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Distribution and Potential Impact of Microplastics in the Ribbed Marsh Mussel Geukensia demissa

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DISTRIBUTION AND POTENTIAL IMPACT OF MICROPLASTICS IN THE RIBBED MARSH MUSSEL *GEUKENSIA DEMISSA*

by

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Abstract

Human activities have generated large quantities of plastics that are actively dumped or indirectly deposited into oceans. In particular, the use of single-use packaging and microplastics in cosmetics and manufacturing has led to significant increases of these contaminants in coastal waters. These plastics, because of their size, can be ingested by filter-, suspension-, and deposit-feeding organisms who coincidentally consume them as potential food sources. As a result, organisms may experience marked reductions in growth and/or health due to the accumulation of these plastics in their digestive tracts. While research has concentrated on the commercially harvested blue mussel *Mytilus edulis*, none have investigated the critically important ribbed marsh mussel *Geukensia demissa*. This study examined microplastic abundances and distribution trends within a bed of *G. demissa* at Sandy Hook, New Jersey, and investigated microplastic ingestion/rejection in a laboratory setting. Results indicate that microplastics in the field ranged from 11,000 pieces/m^2 to 50,000 pieces/m^2. Microplastics were also found in significant abundances down to a sampling depth of 10 cm, which was twice the average sampling depth of other studies. Furthermore, this study confirms that the *G. demissa* ingests polystyrene spherules (5 μm or less), which were histologically observed throughout the digestive system of all experimental mussels. Also, all experimental mussels rejected positively buoyant plastics as negatively buoyant feces and pseudofeces, which may represent a potential source of buoyant microplastics to the benthos.

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Introduction

1.1 Microplastics

Due to their versatility and affordability, the prevalence of plastic compounds in products has increased across varied industries, including commercial, manufacturing, and medical fields. The strength, chemical- and light-resistance, adaptability, and low cost of plastics create a high demand. Worldwide production has grown from 1.7 million tons in 1950 to a staggering 322 million tons in 2015, where single-use plastic packaging makes up the largest market sector demand for plastics production (~ 40%) in Europe (PlasticsEurope, 2016). The durability of these compounds often exceeds the useful life of the product, which allows plastic to enter the environment by accident or through improper disposal. A portion of waste plastic enters the ocean by wind or river transport and accumulates on coastlines and benthic environments, or in ocean gyres. In fact, a report in Science estimated that 4.8 to 12.7 million metric tons of mismanaged plastic in coastal regions ended up in the ocean in 2010 (Jambeck *et al.*, 2015). Recently, scientists have focused on small plastic fragments known as "microplastics" (Moore, 2008). While there is contention over the size of these plastics, the National Oceanic and Atmospheric Administration defines microplastics as those plastics that are less than 5 mm in size (Arthur *et al.*, 2009).

The small size of these particles makes them particularly available to deposit, filter, and suspension feeding invertebrates. Several studies have shown that coral, crustaceans, echinoderms, molluscs, and polychaetes ingest microplastics (Browne *et al.*, 2008; Cole and Galloway, 2015; Cole *et al.*, 2013; Graham and Thompson, 2009; Hall *et al.*, 2015; Murray and Cowie, 2011; Thompson *et al.*, 2004; Wright *et al.*, 2013).

Importantly, there are several studies that have found plastics in the tissues of store-bought seafood (De Witte *et al.*, 2014; Li *et al.*, 2015; Mathalon and Hill, 2014; Rochman *et al.*, 2015; Van Cauwenberghe and Janssen, 2014). The overall consequences of plastic ingestion are under investigation but researchers speculate that these particles may block important physiological processes and leach toxic plasticizers into the organism (Cole *et al.*, 2013; Teuten *et al.*, 2009). More recent research has shown that oyster larvae (Cole and Galloway, 2015), adult oysters (Sussarellu *et al.*, 2016), larval fish (Lönnstedt and Eklöv, 2016) and adult fish (Rochman *et al.*, 2014) had a negative response to virgin plastic concentrations. Furthermore, wayward plastic has been shown to adsorb dangerous levels of PCBs, POPs, DDE, nonylphenols, and a number of other chemicals that ultimately may harm the organisms that ingest these plastics (Mato *et al.*, 2001; Ogata *et al.,* 2009; Teuten *et al.*, 2007; Teuten *et al.*, 2009). Rochman *et al.* (2014) found that adult fish that ingested plastics sorbed with PCBs, PAHs and PBDEs displayed early signs of endocrine disruption. These concerns have triggered studies across the globe on sandy beaches, estuaries, industrial wastewaters, ocean gyres, and freshwater systems to track the abundance of microplastics (Acosta-Coley and Olivero-Verbel, 2015; Browne *et al.*, 2011; Free *et al.*, 2014; Ng and Obbard, 2006; Thompson *et al.*, 2004). To date, no research has focused on microplastic distribution along coastal New Jersey, USA. Moreover, past research has largely concentrated on microplastic ingestion by the commercially important blue mussel, *Mytilus edulis* Linnaeus, 1758 (Browne *et al.*, 2008). There is no information regarding the common and environmentally critical ribbed mussel, *Geukensia demissa* (Dillwyn, 1817).

1.2 The Ribbed Marsh Mussel Geukensia demissa

Geukensia demissa is a mytilid mussel whose diet consists of phytoplankton and particulate organic matter (POM). Feeding by *G. demissa* reduces water turbidity by exerting top-down control of phytoplankton populations and stabilizing significant loads of particulates in the water column (Jordan and Valiela, 1982; Newell, 2004). The subsequent reduction in turbidity stimulates aquatic plant growth by allowing more light to reach the benthos (Dame, 2011). Also, the deposition of suspended POM on the benthos in biodeposits allows sediments to "entomb" the nutrients, where anaerobic bacteria convert these excess nutrients into an inorganic form before aerobic bacteria remineralize the particulates and create anoxic waters (Newell, 2004). More importantly, *G. demissa* maintains a relatively high clearance rate during summer months when food is abundant and rejects a large number of particles as pseudofeces (Kreeger and Newell, 2001), which results in trapping additional POM from the water column.

Geukensia demissa populations are also commonly associated with dense growths of *Spartina alterniflora* (Loisel) in estuaries and intertidal zones along the Atlantic Coast of North America (Jordan and Valiela, 1982). Ribbed mussels are often found attached by byssal threads to the basal portion of *S. alterniflora*, which acts to bind the roots and stems of the marsh grass together and prevents erosion. *S. alterniflora*, in turn, reduces the flow of water allowing *G. demissa* to feed on suspended particles in the water column and eject sediment-rich pseudofeces atop the root system, thereby further promoting marsh growth by delivering nitrogen, phosphorus, and other nutrients to *S. alterniflora* (Bertness, 1984; Gili and Coma, 1998; Newell, 2004). Overall, the ecological roles

provided by ribbed mussels extend beyond the salt marsh and its importance cannot be understated.

Populations of *Geukensia demissa* live in areas with high concentrations of suspended particles increasing the potential for ingestion of microplastics that similarly are found in these hydrodynamic conditions. As filter-feeders, these bivalves have a mechanism for particle selection, but that process occurs after the particle enters the mantle-cavity and thus non-food material is not immediately rejected. In addition, the particle-selection process is activated by proteins encoded only during periods when food availability is scarce (Espinosa *et al.*, 2008). A past study showed that the reproductive cycle of the closely related blue mussel coincides closely with seasonal blooms of phytoplankton where larval and adult mussels would have plentiful food resources (Newell *et al.*, 1982). This could suggest that *G. demissa* is most apt to ingest microplastics when food availability is high and the mussel is either reproducing or developing. Microplastic ingestion has been shown to shift energy allocation away from reproduction towards growth and maintenance in adult Pacific oysters, *Crassostrea gigas* Thunberg, 1793 (Sussarellu *et al.,* 2016), and reduced feeding rates in their larvae (Cole and Galloway, 2015). Ingestion of these plastic pollutants may induce similar responses in the ribbed mussel. Furthermore, the abundance of *G. demissa* makes the organism an excellent source of energy for predators, such as the commercially harvested blue crab (Seed, 1980), which opens the possibility for biomagnification in a commercially fished species. Though not a commercially harvested species, the green crab *Carcinus maenas* was found to contain microplastics after feeding on contaminated blue mussels in a study by Farrell and Nelson (2013). This process could have severe impacts to fisheries and

human health. In contrast, the rejection of microplastic fragments during particle selection could make suspended microplastics available to the benthic community when *G. demissa* traps rejected particles in pseudofeces, which have been shown to settle up to 40 times faster than normally suspended particles (Widdows *et al.*, 1998). Once the pseudofeces reach the benthic environment they then become available to organisms that feed there, or these bound particles could simply be incorporated into the sediment.

This study examined the distribution of microplastics within *Geukensia demissa* mussel beds located in Sandy Hook Gateway National Recreation Area, in Atlantic Highlands, New Jersey, and experimentally assessed the ingestion and processing of microplastics by *G. demissa*.

I hypothesized that:

(1) Microplastic distribution is homogeneous, regardless of size, throughout the mussel bed;

(2) *G. demissa* ingests 5 μm-sized and smaller microplastic particles;

(3) Specimens of *G. demissa* that ingest these microplastics do not digest these particles and these particles are egested through the rectum as fecal material; and

(4) *G. demissa* rejects microplastic particles sized greater than 250 μm as pseudofeces.

Methods

2.1 Field Study Location

Plum Island is a remnant spillover fan located at the mouth of the Navesink River in Raritan Bay along the western shoreline of Sandy Hook Gateway National Recreation

Area (Fig. 1). The island is fed by sediments from the Navesink and Shrewsbury Rivers ("Sandy Hook," 2016) and is exposed to the strong tidal currents of the bay. The predominant summer winds blow in from the southwest ("Geologic Setting of the Modern Shore," 2016). The westernmost edge of Plum Island serves as a protective barrier to a north and south salt marsh that are divided longitudinally across the center of the island by a land bridge. The north and south marshes are each exposed on one side to the tidal currents of Raritan Bay from the north and south, respectively. Both marshes are fringed by beds of *Geukensia demissa* and *Spartina alterniflora* (Fig. 1c).

2.2 Sediment Sampling

Sediment cores (n = 36) were collected on June 27, 2014 along four transects. Two transects (1 and 2) were in the north marsh (NM) and two transects (3 and 4) were in the south marsh (SM). Each transect spanned from the mussel bed's leading edge to the back edge. Three sediment cores were randomly taken 1 m from the leading and back edges, and an area between the other two for a total of 9 cores per transect. Also, a 0.25 m^2 quadrat was randomly laid down in each of the sampling areas and the number of mussels within the quadrat was counted. All samples were frozen at -16 ºC and stored until processing.

Sediment cores were split to differentiate plastics in the top 6 cm of sediment versus plastics between 6-10 cm. Microplastic debris was extracted from each cross section using methods similar to Thompson *et al.* (2004). This method separates microplastic from sediments using a filtered super-saline solution of NaCl at 200 ppt. The solution was poured through a series of standard sieves to separate the debris by size class. The mesh sizes were 4 mm, 2 mm, 1 mm, 500 μm, and 250 μm. Using a dissecting

scope, microplastics were picked directly off the sieves, counted, and separated by size. The plastic counts were later pooled into two size classes; plastics that were 1 mm or larger (up to 5 mm) and plastics less than 1 mm in size, because it was assumed that the smaller sized plastics could enter the mantle cavity of the mussels and affect their health.

2.3 Ingestion by Geukensia demissa and Pseudofeces Collection

Twenty-four specimens of *Geukensia demissa*, approximate lengths from 5-7 cm, were collected from Plum Island. These mussels were scrub-cleaned of epifauna and placed in 5-μm-filtered artificial seawater (Instant Ocean®) at 25 ppt, which approximated the site salinity (23.5 ppt) during collection activities. Individuals were then allowed to acclimate for one week prior to experiments. All mussels were fed a 2 mL blend of *Isochrysis* sp. and *Tetraselmis* sp. (~ 5-6 μm; avg. cell count 4.1 x 10^9/mL) daily. After acclimatization, twelve mussels were placed into 3 L of filtered seawater in a 3.785 L glass jar. These mussels were separated by tubes made with 1 mm aluminum-mesh that were evenly distributed around a Hydor Koralia® 425 wave pump. These tubes were constructed to keep the mussels in an "upright" position with their siphons directed towards the water surface (i.e. in natural position). The wave pump flow was directed straight upwards to create an umbrella-like flow, and was regulated by a Lab-Volt® rheostat (model 193P) (see Fig. 2). These twelve mussels were exposed to 0.167 g/L of 5 μm Visiblex® red-color-dyed polystyrene spherules (sodium azide removed; Phosphorex Inc., Hopkinton, MA) and 3.3 g/L of 250-300 μm red-color-dyed polyethylene microspheres (Cospheric LLC, Santa Barbara, CA). These larger plastics were selected because there was a higher abundance of plastics in this size class recovered from the sediment study. The remaining twelve mussels served as a control and were not exposed

to microplastic. Each group of mussels were fed 1 mL of their daily phytoplankton blend for two hours. Immediately after feeding for two hours, each mussel was placed in separate sealed 1 L glass jars with 600 mL of filtered artificial seawater and an air-stone (see Fig. 3). Four hours post-feeding, 4 exposed specimens of *G. demissa* and 4 controls were preserved in 70% ethanol. Another 4 exposed mussels and 4 control mussels were preserved 12 hours after feeding, and the remaining 4 specimens from each group were preserved 24 hours after feeding. All jars along with seawater were stored at 1°C to preserve feces and pseudofeces for later observation and measurement using light microscopy. All mussels were later deshelled, rinsed with ethanol to remove foreign debris, and dissected to remove the digestive system. These tissues were then prepared using standard histological techniques to examine the digestive glands (Humason, 1979). Tissues were dehydrated through a series of ethanols up to 100%, then a 50:50 mixture of terpineol:toluene, and finally pure toluene. Samples were then processed through two changes of molten paraffin before embedding for histological sectioning. Serial sections were taken at 7 microns, which were then mounted on glass slides and stained with an aqueous solution of toluidine blue. Sections were observed under light microscopy to determine the distribution of microplastics within the digestive system, including the digestive glands (tertiary tubules).

2.4 Statistical Analysis

Two-way ANOVAs, where the edge location and east/west position served as independent factors, were used to determine any differences in microplastic distribution. The dependent factors were the number of plastics recovered and included the total

number of plastics, plastics less than 1 mm, plastics 1 mm or greater, plastics above 6 cm, plastics below 6 cm, plastics less than 1 mm above 6 cm, and plastics less than 1 mm below 6 cm. Tukey post-hoc tests were used to further analyze any differences found in the above two-way ANOVAs. A linear regression was used to compare the number of plastics recovered (total core, above 6 cm, and below 6 cm) against the proportion of sand (total core, above 6 cm, and below 6 cm), and plastics 1 mm or greater against plastics less than 1 mm. A final linear regression was used to evaluate differences in the number of plastic per cm^3 above 6 cm versus the number of plastics per cm^3 below 6 cm^3.

Results

3.1 Sediment Distribution

Samples collected in the field showed wide variation among sediment samples with plastic particle densities ranging from approximately 11,000 to 50,000 pieces/m^2 (see Table 1). A simple linear regression model found that the presence of plastic pieces greater than 1 mm in size is significantly less abundant than the presence of plastic pieces less than 1 mm in size ($F_{33,37.08} = 42.1$ $P < 2.31 \times 10^{-7}$, see Fig. 4), with an R^2 value of 0.561. There are 2.4 pieces of plastic (< 1 mm) for every plastic greater than 1 mm in size. Plastics less than 1 mm in size account for 79.01% of the total number of plastics recovered. In contrast, a simple linear regression found that there is no significant difference between the number of plastic pieces per cm^3 above 6 cm and number of plastic pieces per cm^3 below 6 cm ($F_{33,0.19} = 3.34$ $P < 0.077$).

A two-way ANOVA showed that edge location has a significant effect on the distribution of all the plastic recovered ($F_{2,29} = 4.05$ $P < 0.028$). Also, position ($F_{1,29} = 7.2$ $P < 0.012$) and the interaction between edge and position ($F_{2,29} = 5.29$ $P < 0.011$) had a

significant effect on the distribution. A Tukey's post-hoc test showed that there was a significant difference in means between the middle (B) sites and the back edge (C; C-B P = 0.026) when controlling for edge, and a significant difference in means between the east and west ($P < 0.012$) when controlling for position. A different two-way ANOVA for plastics less than 1 mm produced similar results where edge location ($F_{2,29} = 5.09$ $P < 0.013$), position ($F_{1,29} = 7.30$ $P < 0.011$), and the interaction between edge and position ($F_{2,29} = 6.33$ $P < 0.005$) had a significant effect on plastic distribution. For these plastics (< 1 mm), a Tukey's post-hoc test found that middle (B) sites' means also significantly differed from the back edge (C; C-B $P < 0.011$) when controlling for edge, and that means differed significantly between eastern and western sites ($P < 0.011$). For plastics 1 mm or greater in diameter, neither of these factors (edge and position) had a significant effect on their distribution ($F_{2,29} = 0.82$ $P < 0.449$ and $F_{1,29} = 3.99$ $P < 0.05$, respectively). For plastics (< 1 mm) above 6 cm, a two-way ANOVA showed that distribution was significantly affected by east/west position ($F_{1,29} = 8.68$ $P < 0.006$) where a Tukey's post-hoc analysis revealed a significant difference in means ($P < 0.006$) when controlling for position. In contrast, plastics (< 1 mm) below 6 cm were significantly affected by the edge location ($F_{2,29} = 5.33$ $P < 0.011$). A Tukey's post-hoc test showed that there was a significant difference in means between the middle (B) sites versus the back edge (C; C-B $P < 0.008$) when controlling for edge. In short, plastics (< 1 mm) were significantly affected by the edge location and east/west position, but the influence of these factors differed based on the depth of the plastic.

All samples' sediments mainly consisted of sand-sized grains (90% of the total sediment dry-weight) with exception to transect 1 quadrats A and B, 74% and 88.83%,

respectively. A simple linear regression was calculated to predict the abundance of plastic based on the proportion of sand grains. No significant regression equation was found ($F_{33,69.74} = 0.078$ $P < 0.782$), with an R^2 of 0.002. Overall, the distribution of plastic was not homogenous and there were differences based on location within the marsh.

3.2 Ingestion by Geukensia demissa and Incorporation in Pseudofeces

Serial sections of experimental individuals showed that microplastic spheres (5 μm) were found throughout the stomach, intestine, and primary and secondary digestive glands of all specimens indicating that 100% of these mussels ingested microplastics (see Figs. 5, 6 and 7 for examples). Furthermore, these 5 μm particles were found in the digestive tubules of all specimens in the 12-hour and 24-hour post-feeding groups, and in 75% of the specimens in the 4-hour post-feeding group (see Fig. 8 for example). Microplastic spheres were not found in any of the sectioned control specimens.

Polyethylene spheres sized between 250-300 μm were found in the tissue sections of 50% of the specimens from the 4-hour post-feeding group but none of the 12-hour or 24-hour post-feeding groups. These plastics were observed only during sectioning in wax embedded specimens (see Fig. 9) but dislodged upon contact with the microtome blade making identification of the location within the mussels impossible.

Examination of the experimental specimens' waste also revealed these larger plastic spheres in the feces of several specimens. All experimental mussels ejected both size classes of microplastics as feces and pseudofeces (see Fig. 10), and waste production was observably greater than the control groups.

Discussion

4.1 Sediment Distribution

Plastic distribution is not homogenous in the Plum Island marsh. The density of microplastics ranged from approximately 11,000 pieces/m^2 to 50,000 pieces/m^2, which is within the range of other studies reviewed by Hidalgo-Ruz *et al.* (2012) who reported a range of 0.21 to 77,000 pieces/m^2. However, the estimates used in the present study were based on sediment cores that were twice the depth reported in most of the studies reviewed by Hidalgo-Ruz *et al* (2012). For a more analogous comparison with previous studies, estimates were produced from plastics found in the top 6 cm, which yielded a range of approximately 4,500 to 35,000 pieces/m^2. The relative abundance of plastics sized less than 1 mm in diameter accounted for approximately 79% of the total proportion of recovered plastics and is similar to results from Browne *et al.* (2010) who reported that these particles accounted for 65% of their total plastic debris. They suggested that the greater number of smaller plastics could have been the result of abrasion with sediment particles and strong wave-action. The present study site did not have direct contact with a strong wave front, but it is possible that larger plastics fragmented in the areas adjacent to Plum Island before translocating and settling in the marsh's relatively calm waters. Weinstein *et al.* (2016) demonstrated that high-density polyethylene (HDPE), polypropylene (PP) and polystyrene (PS) plastics broke down more rapidly into microplastics (8 weeks) in a salt marsh presumably because of microbial degradation, detritivore feeding activity, and micro-abrasion caused by repeated drying and rehydrating of biofilms that formed on the plastics. This could also explain the higher abundance of smaller plastics in both the present study and Browne *et al.*'s 2010 study.

Additionally, while processing sediment cores in the present study, macro-plastics were observed tangled in the roots of the marsh cordgrass *Spartina alterniflora* that appeared to be breaking down into fragments (see Fig. 11).

When the total number of plastics recovered was considered, edge location significantly affected distribution (see Table 1). These trends are all consistent when comparing only the plastics that are less than 1 mm in size. In comparison, edge location did not affect the distribution of plastics 1 mm or larger. Thus, it appears that the distributions observed in the present study are affected by the size of the particle. Using particle-size to describe distribution patterns is supported by Kowalski *et al.* (2016), where they found that the settling velocity of several plastic polymer-types increased as the size of the particle increased. Khatmullina and Isachenko (2016) observed that plastic particles with greater angularity and smaller sizes and polymer type, decreased settling velocity. Still, if particle size was the only factor affecting plastic distribution we should expect a significant linear equation for the number of plastics based on the type of sediment (i.e. clay, silt, sand, etc.). In this case, most cores contained a high proportion of sand (see Table 2), which did not significantly affect the distribution of plastics, in concurrence with Browne *et al.* (2010) and Mathalon and Hill (2014). More importantly, the present study would have identified differences in distribution for plastics 1 mm or larger if size was the only factor.

This study differentiated between plastics in the top 6 cm and plastics below 6 cm because it was determined that the mussels at Plum Island were found approximately 6 cm deep in the sediments and that the distribution of plastics could be influenced by these mussels. The distribution of plastics above 6 cm is highly variable between edge

locations (see Fig. 13) and does not appear to be influenced by mussel distributions (see Fig. 14). In other words, plastic abundances in the top 6 cm do not appear to increase in areas with higher abundances of mussels, which is supported by previous studies (Ertman and Jumars, 1988; Santana *et al.*, 2016). Conversely, the distribution of plastics (< 1 mm) above 6 cm are significantly affected by their east/west position in the marsh, where eastern sites have higher abundances of plastics. The distribution of these plastics is likely influenced by particle resuspension from wind/wave currents at the surface, as suggested in a recent publication by Critchell and Lambrechts (2016). The present study sought only to provide evidence of microplastic abundance and did not measure wind and water currents or particle densities and shapes, however, previous studies have shown that plastic density/polymer, particle shape, water density, hydrodynamics, wind, and proximity to inputs all affect the distribution of microplastics (Browne *et al.,* 2010; Browne *et al.*, 2011; Chubarenko *et al.*, 2016; Critichell and Lambrechts, 2016; Jambeck *et al.*, 2015; Khatmullina and Isachenko, 2016; Kowalski *et al.*, 2016; Mathalon and Hill, 2014; Wessel *et al.*, 2016). It should be noted that the eastern side of Plum Island is nearest to a heavily used roadway, situated in a raised position relative to the marsh, that could be another source of plastic input. These factors may help explain the much greater abundance of plastics in the eastern transects, but future research will need to include more precise measurements to identify the definitive factors involved.

In contrast, the distribution of plastics (< 1 mm) below 6 cm are significantly affected by their edge location (see Fig. 12). These plastics were found in higher abundances along the back edges of Plum Island. This is likely due to plastics' tendency to settle along the high strandline, which is why several studies focused on these areas

(Browne *et al.*, 2010; Corcoran *et al.*, 2009; Costa *et al.*, 2010; Silva-Cavalcanti *et al.*, 2009). For plastics (< 1 mm) below 6 cm there is less variation in distribution, possibly because the mussels provide a physical barrier from the hydrodynamic forces that affect the benthic surface. In general, mussels have been shown to provide sediment stability (Bertness, 1984). This stability could explain the similar abundances of plastics that settled in the other edge locations (A and B). On the other hand, the leading edge at transect 2 has the highest abundance of both plastics and mussels. This could suggest that the mussels directly affected the local settlement of plastics (< 1 mm), which contrasts with findings from Santana *et al.* (2016) and Ertman and Jumars (1988). However, Santana *et al.* (2016) sampled the brown mussel *Perna perna* L. (1758) for the presence of plastics and did not account for sediment quality. Ertman and Jumars (1988) recorded where polystyrene (PS) spherules, used to mimic bivalve larvae, had settled. That study used positively buoyant PS ($d = 1.06$ g cm^{-3}) and results only included the particles that settled within the sampling area. It is therefore possible that these particles were rejected in the negatively buoyant feces/pseudofeces of the cockle *Clinocardium nuttalli* (Conrad, 1837), which Lobelle and Cunliffe (2011) suggested could affect the density of microplastics. This is supported by observations made during the feeding experiments of the present study, where buoyant plastics became negatively buoyant after rejection as feces or pseudofeces. Furthermore, Ertman and Jumars (1988) suggested that at least 1,000 bivalves/m^2 would be required to increase larval settlement, which is similar to the bivalve density observed along the leading edge at transect 2 in the present study (783 mussels/m^2). Thus, mussels may have an influence on plastic settlement, but the present study did not measure densities of recovered plastics and thus cannot quantitatively state

that less-dense particles settled in the leading (A) and middle (B) sites. Future studies will need to include spectral analysis of recovered plastics to determine density distribution trends, which may help to determine if bivalve biodeposits affect microplastic distribution.

Despite the distributional differences between plastics above and below 6 cm, there was no difference in the number of pieces per cubic centimeter. These results suggest that the relative abundances of plastics are similar and that only the factors affecting their distribution are different. There was also no significant trends in microplastic abundances by depth in this study, however, Turra *et al.* (2014) found that microplastic peak abundances became more shallow in depth with closer proximity to the water's edge. Nevertheless, that study sampled to a depth of 2 m and found significant abundances of plastic pellets throughout their sampling depth. Both the results of the Turra *et al.* (2014) study and the present study suggest that microplastics can be found in significant abundances below the 5 cm sampling depth used in most of the studies reviewed by Hidalgo-Ruz *et al.* (2012). Overall, a much more extensive study will need to be conducted in the future to clarify the trends in microplastic distribution at Plum Island.

4.2 Ingestion by Geukensia demissa and Pseudofeces Production

This study confirms the ability of the ribbed marsh mussel to ingest polystyrene microplastics (5 μm or less) with plastic spherules found in the stomach, digestive tubules and intestine. These results concur with other studies that found that the related blue mussel *Mytilus edulis* ingested similar sized and shaped microplastics (Browne *et*

al., 2008; von Moos *et al.*, 2012; Wegner *et al.*, 2012). Particles reside in the digestive system of *Geukensia demissa* for at least 24 hours, but further research needs to be conducted to determine the average residency time. In a study conducted by Browne *et al.* (2008) microplastic spherules remained in the digestive glands of *M. edulis* for 3 days before translocating into the mussels' circulatory fluids, where the plastics persisted for over 40 days. The present study was performed on a much shorter time frame, but polystyrene particles were observed in both active and non-active digestive phases of the digestive tubules in all experimental groups. This suggests that the plastic particles may become lodged in these tubules, which may result in a disruption of normal digestive processes. A study conducted by Van Cauwenberghe *et al.* (2015) found that blue mussels that ingested polystyrene particles had a 25% increase in digestive gland energy consumption, but there was no net change to the mussels' overall cellular energy allocation. That study, however, was performed using sterile polystyrene spherules. Other studies have shown that microplastics can adsorb chemicals from the surrounding environment that are toxic to marine organisms (e.g. DDEs, DDTs, PAHs, PCBs, Phe, and POPs; Bakir *et al.*, 2014; Ogata *et al.*, 2009; Teuten *et al.*, 2009) and that exposure to gut surfactants can increase the rate of desorption of these chemicals (Bakir *et al.*, 2014; Teuten *et al.*, 2007). Likewise, microplastics have been shown to host harmful colonies of pathogens that differ from the surrounding environment (McCormick *et al.*, 2014). Also, the presence of plastic particles in the digestive tubules may represent one way for these plastics to enter the circulatory system of the mussel, as noted by Browne *et al.* (2008). Multiple studies suggest that these plastics could translocate to the hemolymph via ingestion and transportation into the gastrointestinal tract where they are incorporated

into the digestive epithelial cells via endocytosis (Browne *et al.*, 2008; von Moos *et al.*, 2012). A study conducted by Avio *et al.* (2015) found magnified traces of desorbed pyrene in these tissues leading to the hemolymph, which appears to confirm this mode of translocation.

Additionally, this study confirms that polyethylene plastics (greater than 250 microns) can enter the mantle cavity and be rejected as pseudofeces, and can also enter the stomach and exit through the intestine to be rejected as feces. These larger polyethylene plastics appear to be completely rejected from the digestive glands after 4 hours post-feeding and some time before 12 hours post-feeding. Observing these larger plastics in the gut and feces was not expected because of the mussels' particle selection size range. For example, *Mytilus edulis* has been repeatedly shown to only ingest particles sized between 4-23 microns (Prins *et al.*, 1991; Ward and Shumway, 2004), however, it is likely that the high concentration of these larger plastics in the experiments led to the ingestion and rejection of these large particles. Future research should consider "natural" concentrations of these plastics to see if this observation persists. Feces and pseudofeces from all experimental mussels contained both sizes and types of plastics, and waste production was observably higher in experimental groups than in control groups. The latter observation is supported by a study conducted on the blue mussel by Wegner *et al.* (2012) where a linear relationship between nanopolystyrene concentration and feces/pseudofeces production was documented, as well as a reduction in filtering activity. They speculated that the additional waste production increases energy expenditure, which, when combined with decreased feeding activity, can lead to starvation of the mussel (Wegner *et al.,* 2012). Moreover, these rejected plastics are aggregated into a

biofilm that may change their characteristics. A study by Lobelle and Cunliffe (2011) suggested that biofilms could increase plastic densities and decrease their buoyancy. This suggestion is supported by the present study, which observed buoyant plastics become negatively buoyant when contained in feces/pseudofeces. This could mean that mussels are a source of microplastics that become available to other benthic organisms or that mussel beds can serve as a sink for microplastic pollutants.

Overall, no discernable difference in tissue health was observed between control and experimental specimens, however, this study was not designed to observe changes in animal health and thus cannot determine if *Geukensia demissa* is affected by plastic ingestion, although other studies have demonstrated issues. For instance, when exposed to microplastics, specimens of *Mytilus edulis* reduced filtering activity, increased waste production (Wegner *et al.*, 2012), and formed granulocytomas (inflammatory response) while the lysosomal membrane degraded (von Moos *et al.*, 2012). The adult Pacific oyster *Crassostrea gigas* displayed significant decreases in oocyte quantity and size, and reduced sperm velocity when exposed to microplastics for 2 months, while the development and number of viable offspring declined (Sussarellu *et al.*, 2016). Additional studies will need to be performed to identify any possible adverse effects that ingested microplastics may have on adult and juvenile ribbed marsh mussels. Furthermore, most studies, including the present study, performed analyses using polystyrene spherules. A recent study conducted by Li *et al.* (2015) found that the leachates of various types of plastics had different toxicity levels to the nauplii of the barnacle *Amphibalanus amphitrite* (Darwin, 1854). This finding suggests that

microplastic ingestion is far more complex than previously thought and that the types of plastics used in each study will need to be considered going forward.

Conclusions

Microplastic distribution is complex and highly variable over relatively short spatial scales. This study showed that microplastics can be found in abundance beyond the sediment depth sampled in most other studies. The full spectrum of factors responsible for the distribution of microplastics in this study remains unclear given the limited metrics used. Past studies have confirmed that wind/water currents, water density, inputs, and topography can have a significant effect on microplastic distribution (Browne *et al.*, 2010; Chubarenko *et al.*, 2016; Jambeck *et al.*, 2015; Wessel *et al.*, 2016). Furthermore, this study only used a microscope to identify all recovered plastics, which Song *et al.* (2015) suggested leads to a significant underestimation of plastic abundances. Future work will need to include spectral analysis of recovered plastics to improve abundance estimates and to distinguish between changes in plastic distribution due to differences in the polymer densities, which has been documented in other studies (Browne *et al.*, 2010; Khatmullina and Isachenko, 2016; Kowalski *et al.*, 2016). Also, future studies will need to include more measurements of the local environmental conditions to better understand microplastic distributions, and more sites need to be considered to determine if mussel beds differ from each other, as well as other habitats in microplastic abundances and distribution trends. Most importantly, we need to standardize sampling and reporting protocols (e.g. equipment, depth, metrics, etc.) to ensure comparability between studies.

Geukensia demissa ingests plastics (5 μm or less and between 250-300 μm) and these plastics can be either rejected as pseudofeces or passed through the digestive system and ejected in feces. Plastics (5 μm or less) were observed throughout the digestive system during the entire length of the experiment (24 hours). Waste production in the experimental mussels was observably increased in comparison to the control mussels, however, there were no changes in health noted. Nevertheless, the sole intention of this study was to demonstrate that the environmentally critical ribbed marsh mussel is at potential risk from microplastic pollution, which was accomplished. Future studies will need to consider using more "natural" concentrations of plastics to eliminate accidental ingestion of plastics. Also, future work will need to increase the duration of the experiments and record changes in behavior/health to gain better insights into microplastic residence times and potential health risks to these mussels. Overall, this study demonstrated that the ribbed marsh mussel can reject plastics in their pseudofeces and feces, which alters the density of plastics enough to make them negatively buoyant. Also, this study proved that plastics can be found in significant abundances beneath populations of *G. demissa*, and that distribution of plastics differs above and below these mussels.

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Figures

Fig. 1. (a) Map showing the location of Plum Island within Sandy Hook Gateway National Recreation Area (b) Map showing the sample site's position at Plum Island (c) An illustration of the transect and sampling/quadrat locations.

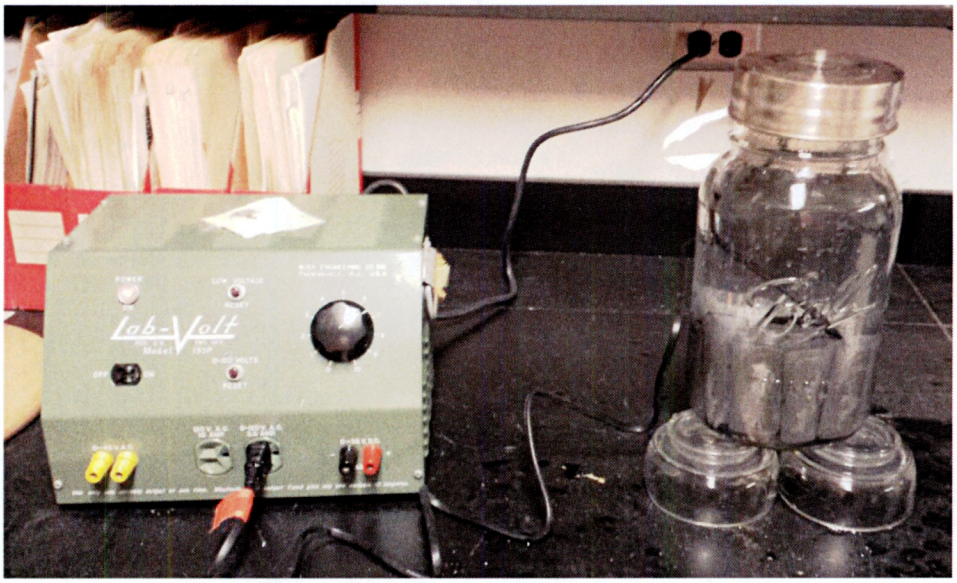

Fig. 2. Image of the microplastic feeding setup. A 3.785 L glass jar with 3.0 L of artificial seawater (25 ppt). A wave pump is directed toward the water surface and centered at the bottom of the jar with 12 metal-mesh tubes surrounding the pump. The tubes were used

to keep the mussels in an upright position. The device in the left-portion of the photo is a rheostat and it was used to regulate the flow of the wave pump, which was set to approximately 65%.

Fig. 3. Image of the control group mussels separated post-feeding. Each jar was 1 L in size and contained 600 mL of artificial seawater (25 ppt) and an air stone. The top of the jars was sealed with plastic wrap. The same setup was used for the experimental mussels.

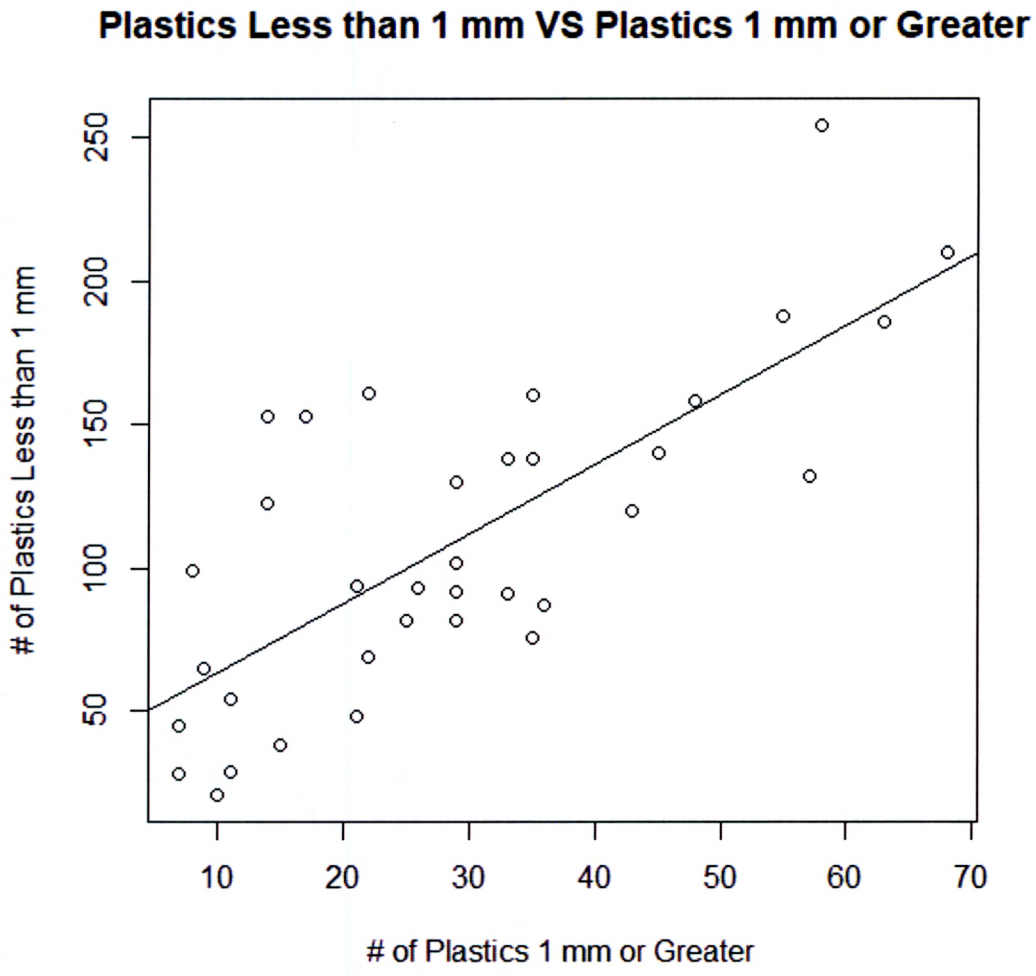

Fig. 4. A simple linear regression comparison of recovered plastic averages by diameter, where the x-axis represents plastics 1 mm or greater and the y-axis represents plastics less than 1 mm. There are approximately 2.4 pieces of plastic (< 1 mm) for ever plastic piece 1 mm or larger.

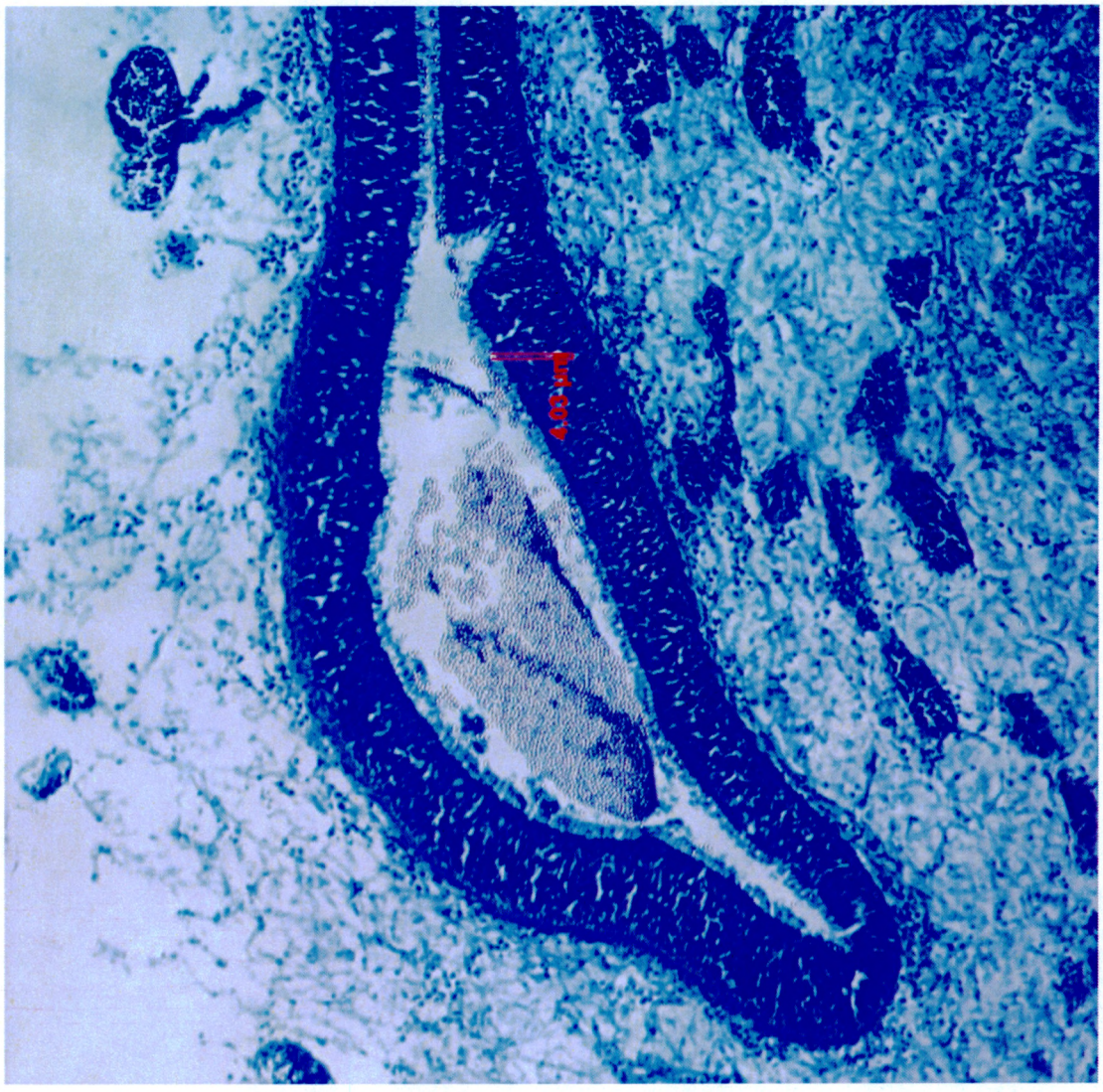

Fig. 5. A primary duct filled with polystyrene spherules, which are the countless clear orbs throughout the duct. Please note that the annotation reads "4.03 µm."

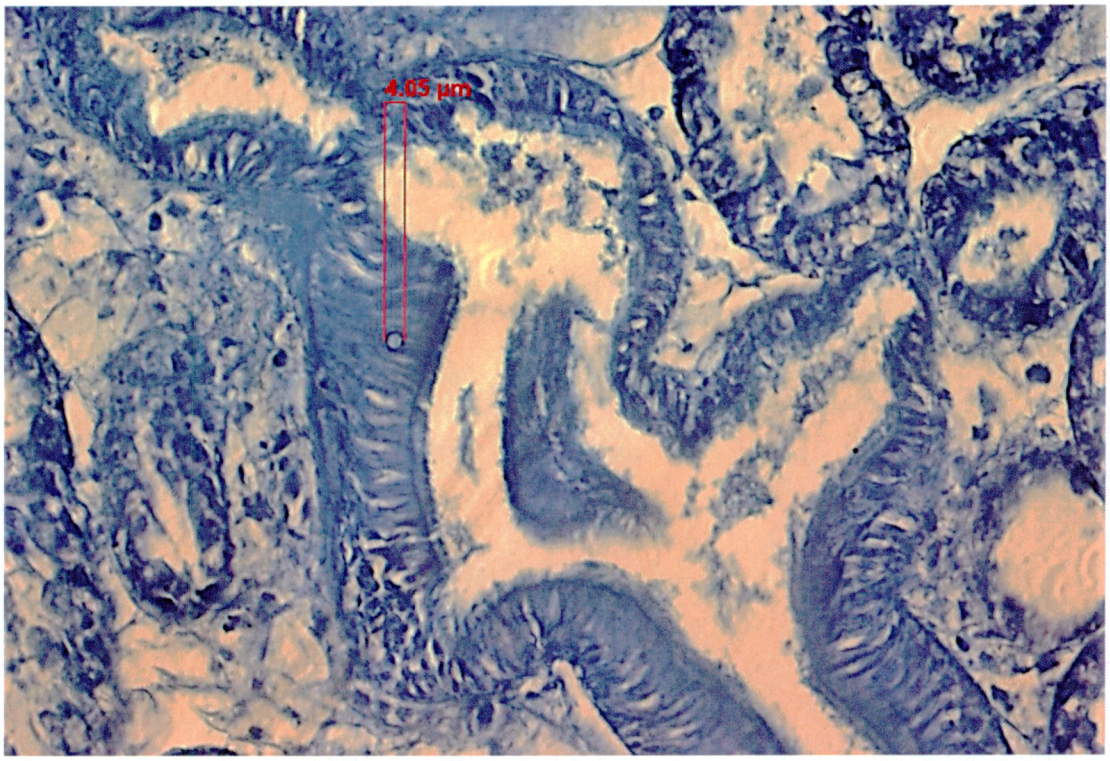

Fig. 6. A polystyrene spherule lodged in the epithelial lining of a secondary duct found in a 12-hour post-feeding experimental mussel.

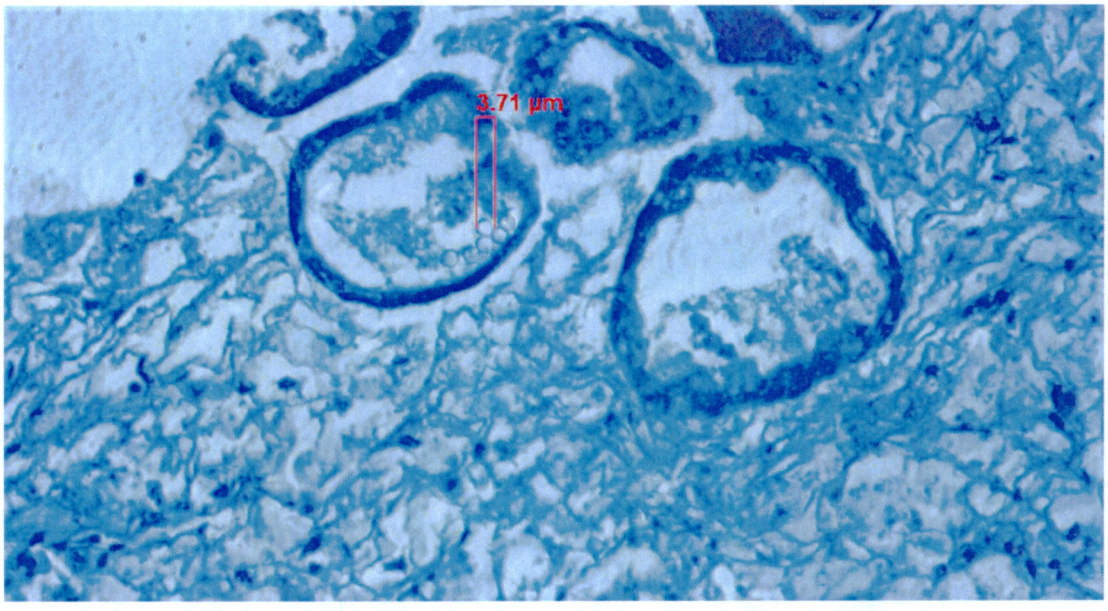

Fig. 7. Six polystyrene spherules (≤ 5 µm) inside a digestive tubule in a 24-hour post-feeding experimental mussel.

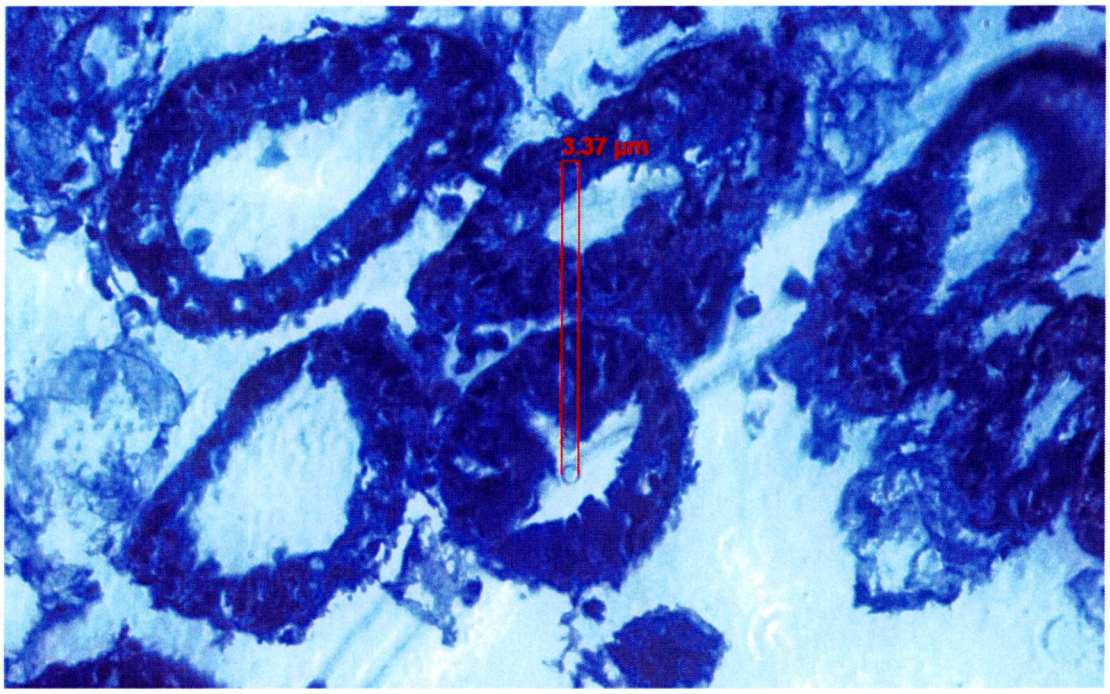

Fig. 8. A polystyrene spherule in an active digestive tubule of a 4-hour post-feeding experimental mussel.

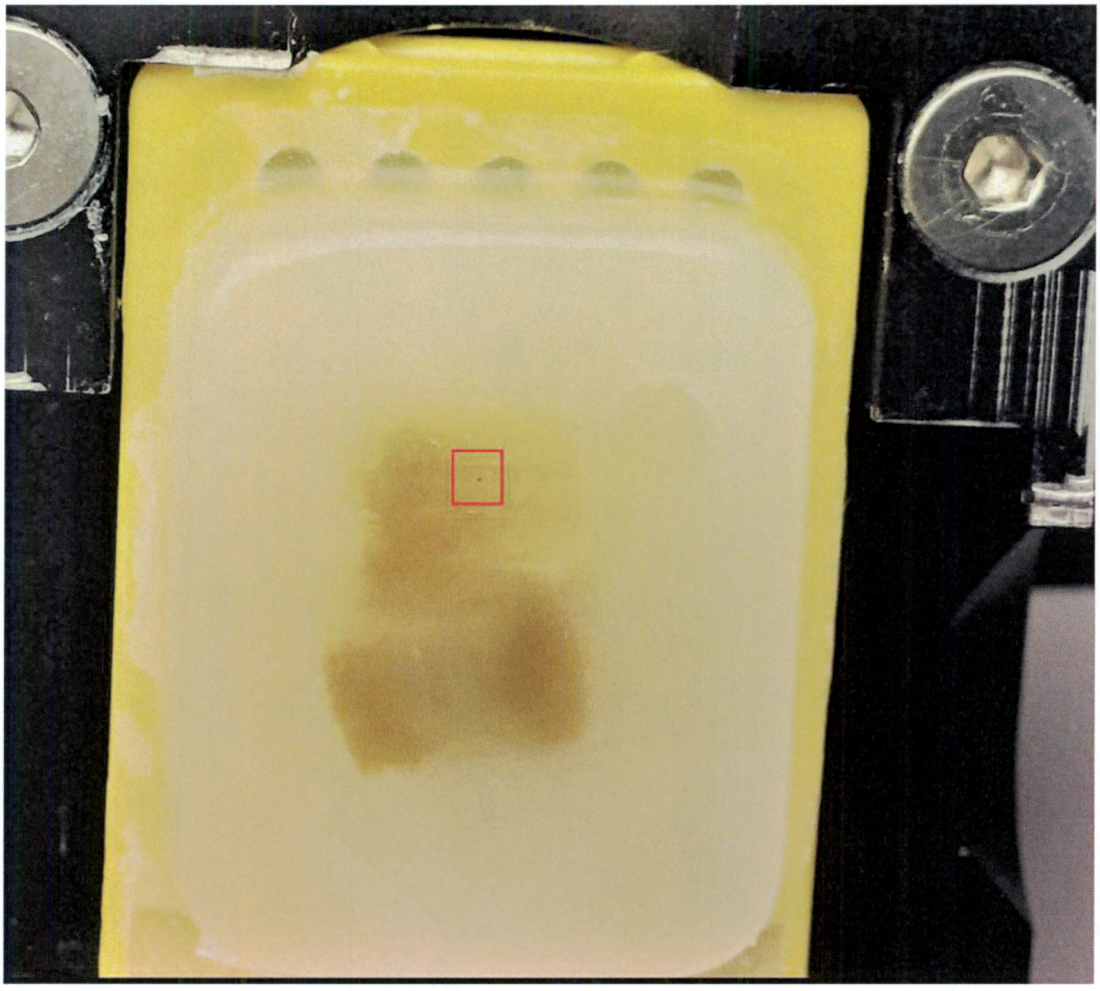

Fig. 9. A polyethylene spherule (≥ 250 μm) inside an experimental mussel. The location of this spherule within the mussel could not be identified because these plastics became dislodged when contacted by the microtome blade.

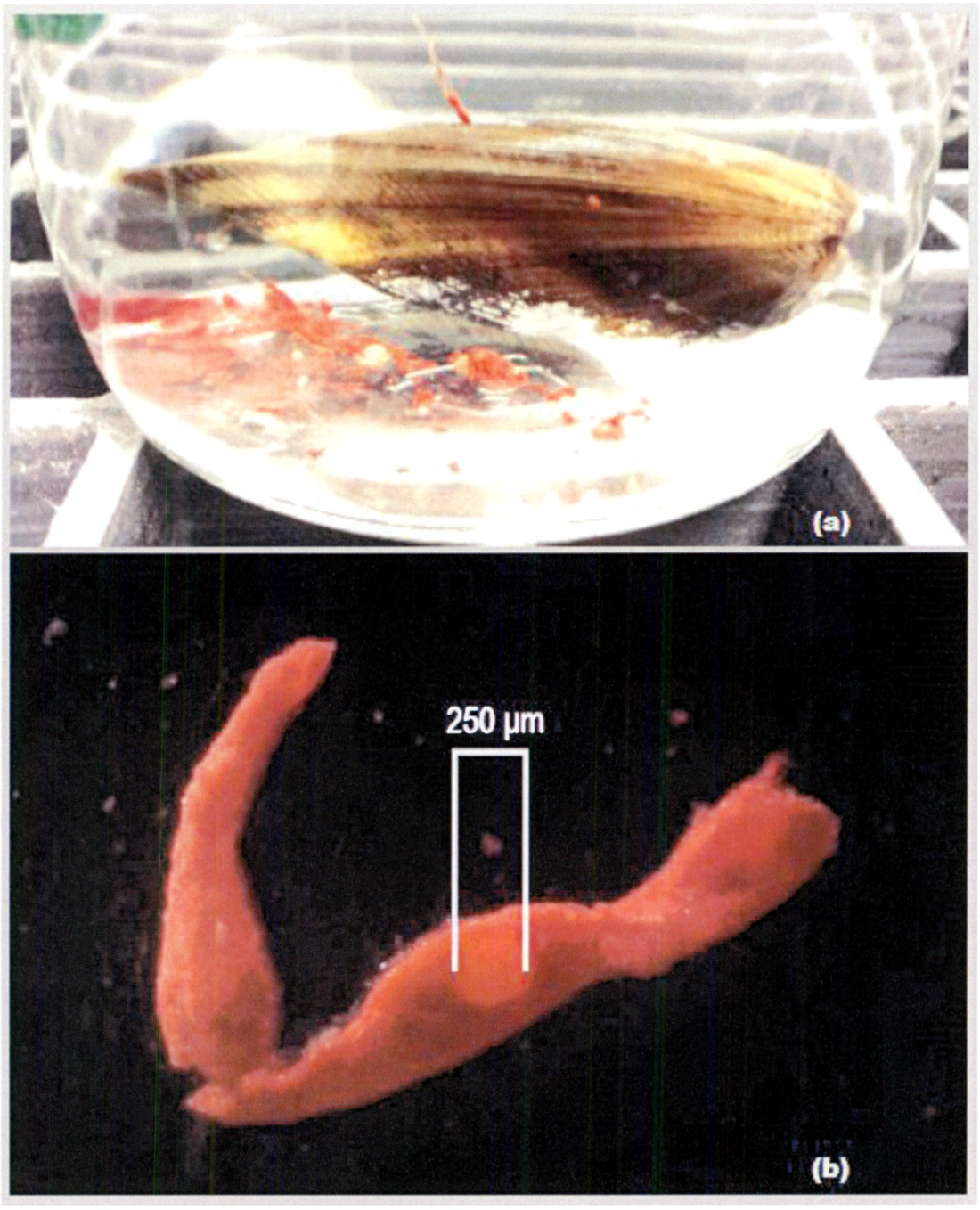

Fig. 10. (a) Polystyrene (≤ 5 µm) and polyethylene (≥ 250 µm) spherules in the feces and pseudofeces of an experimental mussel. (b) A polyethylene spherule (≥ 250 µm) in the fecal waste.

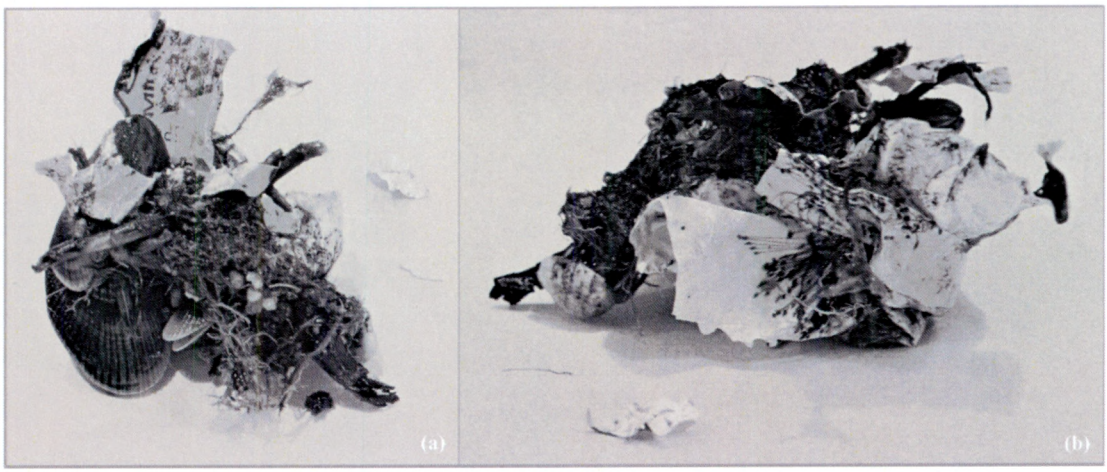

Fig. 11. Macroplastic caught in the root structure of *Spartina alterniflora*. (a) Top down view of a plastic wrapper entangled in the root structure of *Spartina alterniflora* and the byssal threads of *Geukensia demissa.* (b) A frontal view of the same plastic wrapper. Byssal threads can be seen attached to the wrapper and roots can be seen passing through the wrapper. Also, a fragment of the wrapper can be seen in the foreground.

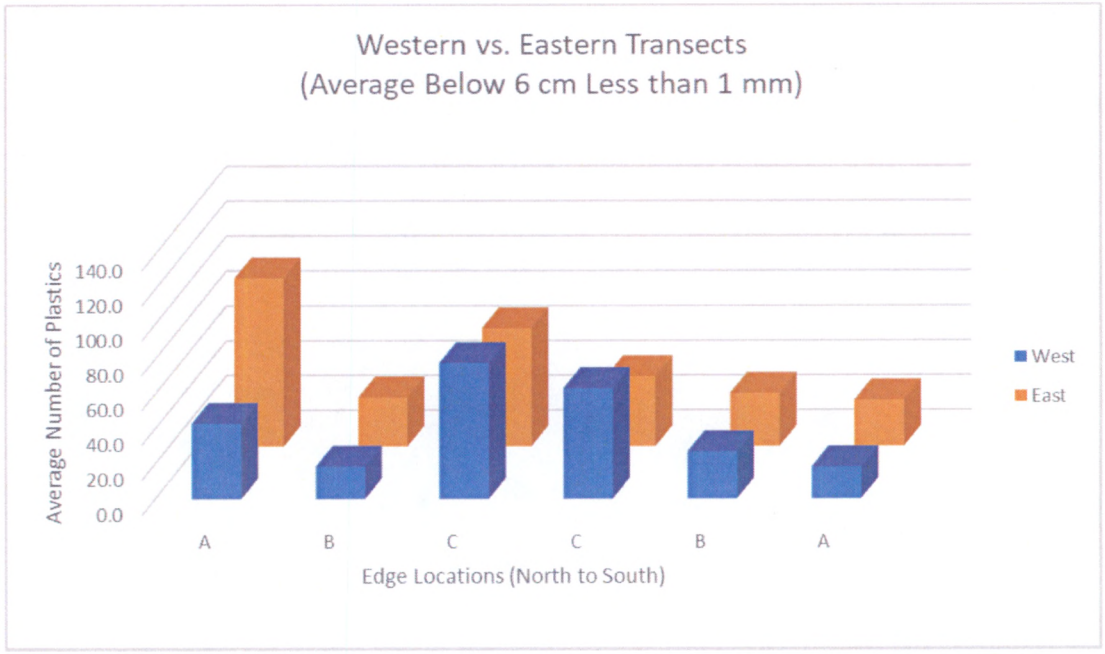

Fig. 12. A boxplot from a two-way ANOVA testing plastics (< 1 mm) below 6 cm against the edge location and east/west position. Edge location had a significant effect on plastic distribution ($F_{2,29} = 5.33$ $P = 0.011$) and a Tukey post-hoc comparison of means

showed that the back edges (C) had significantly higher abundances of plastics than the middle (B) sites (C; C-B $P < 0.008$). Plastic abundance is highest along the leading edge (A) at transect 2 in the east where the highest density of mussels was recorded. Also, the abundances of plastics between sites appears to be more stable than plastics above 6 cm.

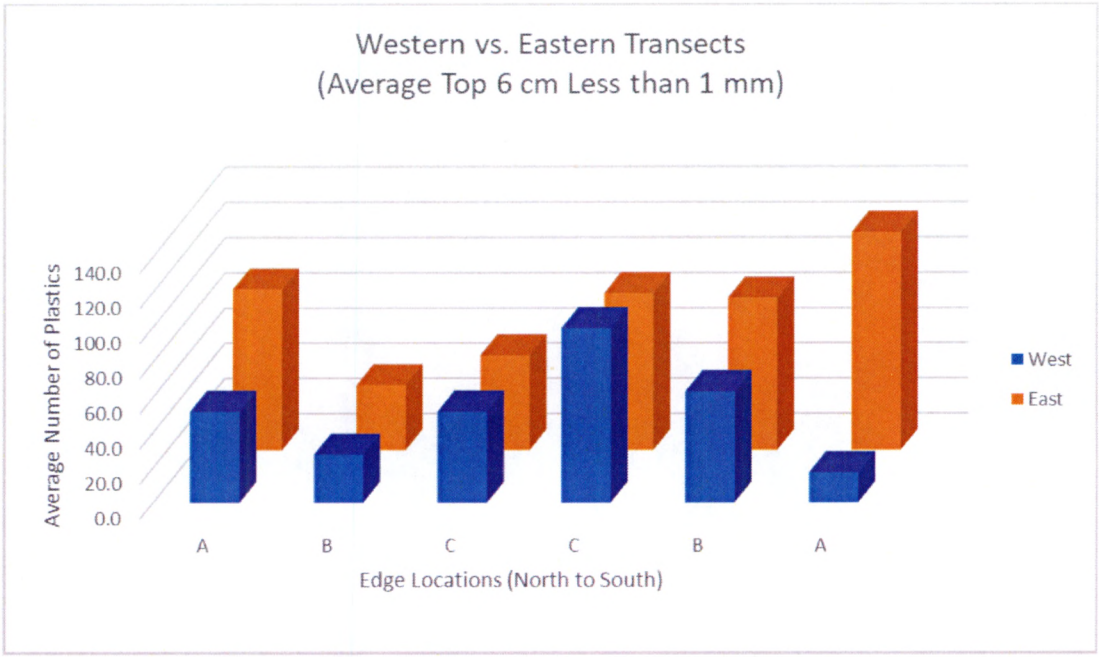

Fig. 13. A boxplot from a two-way ANOVA testing plastics (< 1 mm) above 6 cm against the edge location and east/west position. The distribution of plastics is significantly affected by their east/west position in the marsh ($F_{1,29} = 8.68$ $P < 0.006$), where eastern transects (2 and 4) had a higher abundance of microplastics. Plastic distribution is also more variable between sites than the plastics distributed below 6 cm.

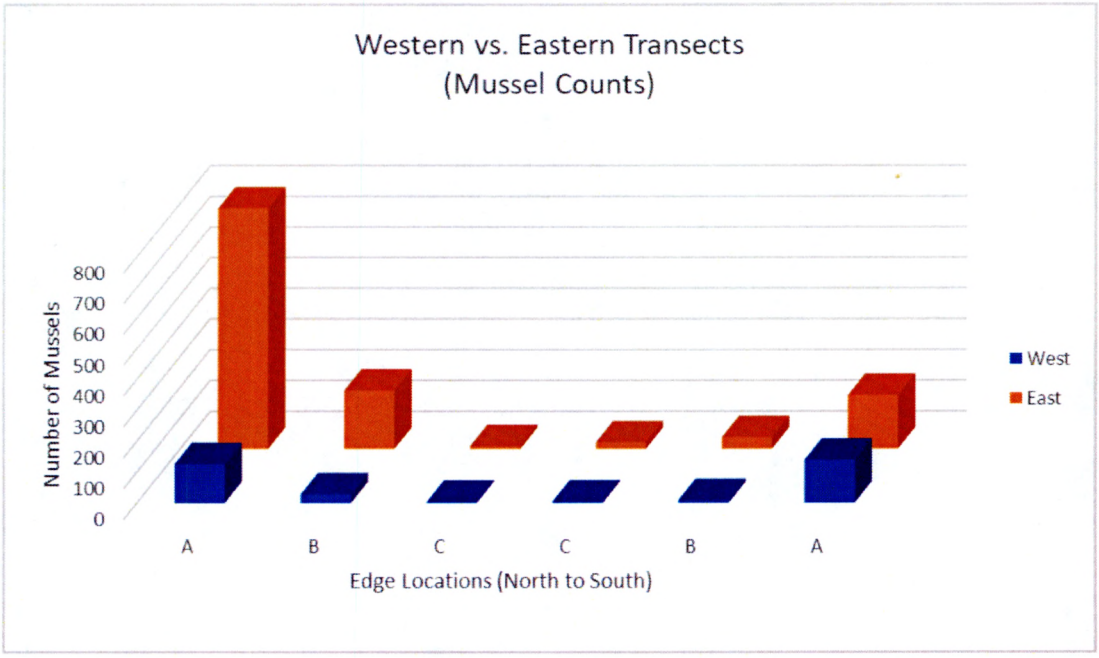

Fig. 14. A plot of the mussel densities recorded at each site. From left to right is north to south. Mussel density was highest along the leading edge (A) of transect 2 in the east (783 mussels/m^2).

Tables

Trans.	Edge	Avg. Plastic	Est. m^2 (Total Core)	Liter (Total Core)	Avg. Plastic (Top 6 cm)	Est. m^2 (Top 6 cm)
1	A	117.67	25,805	258.05	64.33	14,107.57
1	B	55.33	12,133	121.34	32	7,017.60
1	C	148.33	32,528	325.29	57.33	12,572.47
2	A	229.33	50,292	502.92	110.33	24,195.37
2	B	89	19,517	195.18	48.67	10,673.33
2	C	143.67	31,506	315.07	63.33	13,888.27
3	A	51	11,184	111.84	20.67	4,532.93
3	B	122	26,754	267.55	85.33	18,712.87
3	C	217	47,588	475.89	137.33	30,116.47
4	A	193.33	42,397	423.97	159.33	34,941.07
4	B	151	33,114	331.14	107.33	23,537.47
4	C	174.33	38,230	382.31	113.33	24,853.27

Table 1. Microplastic averaged abundances and estimates. The first estimate is based on the average of all the plastic recovered in each core and the surface area of the core (A = 45.6 cm^2). The second estimate is based on the average of all the plastic recovered in each core and the total volume of each core (V = 70.69 cm^3). The last estimate is based on the average of the plastics recovered in the top 6 cm of sediment and the surface area of the core (A = 45.6 cm^2). This last estimate was performed to provide better comparison to other studies, which sample to a depth of 5 cm on average.

Transect	Edge	Position	Avg. Plastic	Sand
1	A	West	117.67	74%
1	B	West	55.33	89%
1	C	West	148.33	97%
2	A	East	229.33	91%
2	B	East	89	94%
2	C	East	143.67	95%
3	A	West	51	97%
3	B	West	122	91%
3	C	West	217	91%
4	A	East	193.33	93%
4	B	East	151	93%
4	C	East	174.33	95%

Table 2. The proportion of sand at each location was based on sediment analysis from a randomly selected core from each site. All sediments consisted of sand with proportions greater than 90%, except for transect 1 edges A and B, where proportions were 74% and 89%, respectively. A simple linear regression comparing the proportion of sand against the number of plastics recovered at each site yielded no significance ($F_{33,69.74} = 0.078$ $P < 0.782$).

Appendix

Transect	Edge	Marsh	Position	# of Mussels	All Plastics	Plastics (≥ 1 mm)	Plastics (< 1 mm)	Top 6 cm (< 1 mm)	Top 6 cm (< 1 mm)	Top 6 cm Totals
1	A	North	West	126	115	21	94	12	58	70
1	A	North	West	126	65	11	54	3	37	40
1	A	North	West	126	173	35	138	22	61	83
1	B	North	West	28	40	11	29	7	21	28
1	B	North	West	28	74	9	65	2	27	29
1	B	North	West	28	52	7	45	4	35	39
1	C	North	West	4	167	14	153	5	68	73
1	C	North	West	4	107	8	99	4	42	46
1	C	North	West	4	171	33	138	7	46	53
2	A	North	East	783	170	17	153	8	75	83
2	A	North	East	783	312	58	254	30	129	159
2	A	North	East	783	206	48	158	18	71	89
2	B	North	East	189	121	29	92	14	62	76
2	B	North	East	189	35	7	28	5	21	26
2	B	North	East	189	111	35	76	16	28	44
2	C	North	East	9	137	14	123	7	66	73
2	C	North	East	9	111	29	82	16	45	61
2	C	North	East	9	183	22	161	5	51	56
3	A	South	West	139	53	15	38	1	13	14
3	A	South	West	139	69	21	48	8	28	36
3	A	South	West	139	31	10	21	1	11	12
3	B	South	West	9	124	33	91	21	71	92
3	B	South	West	9	123	36	87	27	61	88
3	B	South	West	9	119	26	93	18	58	76
3	C	South	West	5	185	45	140	31	91	122
3	C	South	West	5	249	63	186	44	108	152

3	C	South	West	5	N/A	N/A	N/A	26	112	138
4	A	South	East	174	107	25	82	20	65	85
4	A	South	East	174	195	35	160	30	144	174
4	A	South	East	174	278	68	210	56	163	219
4	B	South	East	38	159	29	130	22	106	128
4	B	South	East	38	163	43	120	20	70	90
4	B	South	East	38	131	29	102	20	84	104
4	C	South	East	22	91	22	69	8	40	48
4	C	South	East	22	189	57	132	32	105	137
4	C	South	East	22	243	55	188	32	123	155

Appendix Table 1a – A portion of the original datasheet. Mussel counts are repeated because there was only one count performed at each sample location. All columns following "All Plastics" are a portion of the total number of plastics, and not additional recovered plastics. For example, "Plastics (≥ 1 mm)" represents all the plastics that were 1 mm or greater in diameter from the total number of plastics recovered ("All Plastics") from each core. Also, "Top 6 cm" refers to plastics extracted from the sediment in the top 6 cm of each core. Please note that one core from transect 3 edge C did not fully extend the full 10 cm of sampling depth and therefore portions of the data were omitted.

Transect	Edge	Marsh	Position	# of Mussels	Below 6 cm (≥ 1 mm)	Below 6 cm (< 1 mm)	Below 6 cm Totals
1	A	North	West	126	9	36	45
1	A	North	West	126	8	17	25
1	A	North	West	126	13	77	90
1	B	North	West	28	4	8	12
1	B	North	West	28	7	38	45
1	B	North	West	28	3	10	13
1	C	North	West	4	9	85	94
1	C	North	West	4	4	57	61
1	C	North	West	4	26	92	118
2	A	North	East	783	9	78	87
2	A	North	East	783	28	125	153
2	A	North	East	783	30	87	117

2	B	North	East	189	15	30	45
2	B	North	East	189	2	7	9
2	B	North	East	189	19	48	67
2	C	North	East	9	7	57	64
2	C	North	East	9	13	37	50
2	C	North	East	9	17	110	127
3	A	South	West	139	14	25	39
3	A	South	West	139	13	20	33
3	A	South	West	139	9	10	19
3	B	South	West	9	12	20	32
3	B	South	West	9	9	26	35
3	B	South	West	9	8	35	43
3	C	South	West	5	14	49	63
3	C	South	West	5	19	78	97
3	C	South	West	5	N/A	N/A	N/A
4	A	South	East	174	5	17	22
4	A	South	East	174	5	16	21
4	A	South	East	174	12	47	59
4	B	South	East	38	7	24	31
4	B	South	East	38	23	50	73
4	B	South	East	38	9	18	27
4	C	South	East	22	14	29	43
4	C	South	East	22	25	27	52
4	C	South	East	22	23	65	88

Appendix Table 1b – Continuation of the original datasheet. The first five columns were recopied for easier comparison. This data includes the plastics recovered below 6 cm in depth, which is the portion of plastics recovered from the bottom 4 cm of each core (each core was taken to a depth of 10 cm and split into the top 6 cm and below 6 cm). The data from one core at transect 3 edge C was omitted because the sampling core did not extend the full 10 cm sampling depth.