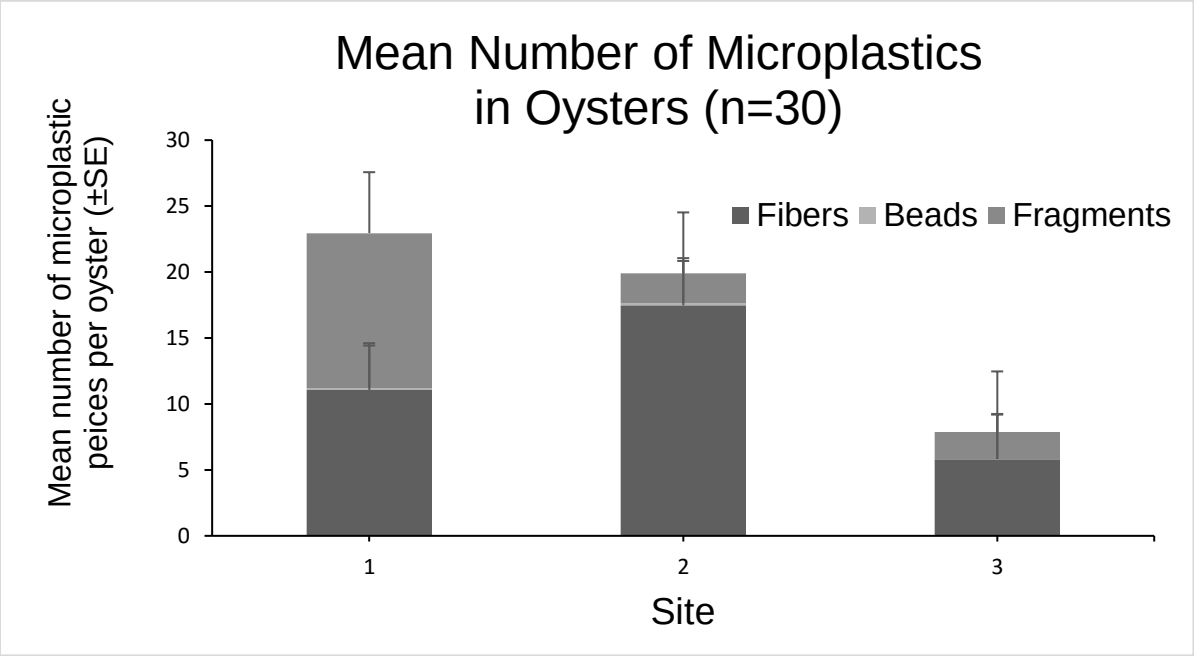

Figure 4 The mean number of microplastic pieces per oyster for each site (n=30).

Table 3: Two-way ANCOVA results analyzing site and type for oysters. Mass was used as a covariate.

	Degrees of freedom	Mean of Squares	F value	p value
mass	1	140.8	7.552	0.00641
site	2	547.9	29.394	3.1 e-12
type	2	2679.7	143.770	< 2e-16
site:type	4	628.3	33.710	< 2e-16

Crabs

The carapace width and weight of organic tissue digested was measured for each individual crab for all three sites. The mean weight in grams and carapace width in millimeters were calculated for all crabs and each site (**Table 4**). The carapace widths

on average were larger at Site 1 than the other two sites. The mean weights, however, were largest at Site 2. Analysis of crab data found a statistical variation in the mass of organic tissue of all crab samples (**Table 5**).

Table 4 Mean soft organic tissue weights and mean carapace width for crabs at each site (n=30). Soft tissue refers to the organic tissue that was digested and filtered for microplastics.

Site	Mean weight of soft tissue (g)*	Mean carapace width (mm)*
1	0.16 ± 0.12	15.7 ± 4.2
2	0.32 ± 0.49	12.1 ± 3.6
3	0.10 ± 0.18	10.9 ± 4.2
Total	0.19 ± 0.32	12.9 ± 4.5

*Mean ± standard deviation

The number and type of microplastic pieces were recorded for both the water in which crabs were contained in for five days before freezing (tank) and the digested soft organic tissue (tissue). The mean number of microplastic pieces and type were calculated for each site and for each origin type, Tank or Tissue (**Figure 5**). A plastic type effect ($p < 2e\text{-}16$; **Table 5**) was observed with fibers dominating in all three sites. The majority of fibers were a dark blue color (87%) and the majority of fragments were clear (76%). Beads were only found in crabs from Site 1 at very low concentrations. A site effect was also observed ($p < 0.000176$; **Table 5**). Sites 2 and 3 contained more microplastic pieces overall, however, more fragments were found at Site 3 than Site 2. An origin effect ($p < 2e\text{-}16$; **Table 5**) found more microplastic pieces in the Tank data than Tissue. However, there was a significant interaction effect, Site:PlasticType:Origin

(p= 8.07e-15; **Table 5**).

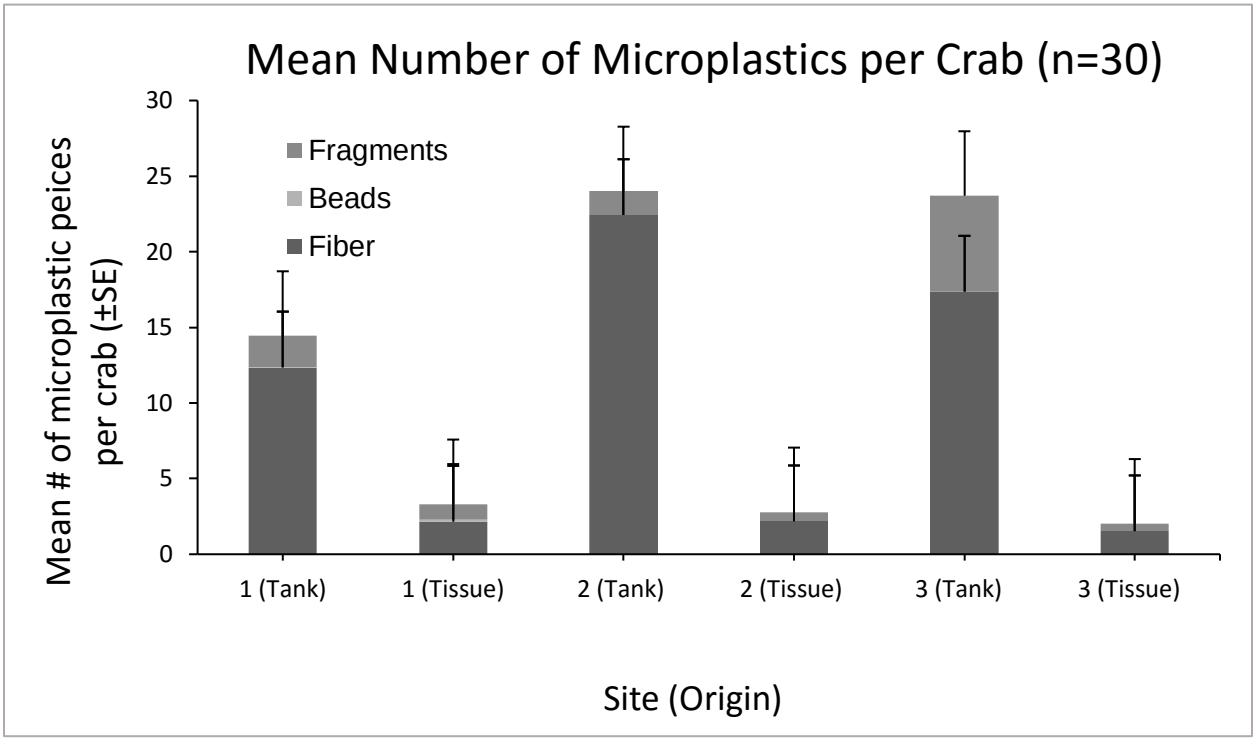

Figure 5 Mean number of microplastic pieces per crab for each site is shown above. The site number is designated by the number written before the origin type in parentheses.

Table 5: Three-way ANCOVA results analyzing the site, type and origin for crabs. Mass was used as a covariate.

	Degrees of freedom	Mean of Squares	F value	p value
mass	1	1164	149.405	< 2e-16
site	2	68	8.789	0.000176
type	2	4398	564.581	< 2e-16
origin	1	3357	430.937	< 2e-16
site:type	4	158	20.326	1.42e-15
site:origin	2	204	26.203	1.44e-11
type:origin	3	2925	375.584	< 2e-16
site:type:origin	4	150	19.300	8.07e-15

A Comparison of Species

The total number and types of microplastic pieces for oysters and crabs were calculated (**Table 6**). Overall, there were higher abundances of microplastic pieces in *P. herbstii* samples than in *C. virginica*. The types of microplastics found in each species varied. Fibers were more common in crab samples, but beads and fragments were more common in oyster samples when compared to crab samples. The mean microplastic pieces per oyster and per crab were calculated (**Figure 6**). Data from all three sites for oysters were combined and averages for each microplastic type were calculated (n_1=90). Data from all three sites for crabs were also combined and averages for each microplastic type was calculated (n_2=90). Crabs had a larger mean number of microplastic pieces per crab than mean number of microplastic pieces per oyster. Although crabs had a higher mean of fibers per crab, oysters had a higher mean number of fragments per oyster than crabs. Beads were uncommon in both species.

Table 6 A summary of total microplastics in all *C. virginica* (n1=90) and *P. herbstii* (n2=90) collected.

	C. virginica	*P. herbstii*
Fibers	991	1,672
Beads	9	2
Fragments	482	305
Total	1,482	1,979

The number of microplastic pieces per gram was calculated for every individual crab or oyster at every site. The average of those values for crabs and oysters were calculated (**Table 7**). Due to the small mass of the organic tissue of crabs, the total mean number of microplastic pieces per gram of soft organic tissue for *P. herbstii* was about 350 times

higher than *C. virginca*. The microplastic pieces in the tank water reflect the plastics in the crab's gills and in turn those plastics found in the water column. To isolate what the crabs may be ingesting from their prey, the mean number of microplastics pieces per gram was also calculated with only the microplastics found in the crab's organic tissue (**Table 7**). This value was lower than when the total amount of microplastics for each crab was used, however, the value was still about 77 times greater than that of oysters.

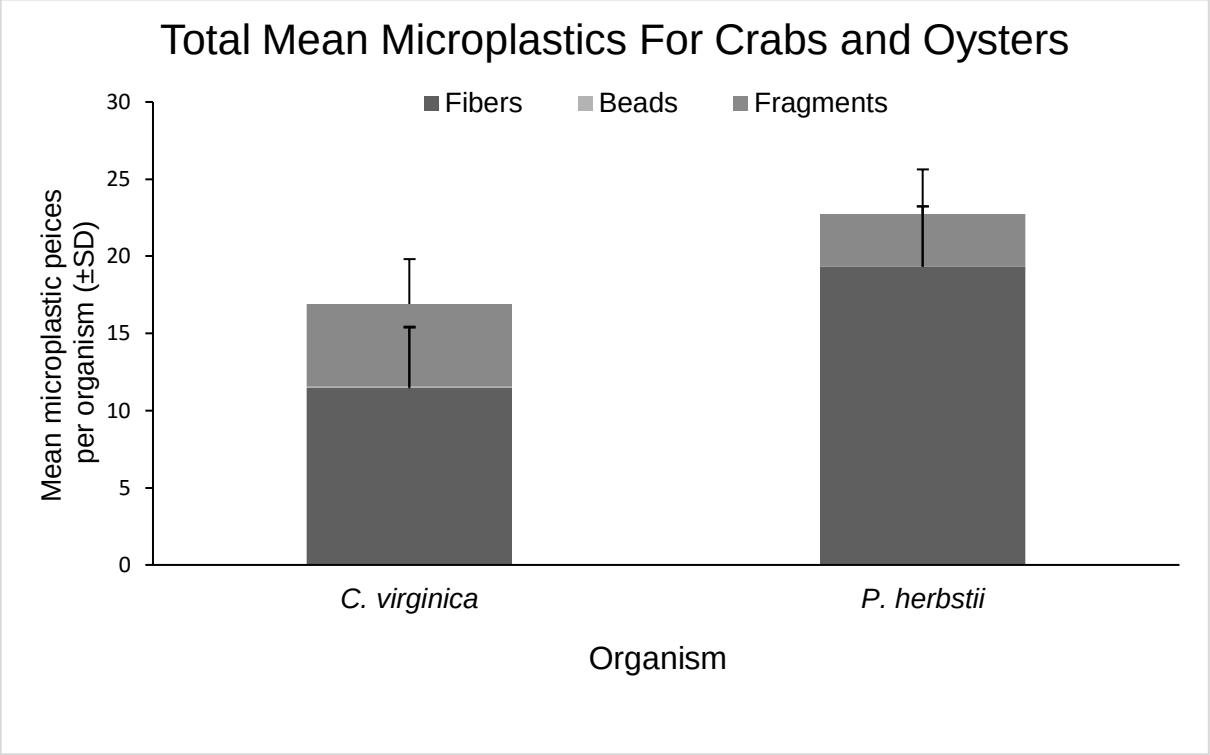

Figure 6 Mean number of microplastic pieces per crab and per oyster for all three sites combined.

Table 7 A summary of microplastic in all *C. virginica* (n1=90) and *P. herbstii* (n2=90) collected per gram of organic tissue.

	Total mean number of microplastic pieces per gram of organic tissue
C. virginica	3.84 ± 3.39
P. herbstii (Total)*	1,361.61 ± 4,928.13
P. herbstii (Tissue only)	297.74 ± 1,178.75

*Both Tissue and Tank are including in this calculation

CHAPTER 4: DISCUSSION

Microplastics are ubiquitous and are found in a variety of environments from estuaries to the deep sea (Andrady 2011). Estuaries are particularly vulnerable to microplastics due to the proximity of anthropogenic sources and urbanization (Andrady 2011). This proximity leads to increased inflow of runoff, sewage, and other sources of pollution (Andrady 2011). There is a dearth in knowledge of the quantity and diversity of microplastics in the Indian River Lagoon system. This study investigated the microplastics found in the northern Indian River Lagoon in Mosquito Lagoon.

Water samples collected from each location had means that ranged from 15 to 33 microplastic pieces per liter (**Figure 3**). There was a significant Site:PlasticType interaction. This implies that site and plastic type both influence the amount of microplastic pieces present in the water column at the different locations; one factor alone cannot explain the variance in amount of microplastics. The amount of microplastic pieces per liter were relatively high compared to other studies in global estuaries. A Chinese estuary had between 5 and 13 microplastic pieces per liter in water samples collected (Zhao *et al.* 2014). Surface water samples in the northeastern Pacific Ocean and coastal British Columbia found mean concentrations of microplastic particles to vary from .008 to 9.18 particles per liter (Desforges *et al.* 2014). Similarly, water samples found in the marine waters of Qatar's Exclusive Economic Zone contained a mean of 0 to 0.003 pieces per liter (Castillo *et al.* 2016).

Elevated concentrations of microplastics in Mosquito Lagoon may be due to higher pollution inputs or higher retention times. The Indian River Lagoon and the

Mosquito Lagoon have seen increases in pollution over the last few decades (Kang *et al.* 2015). Originally, freshwater flowed into the lagoon via slow and meandering streams and rivers (Lapointe *et al.* 2015). Now, land in the watershed has shifted to canals, drainage for agriculture and mosquito control, and more (Lapointe *et al.* 2015). Additionally, there has been a dramatic increase in human settlement along the lagoon's shore (Browne 2011). With an increase in urbanization, the lagoon has experienced an increase in pollution from nonpoint sources such as septic tanks and wastewater drainage (Browne 2011).

In addition to higher amounts of pollution, it is also possible that water flow in the Indian River Lagoon impacts the high concentrations of microplastics found. The Mosquito Lagoon is an enclosed and poorly drained estuary (Lapointe *et al.* 2015). Therefore, flushing of suspended and dissolved particles is essential in water quality (Smith 1993); however, tidal flushing is negligible in the northern basin of the Indian River Lagoon including Mosquito Lagoon (Smith 1993). Mosquito Lagoon is instead impacted more by nontidal flushing mechanisms including local wind forcing and rainfall or extreme weather events, but are less frequent than tidal flushing (Smith 1993). Therefore, it is estimated a 50% renewal of water takes between 200 and 300 days in the northern basin of the Indian River Lagoon (Mosquito Lagoon), compared to about a week in the southern lagoon or one tidal cycle in inlets near the Indian River Lagoon estuaries (Smith 2016). This means that microplastics which enter the lagoon will reside in the lagoon for long periods and allow for the accumulation of plastics.

Of the three types of microplastics found in the water samples (fibers, fragments, and beads), fibers were the most common type of microplastic. This is consistent with other estuarine studies where fibers were also the most common type of microplastic (Chubarenko *et al.* 2016). Possible sources of the fibers include boats ropes, synthetic clothing, and fishing equipment (Andrady 2011; Beaman *et al.* 2016). Clothing fibers usually originate from wastewater and septic tank drainage where laundry water is discharged (Browne 2011). Fragments may originate from the degradation of macroplastic pollution and beads, although uncommon, originate from personal care products such as body wash (Andrady 2011; Vermeiren *et al.* 2016).

Preliminary trials were conducted to determine the recovery rates of microplastics using the hydrogen peroxide treatment and under laboratory conditions. Nylon and polypropylene fibers, two of the most common types of microplastics in the environment, were used to test the digestion process of the experimental design for this study (Chubarenko *et al.* 2016). Preliminary trials determined there to be a high percent recovery for added polypropylene and nylon fibers. This digestive technique is used by NOAA (Masura *et al*. 2015) and is currently the most effective method for extraction of microplastics from the organic tissue of wild organisms (Avio et al. 2015). Although some plastics may be lost in the digestion, experiments using this technique can only underestimate microplastic abundance (Avio *et al.* 2015).

Similar to water sample data, the mean microplastic pieces found in the oysters were higher than the amount of microplastic pieces found per oyster in previous studies (**Figure 4**). For example, *Crassostrea gigas* bought from a French supermarket had an

average of about 2 microplastic pieces per oyster (von Cauwenberghe and Janssen 2014). The average number of microplastic pieces in a commercial clam (*Scapharca subcrenata*) from China similar in size as *C. virginica* had 13 pieces per clam (Li *et al.* 2015). However, only a few studies have looked at the abundance in wild organisms. Wild mussels, *Mytilus edilus*, along the China coastline had from 2 to 8 pieces of microplastics per mussel (Li *et al.* 2016). It was also found that wild mussels had more microplastic pieces than farmed mussels (Li *et al.* 2016). Thus, oysters in the natural areas may see higher amount of microplastics depending on their location or other factors. In addition, higher concentrations in this study may result from pollution and higher retention rates in the Indian River Lagoon system as mentioned before for water samples.

There was a significant interaction for Site:PlasticType for the microplastics in oysters. This indicated that both site and plastic type influenced the variation in number of microplastics. Microplastic abundance differences in the lagoon could result from several factors including wind driven transportation, proximity to pollution inputs or anthropogenic activities like marinas. Additionally, the types of microplastics may be influenced by buoyancy of the plastic type and shape. Less dense microplastics can be found higher in the water column while more dense plastics sink and reside in the soil (Chubarenko *et al.* 2016). Plastics made out of polypropylene and polyethylene are less dense and are commonly found at the surface while plastics types like polystyrene and polyvinyl chloride are more dense and found more commonly in the sediment (Chubarenko *et al.* 2016). Shape of plastics also influences buoyancy; fibers and

fragments with greater surface area are more frequently found in higher in the water column (Chubarenko *et al.* 2016). Fiber were the most common type of microplastics in oysters and beads were the least common. Of those fibers, about 74% were dark blue. This color is consistent with fibers from nylon and polypropylene boat ropes and clothing fibers (Chubarenko *et al.* 2016). Of the fragments found, about 88% were clear. These fragments may originate from secondary plastics degraded from items such as water bottles, packaging, and other types of containers (Zhao *et al.* 2014). Oysters may ingest high amounts of fibers and less dense plastic types due to the buoyancy differences. This could also apply to the type of microplastics found in crabs because crabs are exposed to particles in the water column through their gills as well as those in the sediments of their habitat.

A significant interaction between Site:PlasticType:Origin for crabs indicated that the abundance of microplastics was dependent on all three factors; one factor alone cannot explain the variation. Unsurprisingly, fibers were the most abundant type of microplastic overall in crabs as well with about 87% of fibers being a dark blue color. Crab data revealed an origin effect where the majority of the microplastic pieces recorded were found in the tank water. This suggested that most microplastics in the crabs originated from the gills, but were later expelled. Crabs pump water over their gills for oxygen consumption and plastics can become lodged (Watts *et al.* 2014). Previous lab experiments found that microplastics lodged in the gills were released within 14 (Watts *et al.* 2014) to 21 days (Farrell and Nelson 2013). Release of microplastics may result from a behavior found in decapods called gill grooming (Bauer 1989). Crabs live

in aquatic environments where they are exposed to many microbial fouling organisms (Bauer 1989). Thus, grooming behavior is hypothesized to counteract colonization of microbes in their gills (Bauer 1989). Crabs may also use this behavior to expel microplastics from their gills and may explain the higher concentration of microplastics in the tank water compared to the tissue. Plastics lodged in gills can have negative effects on the crabs including decreasing the crab's ability to respire and osmoregulate (Watts *et al.* 2016). In addition, there is evidence that some microplastics ingested into the digestive system may translocate into the hemolymph and other tissues of crabs (Farrell and Nelson 2013). Thus, it is important for the crabs to expel the microplastic pieces.

Overall, *P. herbstii* had higher mean concentrations of microplastics per individual than *C. virginica*. Most of those microplastics were from tank water and thus, were expelled from the gills. Although oysters filter larger of volumes of water, oysters may expel the microplastics as pseudofeces faster than crabs expel plastics from their gills. It is unknown how long it takes oysters to expel microplastics. Crabs expel microplastics from their gills and digestive tract for up to 21 days in laboratory setting (Farrell and Nelson 2013). Oysters had higher abundances of beads and fragments while crabs had higher abundances of fibers (**Table 6; Figure 6**). It is possible that fibers may become lodged in the crab gills more easily than other types. Crabs are also vulnerable to microplastics in sediment. Sediments have been found to have higher concentrations of microplastics than water samples (Wessel *et al.* 2016). Thus, *P. herbstii* may be exposed to higher concentrations of microplastic pieces than oysters.

Furthermore, the data suggests that microplastics may bioaccumulate in higher trophic levels. Overall, crabs had a much higher total mean microplastic pieces per gram of soft tissue than oysters (**Table 7**). Although few studies have looked at bioaccumulation of microplastics, the potential for microplastic transfer between trophic levels was examined and found to occur in plankton (Setala *et al.* 2014). Another study found an increase in microplastic concentration in lab experiments from the mussel *Mytilus edulis* to a shore crab *Carcinus maenas* (Farrell and Nelson 2013). It is also possible for microplastics to accumulate in top predators like fishes, birds, mammals, and humans (Farrell and Nelson 2013). Bioaccumulation of microplastics may lead to a biomagnification of the toxins, metals, and additives that are associated with microplastics (Ma *et al.* 2016).

Overall, the concentration of microplastics in the organic tissue of oysters and crabs in this study were higher than the previous few studies of other shellfish and crabs (Li et al. 2015; Li et al. 2016; von Cauwenberghe and Janssen 2014). This may be due to the higher pollution and retention rates in Mosquito Lagoon (Lapointe *et al.* 2015; Smith 1993; Smith 2016). Crabs were found to have higher overall concentrations of microplastic pieces than oysters, but the majority were expelled. Fibers were the most common type of microplastic and may originate from laundry water or from recreational activities such as boating ropes, fishing, and clothing (Chubarenko *et al.* 2016). Microplastics can have physical effects such as blockage in the digestive tract or false satiation (Farrell and Nelson 2013) and may accumulate toxins, metals, or additives (Beaman *et al.* 2016). This can lead to biomagnification of toxic chemicals up trophic

levels (Farrell and Nelson 2013). Understanding the types and abundance of microplastics ingested by organisms is essential to then test the effects of the ingestion of microplastics.

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