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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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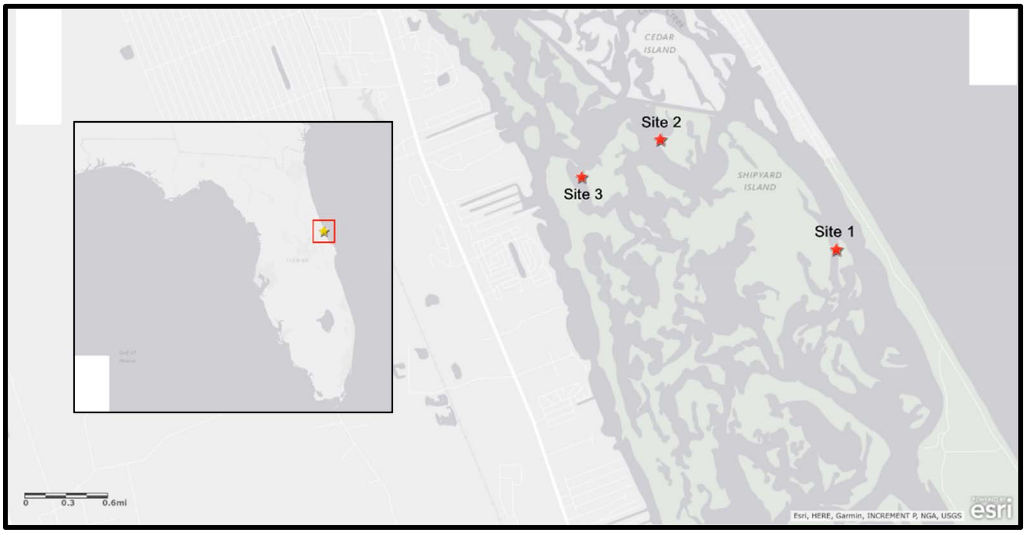

Fig. 1. Study site within the Mosquito Lagoon, in the northern Indian River Lagoon system, Florida.

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