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# Quantity and types of microplastics in the organic tissues of the eastern oyster *Crassostrea virginica* and Atlantic mud crab *Panopeus herbstii* from a Florida estuary

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ABSTRACT

This study determined the quantity and diversity of microplastics in water and soft tissues of eastern oysters (*Crassostrea virginica*) and Atlantic mud crabs (*Panopeus herbstii*) in Mosquito Lagoon, a shallow, microtidal estuary along the east coast of central Florida. One-liter water samples had an average of 23.1 microplastic pieces (n = 15). Crabs (n = 90) had an average of 4.2 pieces in tissues/individual plus an average of 20.3 pieces/individual temporarily entangled in exposed surfaces and released within 5 days in tanks. Adult oysters (n = 90) had an average of 16.5 microplastic pieces/individual. Fibers, mostly royal/dark blue in color, dominated our collections. When compared per gram of tissue, crabs had two orders of magnitude more microplastic pieces than oysters. Our numbers were higher than previous studies on invertebrate microplastics; this is potentially the result of extensive urbanization, limited flushing, and intensive recreational usage of Mosquito Lagoon.

## 1. Introduction

Plastic debris in our oceans has increased in recent decades from approximately 0.5 million tons a year in the 1960s to 30 million tons a year in 2013 (Avio et al., 2016; Beaman et al., 2016). It is estimated that 60 to 80% of marine debris is plastic (Beaman et al., 2016). Microplastics, defined as plastic pieces < 5 mm, are a growing concern as they become increasingly widespread and abundant (Li et al., 2015). Microplastics can 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). They may also originate from larger pieces of plastics mechanically broken down through wave action, sand grinding, and other processes (Barnes et al., 2009). The mechanical action break-down of plastics is further exacerbated by photodegradation, thermal degradation, and biodegradation (Kowalski et al., 2016; Vermeiren et al., 2016). The three most common types of microplastics are fibers, beads, and fragments of irregular shape (Chubarenko et al., 2016). Fibers are the most common microplastic type found in estuaries and subtidal regions (Chubarenko et al., 2016).

Microplastic ingestion has been recorded in > 180 animal species (Wang et al., 2016), with filter-feeding bivalves and crabs being especially vulnerable (Green, 2016). Ingestion of microplastics in bivalves in the laboratory has been shown to negatively affect species richness (Green, 2016), as well as reproductive ability, survival, and larval development (Sussarellu et al., 2015). Microplastics have been found to be absorbed into the digestive tract lining and translocated to other tissues in the mussel *Mytilus edulis* (Wang et al., 2016). Additional studies have found that mussels had significant physiological, histological, and inflammatory responses resulting from ingestion of microplastics (von Moos et al., 2012). The shore crab *Carcinus maenas* took 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). Blockage to the digestive track and false satiation is possible with microplastic ingestion (Farrell and Nelson, 2013). Movement of microplastics through the food web (Vermeiren et al., 2016) and bioaccumulation of plastics is likely (Ma et al., 2016). Additionally, plastics contain polymer additives which may leach when in marine systems or exposed to the digestive tracts of marine organisms (Kowalski et al., 2016). The properties of plastics also 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). These plastics have been found to include biofilms which can carry harmful algal bloom species and pathogenic microbes (Keswani et al., 2016; Vermeiren et al., 2016).

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**Fig. 1.** Study site within the Mosquito Lagoon, in the northern Indian River Lagoon system, Florida.

In addition to studies evaluating the effect of microplastic ingestion on organisms in a laboratory setting using manufactured microplastics (e.g. Green, 2016; Sussarellu et al., 2015; Wang et al., 2016), multiple studies have examined the types and abundance of microplastics present in field-collected individuals. A study on the crustacean, *Nephrops norvegicus*, in the Clyde Sea found balls of plastic in their stomachs (Murray and Cowie, 2011). There have also been several studies on marine bivalves. A study revealed that *Mytilus edulis* and *Crassostrea gigas* in the German North Sea had on average 0.36 and 0.47 microplastics per gram, respectively (van Cauwenberghe and Janssen, 2014). Similarly, Li et al. (2015) found microplastic fibers to be abundant in several species of commercial bivalves in China. Both wild and farmed *M. edulis* in Nova Scotia, Canada ingested between 116 and 178 microfiber pieces per individual (Mathalon and Hill, 2014). Other studies have discovered microplastics in fishes and marine mammals (e.g. Lusher et al., 2013; Eriksson and Burton, 2003).

Previous research suggests oysters and crabs may be at high risk for accumulation of microplastics. Our study expands current knowledge of species effects of microplastics by evaluating organic tissue concentrations of *Crassostrea virginica* (eastern oyster) and *Panopeus herbstii* (Atlantic mud crab), two species playing important roles in Florida's estuaries. Oysters are a keystone species and an ecosystem engineer found in intertidal and subtidal areas of estuaries (e.g. Drexler et al., 2014). Oysters form reef structures that provide habitat for many ecologically and economically important species of decapods, fishes, and bivalves (e.g. Barber et al., 2010; Boudreaux et al., 2006). Oysters, additionally, are economically important shellfish that are harvested for human consumption (Drexler et al., 2014). The eastern oyster, *Crassostrea virginica*, is native to Atlantic seaboard and the average adult shell length of *C. virginica* oyster ranges from 100 to 115 mm (Buroker, 1983). Oysters perform many important functions including water filtration and shoreline stabilization (Drexler et al., 2014; Manis et al., 2014). *Crassostrea virginica* filters 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 gal per day (Newell, 1998).

The Atlantic mud crab, *Panopeus herbstii*, is found along the Atlantic Ocean from South America to New England (Weber and Epifanio, 1996) on intertidal and 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 Epifanio, 1996) with an average carapace width of 3–4 cm (Kaplan, 1988). Decapods, such as *P. herbstii*, actively move water over their gills to absorb dissolved oxygen. *Panopeus herbstii* is carnivorous and primarily consumes 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).

This study aimed to determine: (1) the quantity and diversity of microplastics in water samples and the organic tissues of *C. virginica* and *P. herbstii* in the Mosquito Lagoon; and (2) if location within the estuary affected the types and amounts of microplastics.

## 2. Materials and methods

### 2.1. Study location and collection

This study was conducted in the Indian River Lagoon system (IRL). The IRL is a shallow, narrow estuarine ecosystem located on the east coast of central Florida. It extends for 251 km (Lapointe et al., 2015) with an average water depth of 1 m and a salinity range of 20 to 35 ppt (Hall et al., 2001). Annual water temperatures range from a low of 15 °C to a high of 31 °C (Hall et al., 2001). Freshwater enters the IRL through rainfall, surface water runoff, groundwater and sewage discharge, and inflow from canals (Lapointe et al., 2015). A major threat to the IRL in last few decades has been rapid urbanization (Lapointe et al., 2015), with an increase in human population from about 250,000 people in 1960 to approximately 1.7 million in 2015 (Lapointe et al., 2015). Due to combined effects of urbanization and habitat loss, this lagoon system has experienced high pollution rates and eutrophication (Lapointe et al., 2015). This pollution has led to numerous harmful algal blooms (e.g. Kang et al., 2015; Gobler et al., 2013) which have caused large fish kills, disease outbreaks, and biodiversity loss (Lapointe et al., 2015).

Water samples, oysters, and crabs were collected from three natural intertidal, patch oyster reefs in the northern reaches of the IRL in Mosquito Lagoon (28.8361°N, 80.7990°W; Fig. 1). All collections occurred within the boundaries of Canaveral National Seashore. Within the park, there are 524 reefs of *C. virginica* (Garvis et al., 2015). While recent hurricanes and diseases have had minimal impact on these reefs (Walters et al., 2007), since 1943, 40% of oyster acreage was lost due to anthropogenic impacts (Grizzle et al., 2002; Garvis et al., 2015). Restoration has rapidly improved the functioning of damaged reefs (Chambers et al., 2017). Site 1 was 0.4 km from the eastern boundary of the Mosquito Lagoon, Site 2 was 1.1 km northwest of Site 1, and Site 3 was 2.1 km southwest of Site 2 (Fig. 1).

Five replicate water samples from each site were collected in 1-L plastic bottles using NOAA procedures (Masura et al., 2015). Water samples were collected in 0.5-m depth water, approximately 1 m

seaward of each reef at a depth of 10 cm below the water's surface. Thirty live adult *C. virginica* and thirty *P. herbstii* were haphazardly collected from each reef and separately placed in labeled high density polyethylene (HDPE) plastic buckets, which were rinsed repeatedly prior to use. Collections occurred between November 2016 and January 2017. Samples were transported to the University of Central Florida Biology Field Research Building in Orlando, Florida within 5 h of collection in buckets using portable bubblers (Hush Bubbles™).

### 2.2. Organism-specific details

Whole, intact oysters were placed low-density polyethylene zip lock bags and frozen for a minimum of 24 h before processing. No contamination from the bags was expected as the oyster's soft tissues never contacted the bags. For processing, individuals were thawed, shell lengths measured using Vernier calipers, and shucked. All soft tissue was weighed (g) using a portable balance (Scout Pro) and placed into a labeled 500 mL Erlenmeyer flask. The soft tissue was digested using the technique described below.

*Panopeus herbstii* were placed in clean, individual, covered containers (11.4 cm diameter, 3.8 cm height) with 200 mL of filtered lagoon water ("tank water") to determine if any entangled microplastics were released as in previous studies with crabs (Watts et al., 2014). Oxygen was supplied via bubblers and air-stones. *P. herbstii* were not fed during these 5-day containments. Afterwards, crabs were placed in the freezer in individually-labeled bags for at least 24 h. Again, crabs were placed in the plastic bags only during freezing and no plastic contamination from bags was expected as bags never contacted soft tissues used in the analyses. Tank water was filtered and examined for microplastics as described above.

For microplastic processing of crab tissues, *P. herbstii* were thawed and carapace widths measured using calipers. The digestive tract and gills were removed, weighed, and chemically digested as described below. Microplastics collected from the filtered digested organic tissue of *P. herbstii* were referred to as "tissue".

### 2.3. Chemical digestion and filtration

To avoid microplastic contamination throughout the experiment, all equipment and glassware was rinsed three times with filtered, de-ionized (DI) water. DI water was first filtered through a 0.45 μm nitrocellulose membrane filter paper using vacuum filtration. All filtration in this project used the same pore size (Masura et al., 2015).

Following the chemical digestion techniques of Li et al. (2015) and NOAA (Masura et al., 2015), each individual organism was dissected and placed in separate Erlenmeyer flasks. Then, 30% hydrogen peroxide ($H_2O_2$) was added to each flask at a 40:1 ratio with 200 mL of $H_2O_2$ for every 5 g of organic tissue. The solution was placed in a shaking incubator (311DS Labnet™ Environmental Shaking Incubator) for 24 h at 65 °C and 80 rpm. The solution was next maintained at room temperature 25 °C for 24–48 h, followed by vacuum filtration. Post-filtration, filters were examined for microplastics abundance and diversity using a dissecting microscope at 40× magnification. At this magnification, microplastic pieces as small as 0.001 mm in size were detected and recorded. The colors of microplastic pieces were recorded on a subset of oyster and crab samples (n = 20).

To test the effectiveness of the hydrogen peroxide digestive technique under our laboratory conditions, preliminary trials were conducted. Oyster tissue (n = 10) was placed in Erlenmeyer flasks with known numbers and size fragments of royal blue nylon and bright yellow polypropylene fibers. Fibers were cut from purchased rope and ranged from 0.3 cm to 1.5 cm in length. The oyster tissue and plastics were then digested and filtered using the technique described above. Filter paper was examined for the added fibers and the percent recovery was calculated for both nylon and polypropylene fibers.

### 2.4. Data analyses

Parametric statistical analyses were used throughout as all statistical assumptions of these tests were met. One-way ANOVAs were used to separately compare oyster shell lengths and crab carapace widths among sites. For analysis purposes, plastic type refers to the three common types of microplastics: fiber, bead, or fragment. A two-way, full factorial ANOVA statistical analysis (Site × Plastic Type) was used to compare the number and type of microplastics between sites for water samples. A two-way ANCOVA full factorial analysis (Site × Plastic Type) with mass as the covariate was used to compare the number and type of microplastics for oysters. A three-way ANCOVA full factorial analysis (Site × Plastic Type × Origin) with mass as the covariate 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 source of the microplastics and was either filtered tank water ("tank") which crabs resided in for 5 days before freezing or the filtered digested organic tissue of the crabs ("tissue"). All statistical analyses were run using JMP 13.1 statistical software (JMP, Version 13.1, 2017).

## 3. Results

### 3.1. Preliminary trials

Preliminary trials with oyster tissue found a 91% recovery of nylon fibers and 92% recovery of polypropylene fibers. As this recovery rate was high, we were confident with our methodology.

### 3.2. Water samples

The mean number and types of microplastics per L is presented in Fig. 2A. There was a significant Site × Type interaction ($p = 0.0345$, Fig. 2A). Site 1 had a mean ( ± S.D.) of 33.9 ± 11.6 microplastic pieces per liter, Site 2 had a mean of 15.6 ± 8.4 microplastic pieces per liter, and Site 3 had a mean of 21.6 ± 11.8 microplastic pieces per liter. Site 1 had the most microplastic pieces overall while Site 2 had the least. Fibers were the most common type of microplastic found in the water at all locations and beads the least common (Fig. 2A). More beads were found at Site 2 than other sites; no beads were found at Site 3.

### 3.3. Oysters

For the ninety *C. virginica* collected, the mean ( ± S.D.) shell length was 63.3 ± 17.4 mm and mean weight of organic tissue digested was 5.2 ± 2.0 g (Table 2). Mean shell lengths of *C. virginica* for Site 3 had the largest value of 73.7 ± 24.8 mm, while Sites 1 and 2 had similar mean shell lengths (ANOVA: $p = 0.0002$; Table 1A). Similarly, Site 3 had the largest value of 6.0 ± 2.7 g for the mean weight of soft organic tissue (ANOVA: $p = 0.0235$; Table 1A).

The mean number of each type of microplastic per oyster is presented in Fig. 2B. There was a significant Site X Type interaction (ANCOVA: $p < 0.0001$). Site 1 had more total mean microplastic pieces and relatively more fragments than Sites 2 and 3. Site 1 had a mean ( ± S.D.) of 23.5 ± 6.7 microplastic pieces per oyster, while Sites 2 and 3 had a mean of 19.2 ± 9.3 and 7.6 ± 4.2 microplastic pieces per oyster, respectively. Site 3 had less than half the number of total fragments than Site 1. Consistent with the water samples, beads were not found at Site 3. For all oysters, 88% of the fragments were clear in color and 74% of the fibers were royal or dark blue in color (Fig. 3).

### 3.4. Crabs

The carapace widths of *P. herbstii* were all significantly different between sites (ANOVA: $p < 0.0001$), with the largest crabs collected at

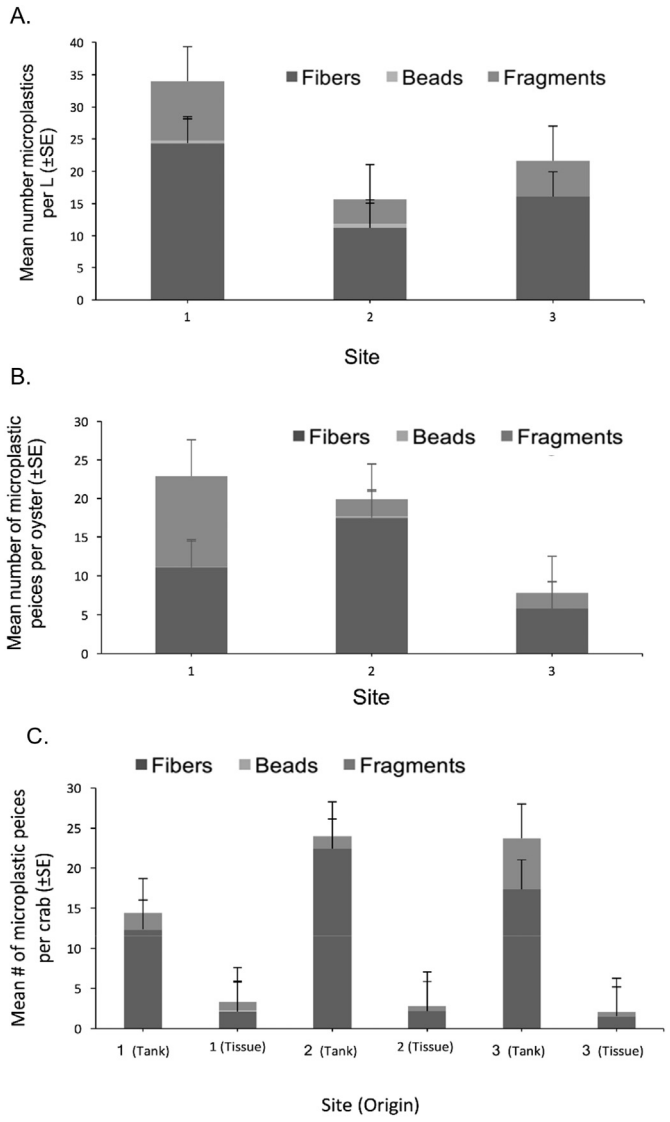


**Fig. 2.** For each site in Mosquito Lagoon and for each type of microplastic (fibers, beads, fragments), (A) the mean number ( ± standard error) of microplastic pieces per 1 L water sample at each site (n = 5); (B) mean number ( ± standard error) of microplastic pieces per oyster for each site (n = 30); (C) mean number ( ± standard error) of microplastic pieces per crab for each site. Origin refers to "tank" or "tissue". Tank refers to the microplastics collected from the tank water that the crabs were held in for 5 days, while tissue refers to microplastic pieces collected from the digested organic material.

**Table 1**
Mean ( ± standard deviation) of soft organic tissue weights in grams and mean shell length/carapace width in mm for (A) oyster and (B) crab, respectively, at each site (n = 30/site). Soft tissue refers to the organic tissue that was digested and filtered for microplastics.

| A. *Crassostrea virginica* | | |
|---|---|---|
| Site | Mean weight of soft tissue (g) | Mean shell length (mm) |
| 1 | 5.2 ± 1.7 | 58.1 ± 6.6 |
| 2 | 4.5 ± 1.2 | 58.0 ± 10.2 |
| 3 | 6.0 ± 2.7 | 73.7 ± 24.8 |
| Overall mean | 5.2 ± 2.0 | 63.3 ± 17.4 |
| B. *Panopeus herbstii* | | |
| Site | Mean weight of soft tissue (g) | Mean carapace width (mm) |
| 1 | 0.2 ± 0.1 | 15.7 ± 4.2 |
| 2 | 0.3 ± 0.5 | 12.1 ± 3.6 |
| 3 | 0.1 ± 0.2 | 10.9 ± 4.2 |
| Overall mean | 0.2 ± 0.3 | 12.9 ± 4.5 |

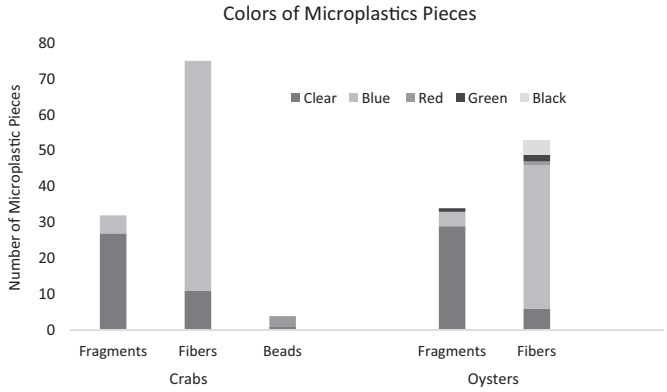


**Fig. 3.** Microplastic colors were recorded on a subset of oysters (n = 10) and crabs (n = 10). The number of fibers, beads, and fragments are shown for both crabs and oysters.

Site 1 with a mean ( ± S.D.) carapace length of 15.7 ± 4.2 mm. Crabs from Site 2, however, had a significantly larger mean mass (12.1 ± 3.6 mm) than crabs from Site 3 (10.9 ± 4.2 mm), with Site 1 crabs not significantly different from Sites 2 and 3 (Table 1).

There was a significant interaction between Site X Type X Origin ("tank" or "tissue") ($p < 0.0001$). More microplastics were collected in tank water than in crab tissues from all sites (Fig. 2C). Site 1 had a total mean ( ± S.D.) of 15.1 ± 3.9 microplastic pieces per crab including both tank and tissue. Site 2 had a total mean of 25.8 ± 7.5 and Site 3 had a total mean of 25.0 ± 7.9 total microplastic pieces per crab. For Site 1, a higher mean amount of microplastic pieces per crab were found in the tank water with 12.0 ± 3.6 pieces compared to 3.1 ± 2.4 pieces in the tissues. Similarly, Site 2 had an average of 23.1 ± 7.0 pieces per crab in the tank water and 2.7 ± 1.3 pieces in the tissues. Site 3 also had a higher average in the tank water with 23.7 ± pieces per crab and 2.7 ± 1.3 pieces in the tissues. As a percentage, fibers dominated at all sites in both the tank water and tissues; in total 85% of microplastics were fibers. Fibers were primarily dark royal blue color (87%) and the majority of fragments were clear in color (76%) (Fig. 3). Beads were only found in crabs from Site 1 in very low numbers.

In summing all 90 oysters, there were a total of 1482 pieces of plastic recorded (Table 2). For the Atlantic mud crab, there were 1979 pieces when all 90 crabs were included (Table 2). Fibers were the most common type of microplastic; 67% of microplastics in oysters were fibers while 85% were fibers in crabs (Table 2). Although *C. virginica* had

**Table 2**
A summary of total microplastics from (A) all *C. virginica* (n = 90) and *P. herbstii* (n = 90), and (B) collected per gram of organic tissue.

| A. Total microplastic pieces by type | | |
|---|---|---|
| | *C. virginica* | *P. herbstii* |
| Fibers | 991 | 1672 |
| Beads | 9 | 2 |
| Fragments | 482 | 305 |
| Total | 1482 | 1979 |

| B. Mean number of microplastic pieces by species per gram of organic tissue | |
|---|---|
| | Pieces per gram of organic tissue |
| *C. virginica* | 3.84 ± 3.39 |
| *P. herbstii* (total)[a] | 1361.61 ± 4928.13 |
| *P. herbstii* (tissue only) | 297.74 ± 1178.75 |

[a] Both Tissue and Tank are including in this calculation. Tank refers to the microplastics in the water crabs were kept in for 5 days. Tissue refers to the microplastics in the digested tissue.

fewer total microplastics compared to *P. herbstii*, the oyster had relatively higher percent of fragments (33% vs 15%, respectively). Adult *C. virginica* weighed more than *P. herbstii* (Table 1). When compared per gram of organic tissue, the differences between the two species were even more striking; crabs had approximately two orders of magnitude more microplastics in their soft tissues (Table 2).

## 4. Discussion

Microplastics were widespread and abundant in both the water and in invertebrate organisms in Mosquito Lagoon (Fig. 2). Overall, mean microplastic density per liter of Mosquito Lagoon water was high compared to other studies in global estuaries (e.g. Zhao et al., 2014; Desforges et al., 2014); Mosquito Lagoon waters had an average ( ± S.D.) of 21.4 ± 13.1 microplastic pieces per liter. Using similar techniques, a Chinese estuary found between 5 and 13 microplastic pieces per liter in water samples (Zhao et al., 2014) and surface water samples in the northeastern Pacific Ocean and coastal British Columbia found mean concentrations of microplastic particles to vary from 0.01 to 9.18 pieces per liter (Desforges et al., 2014). Similarly, water samples found in Qatar's Exclusive Economic Zone, an estuary stretching 32,000 $km^2$, had means between 0 and 0.01 pieces per liter (Castillo et al., 2016). Elevated concentrations of microplastics in Mosquito Lagoon may be due to high inputs, high retention times, and localized weather phenomenon (e.g. high winds prior to sampling). The Indian River Lagoon, including Mosquito Lagoon, have seen increases in pollution over the last few decades (Kang et al., 2015) as human settlement increased along the lagoon's shore (Browne et al., 2011). With increased urbanization, the lagoon has experienced increased pollution from nonpoint sources such as septic tanks and wastewater drainage (Browne et al., 2011). In addition, Mosquito Lagoon is an enclosed and poorly drained estuary with negligible tidal flushing (Smith, 1993; Lapointe et al., 2015). Mosquito Lagoon is instead impacted more by non-tidal flushing mechanisms including local wind forcing and rainfall or extreme weather events (Smith, 1993). It has been estimated that 50% renewal of water takes between 200 and 300 days in Mosquito Lagoon, compared to about a week in the southern lagoon or one tidal cycle near inlets (Smith, 2016). Additional factors could affect the abundance and distribution of microplastics on a daily basis including wind- and boat-wake driven patterns of water motion, and anthropogenic activities, such as fishing, paddling or sailing. Mosquito Lagoon is considered by many to be the “redfish capitol of the world” and is a very popular location for outdoor recreation (Scheidt and Garreau, 2007). Thus, microplastics that enter the lagoon from external sources may reside in the lagoon for long periods, and simultaneously, through recreational, commercial and day-to-day activities, humans are actively and passively enabling additional microplastic deposition within the lagoon boundaries.

The variation of types and abundance of microplastic pieces observed between oyster reef sites suggest spatial variability must also be considered in understanding this issue in Mosquito Lagoon. It is possible that some sites are more exposed to microplastics through higher recreational or boating activity as discussed above. For example, Site 1 had higher concentration of microplastic pieces for both water samples and oysters; a possible explanation is that Site 1 is closer to the eastern border of the lagoon and along a primary boat route. Other factors such as wind and water flow patterns may impact concentration and types of microplastics.

Fibers were the most common type of microplastic in Mosquito Lagoon (Table 2). This is consistent with many other estuarine studies (e.g. Li et al., 2015; Chubarenko et al., 2016; Li et al., 2016). Li et al. (2015), for example, examined microplastics in the organic tissue of nine commercial bivalves in China; over half of microplastics found in all species tested were fibers. Most Mosquito Lagoon fibers were royal/dark blue in color, which is consistent with fibers originating from nylon and polypropylene boat ropes or clothing (Chubarenko et al., 2016). Clothing fibers usually originate from wastewater and septic tank drainage where laundry water is discharged (Browne et al., 2011). It is estimated there are between 1000 and 3000 septic tanks in Volusia County in which Mosquito Lagoon is located (Jones Edmunds, and Associates, Inc., 2017). Other possible sources of the fibers include equipment used in boating and other recreational activities (Andrady, 2011; Beaman et al., 2016). Boating and fishing activity in Mosquito Lagoon is extensive; according to the Florida Fish and Wildlife Conservation Commission, there were 26,573 registered recreational vessels in Volusia County in 2016 (FWC, 2017).

The types of microplastics found may have been influenced by buoyancy of the plastic type and shape. Less dense polypropylene and polyethylene microplastics can be found higher in the water column while denser plastics like polystyrene and polyvinyl chloride sink and reside primarily in the sediment (Chubarenko et al., 2016). If we had collected water samples immediately above the benthos, it is possible more fragments and beads would have been collected. Additionally, the buoyancy of microplastics can be affected by biofouling and contaminant fouling. Plastics with greater surface area and weathering tend to adsorb more pollutants and accumulate more microorganisms which makes plastics denser (Chubarenko et al., 2016). Shape of plastics also influences buoyancy; fibers and fragments with greater surface area and limited fouling are more frequently found higher in the water column (Chubarenko et al., 2016). Our collections all occurred at low tide when the intertidal oyster reefs were exposed. When collected, all crabs and oysters were exposed on muddy lagoon sediments. By collecting 10 cm below the water surface, our water sampling protocol likely increased our chances of capturing fibers due to their high buoyancy. Similarly, oysters filtering water and crabs actively moving water across their gills should increase the likelihood of collecting fibers. There were, however, hundreds of fragments collected and only 11 beads, 9 of which were obtained from oyster tissue. Hence, the water column in this shallow estuary is mixed enough to enable organisms to encounter all three common types of microplastics.

The mean number of microplastic pieces found in *C. virginica* (16.5 microplastic pieces per oyster with average shell lengths of 63.3 mm) was higher than the amount of microplastic pieces found in other bivalves. *Crassostrea gigas* raised in the Atlantic Ocean off the shore of Brittany, France (average shell length: 9.0 cm) had an average of about 2 microplastic pieces per oyster (van Cauwenberghe and Janssen, 2014). The average number of microplastic pieces in a commercial clam (*Scapharca subcrenata*) from a fishery market of Shanghai, China similar in size to *C. virginica* had, on average, 13 pieces per clam (Li et al., 2015). Wild-harvested blue mussels, *Mytilus edulis*, (average shell length: 4.3 cm) collected from intertidal coastal waters of China had between 2 and 8 pieces of microplastics per mussel (Li et al., 2016). It was also found that wild mussels had more microplastic pieces than mussels farmed on long lines (Li et al., 2016). Again, the high densities of microplastics in Mosquito Lagoon directly relates to the high volumes of plastics in the estuary's waters (Fig. 2, Table 2).

The significant interaction between Site X Plastic Type X Origin for *P. herbstii* indicated that the abundance and diversity of microplastics was highly variable within our study area. Again, buoyancy and plastic composition may be two aspects that could influence the type of microplastics found because *P. herbstii's* gills were exposed to plastics in the water column while foraging should be associated with sediments or accumulation of plastics in prey. Farrell and Nelson (2013) found some microplastics ingested into the digestive system translocated into the hemolymph and other tissues of crabs. Most microplastics, especially fibers, were found in the tank water rather than in soft tissues. This suggested that most microplastics were entangled and expelled within 5 days. Crabs pump water over their gills for oxygen consumption and plastics may become lodged during this process (Watts et al., 2014). Previous laboratory experiments found that microplastics lodged in the gills of many crab species were released within 14 (Watts et al., 2014) to 21 days (Farrell and Nelson, 2013). 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). 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 and grooming behavior is hypothesized to counteract colonization of microbes in their gills (Bauer, 1989) which may potentially aid them in removing microplastics as well.

It is possible that microplastic concentrations were overestimated due to only using microscope observations (Shim et al., 2016; Song et al., 2015). Precautions were taken to ensure as much accuracy as possible. A hot needle test (Karlsson et al., 2017) was used to distinguish between organic materials (i.e. diatoms) and plastics; however, there is currently no low-cost solution to test whether pieces are plastic. Ideally, a Fourier transform infrared spectroscopy (FT-IR) would be used to more accurately determine amount and chemical types of plastic (Song et al., 2015). Nonetheless, preliminary data found over 91% of plastic were recovered. In addition, it is important to acknowledge possible contamination from the use of plastic zip-lock bags and buckets may have occurred, though would likely be minimal because precautions were used to rinse buckets and bags were only for freezing whole animals.

It is not known if the large difference in numbers of the microplastics per gram of soft tissue for *C. virginica* versus *P. herbstii* was the result of the organism's biological processes or bioaccumulation (Table 2). Although oysters are renowned for filtering large volumes of water, individuals may expel microplastics as pseudofeces and feces. Although it is not known how long it takes oysters to expel microplastics, it is common practice in the fishery industry to keep bivalves in filtered water for a minimum of 48 h to remove contaminants in a process called depuration (Lee et al., 2008). Crabs can continue to expel microplastics from their gills and digestive tract for up to 21 days in laboratory setting (Farrell and Nelson, 2013). We limited our entanglement observations to 5 days as all microplastic pieces of this small crab were released within this time frame in preliminary trials (HW, pers. obs.). It is likewise unknown if and how long it would take a crab to remove plastic waste as feces. Although few studies have looked at bioaccumulation of microplastics, the potential for microplastic transfer between trophic levels exists. Setala et al. (2014) observed this with plankton and Farrell and Nelson (2013) recorded an increase in microplastic concentration in laboratory experiments from the mussel *Mytilus edulis* to a shore crab *Carcinus maenas*. 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, additives and other organic compounds that are associated with microplastics (e.g. Beaman et al., 2016; Ma et al., 2016). In Mosquito Lagoon, both crabs and oysters are consumed by a wide variety of predators, including larger invertebrates (e.g. blue crabs), fishes, wading birds, and, for oysters, by humans. Some or all of these organisms may be accumulating microplastics initially consumed by *C. virginica* or *P. herbstii*.

## 5. Conclusions

In summary, the concentration of microplastics in the organic tissue of oysters and crabs from Mosquito Lagoon were higher than in previous studies of field-collected shellfish and crabs (e.g. Li et al., 2015; Li et al., 2016; van Cauwenberghe and Janssen, 2014). This may be due to extensive urbanization, intensive recreational use, weather, and limited flushing in Mosquito Lagoon (Lapointe et al., 2015; Smith, 1993; Smith, 2016). To better understand the global microplastics, we need to continue to increase the number of studies, such as ours, in various habitats and geographies. We, likewise, need to better understand: 1) age-specific, 2) gender-specific, and 3) development stage-specific accumulation by species. Additionally, we need to investigate seasonal differences due to different water inflow patterns as well as bioaccumulation of microplastics and biomagnification of associated contaminants at all levels of the food web. It is important to continue to increase our knowledge of microplastics in a wide variety of species to truly grasp the extent of this anthropogenic problem.

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## Contributions

Waite contributed to experimental design, data collection and analysis, and manuscript preparation. Donnelly contributed to experimental design, data analysis, and manuscript preparation. Walters contributed to experimental design, data collection, and manuscript preparation.

## Conflicts of interest

None.

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