Microplastic in Two South Carolina Estuaries: Occurrence, Distribution, and Composition

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Abstract

Here we report on the distribution of microplastic contamination in two developed estuaries in the Southeastern United States. Average concentration in intertidal sediments of Charleston Harbor and Winyah Bay, both located in South Carolina, U.S.A., was 413.8±76.7 and 221.0±25.6 particles/m^2, respectively. Average concentration in the sea surface microlayer of Charleston Harbor and Winyah Bay was 6.6±1.3 and 30.8±12.1 particles/L, respectively. Concentration in intertidal sediments of the two estuaries was not significantly different ($p=0.58$), however, Winyah Bay contained significantly more microplastics in the sea surface microlayer ($p=0.02$). While microplastic concentration in these estuaries was comparable to that reported for other estuaries worldwide, Charleston Harbor contained a high abundance of black microplastic fragments believed to be tire wear particles. Our research is the first to survey microplastic contamination in Southeastern U.S. estuaries and to provide insight on the nature and extent of contamination in these habitats.

Introduction

Over the past few years, the occurrence of plastic debris in the environment has gained the attention of not just researchers, but also of policy makers, the general public, and various environmental groups. Much of this attention has focused on the presence, abundance, and fate of microplastics, as well as the potential toxic effects of microplastic exposure to organisms. Microplastics are defined as small plastic particles measuring less than 5 mm in dimension (Van Cauwenberghe et al., 2013; Dris et al., 2015). These particles can be directly released into the environment, or can result from the degradation of large plastic debris. While the degradation of plastic in the environment is generally believed to be a slow process (Eerkes-Medrano et al.,

2015), Weinstein et al. (2016) found that plastic debris in a salt marsh habitat can produce microplastics in as little as 8 weeks.

Coastal and marine ecosystems are particularly susceptible to plastic pollution. Microplastics have been found everywhere from populated urban beaches (Vianello et al., 2013) to deep-sea sediments (Van Cauwenberghe et al., 2013). While the ecological and public health effects of microplastics in the environment have yet to be fully elucidated, exposure to and ingestion of microplastics by aquatic organisms has been linked to decreased energy reserves (Wright et al., 2013), decreased growth (Wertz, 2015), and decreased reproductive output (Au et al., 2015). In addition, microplastic ingestion by aquatic organisms is suspected to serve as a route of human exposure through the consumption of seafood (Van Cauwenberghe and Janssen, 2014).

While a great deal of research investigating the occurrence and effects of microplastics in the oceans has been conducted (reviewed by Auta et al., 2017), fewer studies have investigated the presence and abundance of microplastics in estuarine systems that receive water from inland rivers and streams. Microplastic abundance in inland water was found to be positively correlated to population density and urban development (Eriksen et al., 2013; Yonkos et al., 2014). As the communities surrounding estuaries can be densely populated (Kennish, 2002), estuaries receiving water from inland rivers and streams may serve as a sink for microplastic debris, as often occurs with other contaminants such as metals, hydrocarbons, and pesticides.

Estuaries provide several valuable ecosystem services such as protecting the coastline from erosion and wave action, fixing carbon, and recycling nutrients (Schaafsma and Turner, 2015). Estuarine pollution is particularly problematic as estuaries also provide essential habitat for many commercially and recreationally important species such as crabs, fish, and shellfish. In a review by Van Cauwenberghe et al. (2015), researchers detailed the presence of microplastic

46 particles in marine sediments and found that marine organisms residing in estuaries can ingest
47 microplastic particles, mistaking them for a source of food. In addition, several recent studies
48 have assessed the ingestion of microplastic particles by estuarine invertebrates such as grass
49 shrimp, shore crabs, oysters, and clams (Van Cauwenberghe and Janssen, 2014; Watts et al.,
50 2014; Davidson and Dudas, 2016; Gray and Weinstein, 2017). Results from these studies have
51 indicated that commercially and recreationally important estuarine species can ingest
52 microplastics and that this ingestion can result in mortality and uptake into gill appendages and
53 soft tissues.

54 As top consumers of ocean-based food webs, humans likely accumulate contaminants, which
55 may compromise fecundity, reproduction, and other somatic processes (Bergmann et al., 2015).
56 Similarly, it has been suggested that seafood may serve as a route of microplastic exposure and
57 accumulation in humans (Van Cauwenberghe and Janssen, 2014). While the consequences of
58 microplastic ingestion by humans have not been fully elucidated, it is thought that microplastics
59 may pose a variety of risks including oxidative stress, cell damage, inflammation, and leaching
60 of chemical additives and adsorbed contaminants (Vethaak and Leslie, 2016). For these reasons,
61 it is important to investigate the occurrence of microplastics in estuaries in order to better
62 understand how they may affect the ecosystem services, economic value, and environmental and
63 public health in these areas.

64 Charleston Harbor and Winyah Bay are two estuaries that are located on the coast of South
65 Carolina, U.S.A. whose uses span from recreational to agricultural. The present study
66 investigated the abundance, distribution, and composition of microplastics in intertidal sediments
67 and in the sea surface microlayer at both locations. These estuaries are surrounded by coastal
68 communities and may serve as sinks for microplastic pollution originating from a variety of point

and nonpoint sources. Therefore, understanding the abundance of microplastics in these two locations can help identify contributing sources of microplastics as well as inform residents, researchers, and policy makers about their potential hazards.

Materials and Methods

Study sites

Charleston Harbor (32° 49' 7.1" N, 79° 55' 40.41" W) is an inlet of the Atlantic Ocean and is formed by the confluence of the Ashley River, the Cooper River, and the Wando River in Charleston County, SC (population 396,484) (United States Census Bureau, 2016a). It is a partially mixed estuary that serves as part of the intercoastal waterway and has an estuarine drainage area of 3,113 km^2. The population surrounding the entire watershed of Charleston Harbor is 664,607 people (Charleston Waterkeeper, 2014). The harbor has several competing uses including industrial, tourism, commercial, and recreational activities. Along the rivers that drain into the harbor, there are several industrial facilities that include petrochemical, ink and pigment, and paper and packaging manufacturers. Inside the harbor, there are several shipyards that receive contents from cargo ships. In addition, Charleston Harbor is home to the fastest growing U.S. port (South Carolina Ports Authority, 2015).

Winyah Bay (33° 17' 28.32"N, 79° 16' 32.16"W) is the fourth largest estuary on the Eastern coast of the U.S. in terms of discharge rate, with an estuarine drainage area of 24,633 km^2 (Voulgaris et al., 2002) and is the state's largest tidal freshwater wetlands (The Nature Conservancy in South Carolina Winyah Bay, 2015). Winyah Bay is also an inlet of the Atlantic Ocean and is formed by the confluence of the Waccamaw River, Pee Dee River, Black River, and Sampit River in Georgetown County, SC (population 60,804) (United States Census Bureau, 2016b).

The population surrounding the entire watershed of Winyah Bay is 227,200 people (SC DNR, 2009). Winyah Bay has several competing uses including industrial, recreational, and agricultural activities. The five lakes that drain into the watershed are used for industrial and recreational purposes, supplying power, and supplying irrigation (SC DNR, 2009). A majority of the water that drains into Winyah Bay is used for thermoelectric power (83.5%), industry (10.0%) and water supply (6.0%) (SC DNR, 2009).

Sampling for the present study occurred in both Charleston Harbor and Winyah Bay. Intertidal sediment was collected from five sites within Charleston Harbor (Fig.1; Table 1) and five sites within Winyah Bay (Fig. 2; Table 2). Sea surface microlayer samples (n=1) were collected from six sites in Charleston Harbor (Fig. 1; Table 1) and six sites in Winyah Bay (Fig. 2; Table 2). Sample sites were selected to be upstream of the estuary, below the confluence of the rivers feeding the estuary, in the middle of the estuary, and near the mouth of the estuary emptying into the Atlantic Ocean. Sampling in Charleston Harbor and Winyah Bay occurred June through August 2014. The average tidal range of Charleston Harbor and Winyah Bay is 1.5 m and 1.4 m, respectively.

Sediment sampling

At each site, the beach was measured using satellite imagery from Google Earth. Three vertical transects were pre-determined and evenly spaced along the length of the beach. Sampling was conducted at low tide. Transects extended from the low tide line to the supralittoral zone. Sediment was removed from four quadrats (0.25 m x 0.25 m) along each transect within the low intertidal zone, high intertidal zone, high tide line, and the supralittoral zone. At each site, a sample size of n=12 was collected, except for two sites in Charleston Harbor. A supralittoral zone was not present at the Crab Bank and Shute's Folly sample sites in Charleston Harbor and

114 therefore was not sampled, resulting in a sample size of n=9 for those two sites (Table 1). To
115 determine the low intertidal and high intertidal zones, the distance from the water to the high tide
116 line was measured and then divided in half. Quadrats within each zone were selected using a
117 random number generator. A total of 54 intertidal sediment samples were collected from
118 Charleston Harbor and 60 intertidal sediment samples were collected from Winyah Bay.

119 The top 2 cm of sediment was removed from the quadrats using a stainless steel trowel. This
120 sediment depth was within the range of past studies investigating microplastics in sediment,
121 reported in a review by Hidlago-Ruz et al. (2012). At each site, sediment was placed into
122 stainless steel buckets, weighed, and processed according to the density separation procedure
123 reported by Thompson and colleagues (Thompson et al., 2004). Specifically, 4 L of seawater was
124 added to the collected sediment and was mixed with 800 g of NaCl to make a supersaline
125 solution (Fok and Cheung, 2015; Karlsson et al., 2017). The resulting mixture was stirred for 2
126 minutes using a stainless steel trowel and was allowed to settle for 2 minutes. Following the 2-
127 minute settling period, the supernatant was poured through a series of nested sieves (500, 150,
128 and 63 μm). The items retained on the sieves were rinsed into 200 mL amber glass jars and were
129 taken to the laboratory where they were then treated with 10 mL of 30% H_2O_2 and allowed to sit
130 for one week to remove natural organic material (Nuelle et al., 2014). The resulting density of
131 the supersaline solution was 1.16±0.01g/mL. The density of this solution allowed for the
132 recovery of plastics that were less dense such as polyethylene (PE), polystyrene (PS),
133 polypropylene (PP), low density polyethylene (LDPE), high density polyethylene (HDPE), and
134 nylon. Denser plastic polymers such as polyvinyl chloride (PVC) and polyethylene terephthalate
135 (PET) were not likely to be recovered with this protocol.

Following treatment with H_2O_2, each sample was rinsed on a 38 μm sieve, then poured into a glass crystalizing dish and examined under a dissecting microscope. Plastic particles were counted and archived in 20 mL clear glass vials. Color, size (63-149, 150-499, ≥500 μm), and shape were all noted. Shapes that were identified included: fragments, fibers, foam, and spheres. Shapes were classified in accordance with the definitions provided by Hidalgo-Ruz et al. (2012). The polymer composition of a subset of particles (n=80) collected from intertidal sediments was determined using Fourier Transform Infrared Spectroscopy (FT-IR) operating in Attenuated Total Reflectance (ATR) mode and compared to spectra of known plastic polymers using a Bruker ALPHA FT-IR spectrometer (Bruker Optik GmbH, Ettlingen, Germany). The subset of particles was chosen to represent a variety of shapes, sizes, and colors.

Sea surface microlayer

At each site, the sea surface microlayer was sampled for microplastics using a sea surface microlayer collection apparatus (Intergovernmental Oceanographic Commission, 1985). The apparatus consisted of an aluminum frame (0.5 m x 0.5 m) fitted with 2 mm stainless steel mesh. To collect samples, the apparatus was dipped onto the surface of the water and then drained into a stainless steel funnel which emptied into a 4 L amber glass jar. Samples were only collected during calm conditions when the sea surface microlayer was undisturbed. A total of 4 L of sea surface microlayer water was collected from each site, with each dip of the collection apparatus yielding approximately 75 mL of water. One 4-L sea surface microlayer sample was collected from each of the 6 sampling sites within Charleston Harbor and Winyah Bay, resulting in a total of 6 4-L sea surface microlayer samples collected from each estuary. These samples were transported back to the laboratory where the water was then poured through a series of nested sieves (500, 150, and 63 μm). The particles retained on the sieves were processed and

159 enumerated as described for sediment sampling. Because so few particles were collected from
160 the sea surface microlayer, only particles collected from intertidal sediments were analyzed using
161 FT-IR.

162 **Quality assurance/quality control**

163 To minimize contamination at each sampling site, stainless steel and glass equipment was used
164 to collect and store intertidal sediment and sea surface microlayer samples. Sampling was
165 conducted during calm conditions to minimize potential atmospheric deposition. Because water
166 from the field was used during the density separation procedure, potential contamination of
167 plastic particles from the water was quantified. Field-collected blanks (n=14) contained an
168 average (mean ± SE) of 0.54±0.17 particles/L. To minimize contamination within the laboratory,
169 nitrile gloves and white cotton laboratory coats were worn at all times to prevent plastic
170 contamination from clothing. Potential plastic contamination within the laboratory was
171 quantified using blanks. Laboratory blanks (n=46) contained an average (mean ± SE) of
172 0.74±0.16 particles. The data reported hereafter were not corrected for procedural contamination
173 nor were they corrected following FT-IR analysis. To determine the extraction efficiency of the
174 amended density separation procedure from Thompson et al. (2004) used in the present study,
175 extractions in the laboratory were performed with three replicates of sediments spiked with 100
176 polyethylene microbeads (165 μm). This procedure recovered 87.0% of the plastics within the
177 spiked sediment samples.

178

179 **Statistical analyses**

Differences in microplastic abundance among sites within each estuary and between Charleston Harbor and Winyah Bay were analyzed using Kruskal-Wallis and Kruskal-Wallis multiple comparison nonparametric tests. Differences among microplastic size fractions (63-149, 150-499, ≥500 μm), shape (foam, fiber, fragment, sphere), and tidal distribution (low intertidal, high intertidal, high tide, supralittoral) were also analyzed using Kruskal-Wallis and Kruskal-Wallis multiple comparison nonparametric tests. Microplastic concentrations were analyzed as both particles/m^2 and particles/kg wet weight (w.w.). As microplastic concentrations normalized by weight (particles/kg w.w.) corroborated the results of microplastic concentrations per unit area (particles/m^2), only microplastic concentrations per unit area are reported for intertidal sediment samples. Microplastic concentrations per unit volume (particles/L) are reported for sea surface microlayer samples. Unless otherwise indicated, values represent mean ± SE. Statistical analyses were carried out using the statistical software R (version 3.3.3) with $\alpha = 0.05$.

Results

Charleston Harbor

194 Microplastic particles were present in 98.1% of intertidal sediment samples collected in
195 Charleston Harbor, and consisted of a variety of sizes (>63 μm), shapes, and colors (Table 3).
196 The harbor-wide average concentration of microplastic in intertidal sediments was 413.8±76.7
197 particles/m^2. Concentrations ranged from a high of 1195.7±193.9 particles/m^2 at Daniel Island to
198 a low of 42.2±8.5 particles/m^2 at Shute's Folly (Fig. 4A). Daniel Island contained significantly
199 more microplastic particles than Crab Bank, Grice Cove, and Shute's Folly (X_2=36.0, df=4,
200 p<0.0001, Fig. 4A). The high concentration of microplastic at Daniel Island heavily influenced
201 the harbor-wide average of Charleston Harbor.

202 There was no significant difference in the concentration of microplastic among tidal zones within
203 each Charleston Harbor sample site, nor was there a significant difference in the concentration of
204 microplastic among tidal zones for the pooled data (X_2=2.1, df=3, p=0.54, Fig. 6A). At Daniel
205 Island, there was a significant difference in the abundance of microplastic particles among size
206 fractions (63-149, 150-499, ≥500 μm) with significantly more particles in the 150-499 μm size
207 fraction than in either the 63-149 or ≥500 μm size fractions (X_2=16.4, df=2, p=0.0003). When
208 the data were pooled, however, there was no significant difference in microplastic concentration
209 among size fractions in Charleston Harbor (X_2=2.7, df=2, p=0.25, Fig. 6B).

210 Fragments (Fig. 3A) were the most common type of microplastic particles found in Charleston
211 Harbor intertidal sediments, constituting 76.2% of total microplastic collected (Table 3). The
212 majority of fragments were black in color (95.8%) (Table 3). The second most abundant type of
213 particle in Charleston Harbor were foam particles (Fig. 3B), constituting 18.9% of total
214 microplastic collected. Most foam particles (98.0%) were white (Table 3). Fibers (Fig. 3C)
215 constituted 3.9% of total microplastic collected, with blue fibers being the dominant color
216 (54.0%) (Table 3). Spheres (Fig. 3D) constituted 1.0% of total microplastic collected, with green

spheres being the dominant color (88.9%) (Table 3). At each sampling site, the concentration of fragments was significantly higher than the concentration of fibers and spheres. This trend was also evident in the pooled data for the harbor (X_2=91.7, df=3, p<0.0001, Fig. 7). Dominant particle colors included black, blue, colorless (translucent), gray, green, red, and white. Other colors included orange, brown, purple, and yellow, however these colors were observed at lower frequencies (Table 3).

Microplastic particles (>63 µm) were present in 100.0% of sea surface microlayer samples collected in Charleston Harbor. The harbor-wide average concentration of microplastic in the sea surface microlayer was 6.6±1.3 particles/L. Concentrations ranged from a high of 11 particles/L at Cooper River to a low of 3 particles/L at Ashley River (Fig. 5A). The most abundant type of particle in the sea surface microlayer of Charleston Harbor were fibers, constituting 56.0% of total microplastic particles collected (Table 5). Fragments constituted 26.4% of total microplastic collected in the Charleston Harbor sea surface microlayer while foam constituted 15.1% and spheres constituted 2.5% (Table 5). The concentration of fibers was significantly greater than the concentration of spheres (X_2=11.69, df=3, p=0.009, Fig. 8A). There was no significant difference in the abundance of particles among size fractions for the pooled data for the harbor (X_2=4.1, df=2, p=0.13, Fig. 8B).

Winyah Bay

Microplastic particles (>63 µm) were present in 98.3% of sediment samples collected in Winyah Bay (Table 4). The bay-wide average concentration of microplastic in intertidal sediments was 221.0±25.6 particles/m^2. Concentrations ranged from a high of 440.7±71.8 particles/m^2 at Sand Island to a low of 51.3±6.2 particles/m^2 at Malody Bush. Sand Island, Oak Island, and North

239 Island contained significantly more microplastic particles than East Bay Park and Malody Bush
240 (X_2=37.4, df=4, p<0.0001, Fig. 4B).

241 There was no significant difference in the concentration of microplastic among tidal zones for
242 each sample site in Winyah Bay, nor was there a significant difference in the concentration of
243 microplastic among tidal zones for the pooled intertidal sediment data (X_2=1.3, df=3, p=0.74,
244 Fig. 6A). At each sampling site in Winyah Bay, as well as for the pooled Winyah Bay data, the
245 concentration of microplastics in the 63-149 and 150-499 µm size fractions was significantly
246 higher than the concentration of particles ≥500 µm (X_2=40.5, df=2, p<0.0001, Fig. 6B).

247 Fragments were the most common type of microplastic particles found in Winyah Bay intertidal
248 sediments, constituting 77.5% of total microplastic collected (Table 4). The majority of
249 fragments were black in color (90.0%) (Table 4). The second most abundant type of particle in
250 Winyah Bay were fibers, constituting 17.6% of total microplastic collected (Table 4). The
251 majority of fibers (77.4%) were blue (Table 4). Foam particles constituted 3.6% of total
252 microplastic collected, with white foam being the dominant color (99.2%) (Table 4). Spheres
253 constituted 1.2% of total microplastic collected, with red spheres being the dominant color
254 (39.5%) (Table 4). The concentration of fragments was significantly higher than the
255 concentration of foam and spheres at each sampling site, as well as for the pooled intertidal
256 sediment data (X_2=112.1, df=3, p<0.0001, Fig. 7). Dominant particle colors included black, blue,
257 colorless (translucent), gray, green, red, and white. Other colors included orange, brown, purple,
258 and yellow, however these colors were observed at lower frequencies (Table 4).

259 Microplastic particles (>63 µm) were present in 100.0% of sea surface microlayer
260 samples collected in Winyah Bay. The bay-wide average concentration of microplastic in the sea
261 surface microlayer was 30.8±12.1 particles/L. Concentrations ranged from a high of 88

262 particles/L in the middle of the harbor to a low of 6 particles/L at Mud Bank (Fig. 5B). The most
263 abundant type of particle in the sea surface microlayer of Winyah Bay were fragments,
264 constituting 63.4% of total microplastic particles collected (Table 5). Foam particles constituted
265 32.7% of total microplastic collected in the Winyah Bay sea surface microlayer while fibers
266 constituted 3.4% and spheres constituted 0.5% (Table 5). The concentration of fragments was
267 significantly greater than the concentration of spheres (X_2=13.3, df=3, p=0.004, Fig. 8A). There
268 was no significant difference in the abundance of particles among size fractions for the Winyah
269 Bay sea surface microlayer pooled data (X_2=1.1, df=2, p=0.57, Fig. 8B).

270 **Comparison of Winyah Bay and Charleston Harbor**

271 The concentration of intertidal microplastic in Charleston Harbor (414.0±77.0 particles/m^2) was
272 not significantly different than the concentration of intertidal microplastic in Winyah Bay
273 (221.0±26.0 particles/m^2) (X_2=0.30, df=1, p=0.59, Fig. 9). The concentration of microplastic
274 particles in the sea surface microlayer of Winyah Bay, however, was significantly greater than
275 the concentration of microplastic particles in the sea surface microlayer of Charleston Harbor
276 (X_2=5.8, df=1, p=0.02, Fig. 9).

277 **Microplastic composition**

278 A subset of microplastic particles (n=80) collected from Charleston Harbor intertidal sediments
279 was analyzed using FT-IR and compared with reference spectra. Shape characteristics of
280 microplastics identified in the present study were based on the definitions provided by Hidalgo-
281 Ruz et al. (2012) (Fig. 3A-D). Due to the limitations of the instrument, only particles ≥500 µm in
282 dimension were analyzed. Within the subset of samples analyzed with FT-IR, 90% were
283 positively identified as plastic polymers.

284 Most foam particles (n=51, 98.0%) were positively identified as polystyrene. Only one particle
285 that was visually identified as foam was not plastic material. Most of the fragments analyzed
286 (n=27, 95.0%) were positively identified as plastic material. Fragments were a variety of colors
287 including black, blue, colorless, or red. Black fragments (n=16) were positively identified as a
288 polyamide composite or nylon (56.0%), polyester (19.0%), non-plastic material (19.0%), and
289 polyethylene (6.0%). Blue fragments (n=3) were identified as polyethylene (66.0%) and
290 polypropylene (33.0%). Red fragments (n=2) were identified as polypropylene, and colorless
291 fragments (n=6) were identified as both polyethylene (83.0%) and polypropylene (16.0%). Most
292 fibers were too small to identify polymer type. One white fiber was greater than 500 µm and
293 was identified as polyethylene. Similarly, the majority of spheres were too small to identify
294 polymer type using FT-IR. However, one green sphere was analyzed and identified as
295 polyethylene.

296 ***Discussion***

297 Microplastic particles were found in intertidal sediments and in the sea surface microlayer at
298 each sampling site in Charleston Harbor and Winyah Bay. While few studies have investigated
299 the occurrence and distribution of microplastics in U.S. estuaries (McDermid and McMullen,
300 2004; Steve, 2014; Yonkos et al., 2014; Wessel et al., 2016), results from the present study
301 demonstrate that Charleston Harbor and Winyah Bay have similar levels of microplastics relative
302 to other U.S. estuaries. For example, microplastic abundance reported by Wessel et al. (2016) in
303 beach sediments of Mobile Bay, Alabama ranged from 5-117 particles/m^2 with an average of
304 50.6±9.96 particles/m^2 at marine-influenced sites and 13.2±2.96 particles/m^2 at freshwater-
305 dominated sites. By comparison, microplastic abundance in Charleston Harbor intertidal
306 sediments ranged from 0-2524 particles/m^2 with an average of 413.8±76.7 particles/m^2, and

307 microplastic abundance in Winyah Bay ranged from 0-796 particles/m^2 with an average
308 concentration of 221.0±25.6 particles/m^2.

309 The concentration of microplastics in Charleston Harbor and Winyah Bay is also comparable to
310 recent studies investigating the occurrence and distribution of microplastics in estuaries globally.
311 For example, microplastic concentrations in the sea surface microlayer near Goeje Island and
312 Jinhae Bay, South Korea were reported to be 16±14 particles/L and 88±68 particles/L,
313 respectively (Song et al., 2014; Song et al., 2015). By comparison, the concentration of
314 microplastics in the sea surface microlayer of Charleston Harbor ranged from 3-11 particles/L
315 with an average concentration of 6.6±1.3 particles/L, and the concentration of microplastics in
316 the sea surface microlayer of Winyah Bay ranged from 6-88 particles/L with an average
317 concentration of 30.8±12.1 particles/L. In addition, De Carvalho and Neto (2016) investigated
318 beach sediments in Brazil and found microplastic concentrations ranged from 12-1300
319 particles/m^2 which is comparable to Charleston Harbor and Winyah Bay. Furthermore, Sruthy
320 and Ramasamy (2016) reported a mean abundance of 252.80±25.76 particles/m^2 in the sediments
321 of Vembanad Lake, a brackish wetland ecosystem in southern India. Vembanad Lake is the
322 largest wetland system in India, with a surrounding population of 1.6 million people. Given that
323 microplastic concentrations in both Charleston Harbor and Winyah Bay were comparable to
324 Vembanad Lake—a body of water whose surrounding population is 4-27 times greater than the
325 two locations of the present study—suggests that the relative contributions of various sources of
326 microplastic is different between Vembanad Lake and coastal South Carolina. This underscores
327 the fact that population size alone may not determine the level of microplastic pollution within
328 an area. Other factors that may contribute to differences in microplastic abundance among

329 geographic locations include differences in prevailing winds and currents, urbanization,
330 socioeconomics, and solid waste management infrastructure.

331 In the present study, the size of the water shed may be another factor contributing to differences
332 in microplastic abundance between locations. While the population surrounding Charleston
333 Harbor (396,484 people) is greater than that of Winyah Bay (60,804 people), Winyah Bay's
334 watershed is greater than Charleston Harbor's. Winyah Bay has an estuarine drainage area of
335 24,633 km^2 and is the terminus of the Yadkin-Pee Dee River Basin which is the second largest
336 river basin originating in North Carolina (SC DNR, 2009). In comparison, Charleston Harbor has
337 an estuarine drainage area of 3,113 km^2. Although the drainage area of Winyah Bay is greater
338 than Charleston Harbor, we initially suspected that the population of the surrounding areas would
339 be a greater influence to microplastic pollution in each estuary. These results suggest, however,
340 that the contribution of the entire drainage area of an estuary, rather than only the surrounding
341 population, may need to be considered when investigating sources of coastal microplastic
342 pollution. The greater input Winyah Bay receives from the Yadkin-Pee Dee River Basin may
343 account for the significantly higher concentration of microplastics in the sea surface microlayer
344 of Winyah Bay compared to Charleston Harbor.

345 Intertidal sediments contained a greater amount of microplastics than the sea surface
346 microlayer in both Charleston Harbor and Winyah Bay. The differences in microplastic
347 concentration within the intertidal sediments and the sea surface microlayer may be explained by
348 the residence times of microplastics in these two environmental compartments. Microplastics
349 deposited in the sediment along a shoreline would be expected to accumulate over time, such that
350 samples taken there measure the long-term occurrence of microplastics. In contrast,
351 microplastics in estuarine surface waters represent recent, transient inputs of microplastics. For

352 estuaries, the residence time of microplastics in surface waters would be a function of the
353 flushing rate of the estuary. The flushing rate of Charleston Harbor is 5 days, and the flushing
354 rate of Winyah Bay is 7 days (Bricker et al., 1999; Lawrenz et al., 2010). This difference in
355 flushing rate may also influence the difference in microplastic concentration within the sea
356 surface microlayer observed between estuaries.

357 Neither Charleston Harbor nor Winyah Bay exhibited significant differences in the concentration
358 of microplastic particles among tidal zones (low intertidal, high intertidal, high tide,
359 supralittoral). Previous studies have documented higher abundances of microplastics in the
360 supralittoral zone and at the high tide line (Turner and Holmes, 2011; Hidalgo-Ruz et al., 2012).
361 Those previous studies, however, collected samples from high-energy beaches experiencing
362 intense wave action, whereas the sites sampled in Charleston Harbor and Winyah Bay do not
363 typically experience high-energy waves. This difference may account for the uniform
364 distribution of microplastics throughout the intertidal zone observed in the present study.

365 Fragments were the most abundant microplastic particle type recovered in intertidal sediments of
366 Charleston Harbor and Winyah Bay. These findings differ from previous studies that examined
367 harbor or mangrove sediments, where fibers were the most prevalent microplastic particle type
368 (Thompson et al., 2004; Claessens et al., 2011; Nor and Obbard, 2013). Fibers did, however,
369 constitute the most abundant particles recovered from the sea surface microlayer in Charleston
370 Harbor. High fiber-count synthetic materials (such as fleece) can shed greater than 1,900 fibers
371 per garment per machine wash load (Browne et al., 2010). These fibers can pass through
372 wastewater treatment facilities and enter the environment. Charleston Harbor, which has a
373 greater surrounding population density than Winyah Bay, has four NPDES-permitted waste
374 water treatment plant (WWTP) end pipes in the harbor. In contrast, Winyah Bay has only one

NPDES-permitted WWTP end pipe in the bay. As such, it is possible that the fibers collected in the sea surface microlayer of Charleston Harbor were released from WWTPs and that the greater number of WWTPs in Charleston Harbor accounted for the greater abundance of fibers compared to Winyah Bay. These results corroborate those of a recent study investigating the removal of microplastics by WWTPs in Charleston, SC which determined that blue microplastic fibers were most often released from WWTPs (Conley, 2017).

While there is only one permitted NPDES end pipe that drains into Winyah Bay, there are 15 total NPDES permit sites in Georgetown County—the county in which Winyah Bay is located—that discharge effluent into the rivers that ultimately drain into Winyah Bay (6 sites into the Sampit River, 3 sites into the Waccamaw River, 2 sites into the Black River, and 1 site into the North Santee River) (Waccamaw Region Section 208 Water Quality Management Plan, 2011). Beyond Georgetown County, several WWTPs discharge into the watershed including: Conway WWTP, Pawley's Island WWTP, Murrells Inlet WWTP, Schwartz WWTP, North Myrtle Beach Crescent Beach WWTP, and the George R. Vereen WWTP. These WWTPs provide an avenue for microplastic particles to enter Winyah Bay and may also contribute to the greater concentration of microplastics in the sea surface microlayer of Winyah Bay compared to Charleston Harbor

Microplastic concentrations varied among sampling sites within both Charleston Harbor and Winyah Bay. In Charleston Harbor intertidal sediments, microplastic concentration ranged from 42.2±8.5 particles/m^2 at Shute's Folly to 1195.7±193.9 particles/m^2 at Daniel Island. In Winyah Bay intertidal sediments, concentrations ranged from 51.3±6.2 particles/m^2 at Malody Bush to 440.7±71.8 particles/m^2 at Sand Island. In the sea surface microlayer, the concentration of microplastics in Charleston Harbor ranged from 3-11 particles/L and the concentration of

398 microplastics in Winyah Bay ranged from 6-88 particles/L. The greatest microplastic
399 concentrations in intertidal sediments were found at Daniel Island in Charleston Harbor which is
400 situated in the inner harbor at the confluence of the Cooper and Wando Rivers. This variation in
401 microplastic concentration among sampling sites within each estuary may have been a result of
402 differences in currents, winds, or point sources of microplastic input near the sampling site.

403 As previously mentioned, the majority of the microplastics collected in Charleston Harbor and
404 Winyah Bay were fragments. Fragments constituted 76.2% of the total microplastic particles
405 collected in Charleston Harbor, 95.8% of which were black fragments. Similarly, fragments were
406 the dominant particle type in Winyah Bay constituting 77.4% of collected microplastics, 89.9%
407 of which were black fragments. While black fragments were found at all of the sample sites in
408 Charleston Harbor, the abundance of black fragments at Daniel Island was one to two orders of
409 magnitude greater than all of the other sites in Charleston Harbor and Winyah Bay. Polymer
410 analysis of black fragments ≥500 µm (n=16) using FT-IR determined that 56.0% were a
411 polyamide composite (nylon), 19.0% were polyester, 19.0% were non-plastic material, and 6.0%
412 were polyethylene. Only 6.7% of the collected black fragments were within the ≥500 µm size
413 fraction and could be analyzed using FT-IR. The majority of black fragments (62.4%) were
414 within the 150-499 µm size fraction, while 30.8% were within the 63-149 µm size fraction.
415 While instrument limitations allowed only particles ≥500 µm to be analyzed using FT-IR, the
416 evidence suggests that these microplastics (≥500 µm) have a variety of sources in these two
417 estuaries.

418 The macroplastic litter in Charleston Harbor consists of mostly single-use consumer products
419 composed of polystyrene, polyethylene terephthalate, polypropylene, and high-density
420 polyethylene (Wertz, 2015). The polymer analysis of particles ≥500 µm conducted in the present

421 study indicated that a majority of these microplastics were composed of polystyrene,
422 polyethylene, polypropylene, and polyamide composite. That the polymer composition of these
423 microplastics in Charleston Harbor is consistent with the polymer composition of macroplastics
424 in Charleston Harbor suggests that these microplastics are secondary microplastics produced by
425 the fragmentation of macroplastic litter in Charleston Harbor.

426 A remaining mystery, however, is the source and identity of the high proportion of collected
427 black fragments within the 150-499 µm size fraction, which we were unable to analyze using
428 FT-IR due to the particle size limitations of the instrument. The shape and morphological
429 characteristics of the black fragments collected in both Charleston Harbor and Winyah Bay,
430 however, are consistent with tire wear particles produced by the abrasion of tires on roadway
431 surfaces (Wik and Dave, 2009). This is a unique finding because, to the best of our knowledge,
432 these black fragments have not been reported in any microplastic field studies to date in the
433 United States. While the sources of microplastic input into each estuary were not investigated in
434 the present study, these data provide a foundation for future studies to assess the point and
435 nonpoint sources contributing to microplastic pollution, and in particular, the sources and
436 pathways by which these small black fragments enter into these two estuaries.

437 The presence of microplastics within estuaries is a cause for concern for several reasons.
438 Laboratory studies suggest that microplastic exposure and ingestion can alter organism
439 development, induce acute toxicity, and alter organism energetics. For example, Rochman et al.
440 (2014) found that Japanese medaka exhibited altered gene expression when exposed to virgin
441 and marine microplastics and that after exposure, there was a significant down regulation of
442 vitellogenin (Vtg 1). Similarly Sussarellu et al. (2015) investigated the developmental effects of
443 oysters when exposed to polystyrene microbeads and found that after exposure oysters had

444 significant decreases in oocyte number (−38%), oocyte diameter (−5%), and sperm velocity
445 (−23%). Gray and Weinstein (2017) found microplastics of various polymers, shapes, and sizes
446 to be acutely toxic to adult daggerblade grass shrimp when ingested. In addition, Watts et al.
447 (2014) found that shore crabs that ingested microplastic particles showed reduced food
448 consumption and energy. Together, these studies demonstrate that microplastics pose hazards to
449 marine organisms. In the environment, these hazards may translate into population- and
450 ecosystem-level effects such as regime shifts within respective habitats if certain populations of a
451 species decline due to microplastic pollution. These shifts may also alter the ability of the estuary
452 to support the wildlife that reside in that habitat as well as those that frequently visit such as
453 migratory birds.

454 The presence of microplastics in coastal ecosystems also has implications that reach far beyond
455 potential hazards for marine wildlife. Microplastics in the environment can impact ecosystems,
456 economies, and human health. As the population in coastal areas grows and these regions
457 become more developed, there is a potential for their ability to provide valuable ecosystem
458 services to become compromised. Charleston Harbor is currently undergoing a deepening project
459 where the harbor will be deepened from 48 ft to 52 ft, making it the deepest navigation channel
460 on the East Coast of the U.S. In addition, the city of Charleston is undergoing rapid population
461 growth and has recently become South Carolina's largest city. The present study provides
462 baseline data for microplastic abundance in Charleston Harbor and in Winyah Bay. These data
463 can be used to inform our future understanding of how increased population growth and shipping
464 traffic may affect microplastic accumulation in developed coastal environments.

465 Coastal ecosystems that rely on estuaries to support their economy, such as Charleston
466 Harbor and Winyah Bay, can be affected by microplastic pollution in these habitats (Waycott et

al., 2009). As plastic pollution increases, the incidence of microplastic pollution in these habitats will increase. The ingestion of environmental microplastics, subsequent trophic transfer, and potential for human exposure are beginning to be elucidated. For example, microplastics have been shown to be transferred from mussels to crabs through the food chain (Li et at., 2015). In addition, Payton (2017) found that local fish species (croaker, flounder, mullet, red drum, sea trout, spot, and whiting) caught within Charleston Harbor had microplastics present within their intestines. Furthermore, research has shown that nanoplastics can translocate into the tissues of organisms (Bouwmeester et al., 2015; Mattsson et al., 2015). These results suggest that trophic transfer of microplastics through coastal food webs may result in microplastic exposure to consumers. Certainly, the risk associated with such exposures should be further investigated.

While the present study provides the first comprehensive survey of microplastic abundance in Charleston Harbor and in Winyah Bay, several limitations of the study should be noted. The density separation procedure was shown to recover 87.0% of microplastics from sediments, suggesting that this method of extraction likely underestimated the total abundance of microplastics present. Plastic particles such as PVC and PET that were denser than the saturated NaCl solution (1.16 g/mL) were not likely to be recovered through this method. This procedure was used in an effort to remain consistent with previous sampling of microplastics conducted over the past five years in Charleston Harbor. In addition, only particles ≥500 μm were able to be analyzed for their polymer composition using FT-IR. These microplastics that were ≥500 μm do not necessarily represent the smaller size fraction microplastics, and it cannot be assumed that particles <500 μm were of the same polymer composition.

Conclusion

Results from the present study demonstrate that microplastic particles are widely distributed and abundant in the intertidal sediments and sea surface microlayer of two Southeastern U.S. estuaries. This work provides baseline data for monitoring microplastic concentration in Charleston Harbor and Winyah Bay over time, and also serves as a foundation for understanding the sources, fate, and hazards associated with microplastics. Future monitoring of microplastics is especially important in Charleston Harbor as the dredging of the port may affect the concentration of microplastics between the intertidal sediment and sea surface microlayer and may reintroduce buried microplastics into the ecosystem, making them bioavailable to estuarine organisms. This work can help support future studies that investigate the sources that contribute to microplastics in these two areas as well as the potential sources of the small black fragments that were found in high abundance.

Acknowledgments

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Data availability

509 Data, associated metadata, and calculation tools are available on our online GIS inventory
510 database https://www.gisinventory.net/GISI-26305-Oceanographic-Surveys-A-field-survey-of-
511 microplastic-pollution-63-500-microns-in-Charleston-Harbor-and-Winyah-Bay-wa.html

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Figure Legends

Figure 1. Sampling sites in Charleston Harbor. Open circles represent sites where sea surface microlayer samples were collected (1-Cooper River, 2-Ashley River, 3-James Island Creek, 4-Middle of Harbor, 5-Wando River, 6-Shem Creek). Closed circles represent sites where intertidal sediments were collected (1-Daniel Island, 2-Shute's Folly, 3-Crab Bank, 4-Grice Cove, 5-Sullivan's Island). Map made with ArcGIS Map 10.4.1.

Figure 2. Sampling sites in Winyah Bay. Open circles represent sites where sea surface microlayer samples were collected (1-Sampit River, 2-Pee Dee, 3-Waccamaw River, 4-Middle of Harbor, 5-Mudbank, 6-Near Inlet). Closed circles represent sites where intertidal sediments were collected. (1-East Bay Park, 2-Malody Bush, 3-Oak Island, 4-North Island, 5-Sand Island). Map made with ArcGIS Map 10.4.1.

Figure 3. Images of microplastic particles collected from the field. A) Fragment particles visualized using a dissecting microscope. B) Foam particles visualized using a dissecting microscope. C) A fiber particle visualized using scanning electron microscopy (SEM). D) A sphere particle visualized using a dissecting microscope.

688 Figure 4. A) The average concentration of microplastics collected from intertidal sediments (IS)
689 in Charleston Harbor. Daniel Island contained significantly more microplastic particles than Crab
690 Bank, Grice Cove, and Shute's Folly (X_2=36.0, df=4, p<0.0001). B) The average concentration
691 of microplastics collected from intertidal sediments (IS) in Winyah Bay. Sand Island, Oak Island,
692 and North Island contained significantly more microplastic particles than East Bay Park and
693 Malody Bush (X_2=37.4, df=4, p<0.0001). Different letters represent significant differences. Error
694 bars represent standard error.

695 Figure 5. A) The concentration of microplastics collected from the sea surface microlayer (SML)
696 in Charleston Harbor (n=1 per site). B) The concentration of microplastics collected from the
697 SML of Winyah Bay (n=1 per site).

698 Figure 6. A) Average number of microplastic particles in intertidal sediment (IS) in the tidal
699 zones between Charleston Harbor (black bars) and Winyah Bay (white bars). Microplastic
700 concentration did not differ significantly among tidal zones. B) Distribution of microplastic
701 particles among size fractions between Charleston Harbor and Winyah Bay. Different letters
702 represent significant differences within each estuary. The concentration of 63-149 µm particles
703 and 150-499 µm particles was significantly higher than the concentration of ≥500 µm particles in
704 Winyah Bay (X_2=40.5, df=2, p<0.0001). Error bars represent standard error.

705 Figure 7. Average number of microplastic particle types found in the intertidal sediment (IS)
706 between Charleston Harbor (black bars) and Winyah Bay (white bars). Significant differences
707 within Charleston Harbor are represented with letters A and B, while significant differences
708 within Winyah Bay are represented with letters Y and Z. The concentration of fragments was
709 significantly higher than the concentration of fibers and spheres in Charleston Harbor (X_2=91.7,
710 df=3, p<0.0001). The concentration of fragments was significantly higher than the concentration

711 of foam and spheres in Winyah Bay (X_2=112.1, df=3, p<0.0001). Error bars represent standard
712 error.

713 Figure 8. A) Average number of microplastic particle types in the sea surface microlayer of
714 Charleston Harbor (black bars) and Winyah Bay (white bars). The concentration of fibers was
715 significantly greater than the concentration of spheres in the Charleston Harbor sea surface
716 microlayer (X_2=11.69, df=3, p=0.009). The concentration of fragments was significantly greater
717 than the concentration of spheres (X_2=13.3, df=3, p=0.004) in the Winyah Bay sea surface
718 microlayer. Significant differences within Charleston Harbor are represented with letters A and
719 B, while significant differences within Winyah Bay are represented with letters Y and Z. B)
720 Average number of microplastic particles among size fractions (63-149, 150-499, ≥500 μm)
721 collected from the sea surface microlayer of Charleston Harbor and Winyah Bay. Microplastic
722 concentration did not differ significantly among size fractions.

723 Figure 9. Average number of microplastic particles collected from intertidal sediments (black
724 bars) and the sea surface microlayer (white bars) from Charleston Harbor and Winyah Bay.
725 Primary axis represents average number of microplastics from intertidal sediments. Secondary
726 axis represents average number of microplastics in the sea surface microlayer. Different letters
727 represent significant differences within each sample type. Error bars represent standard error

Figures

Figure 1.

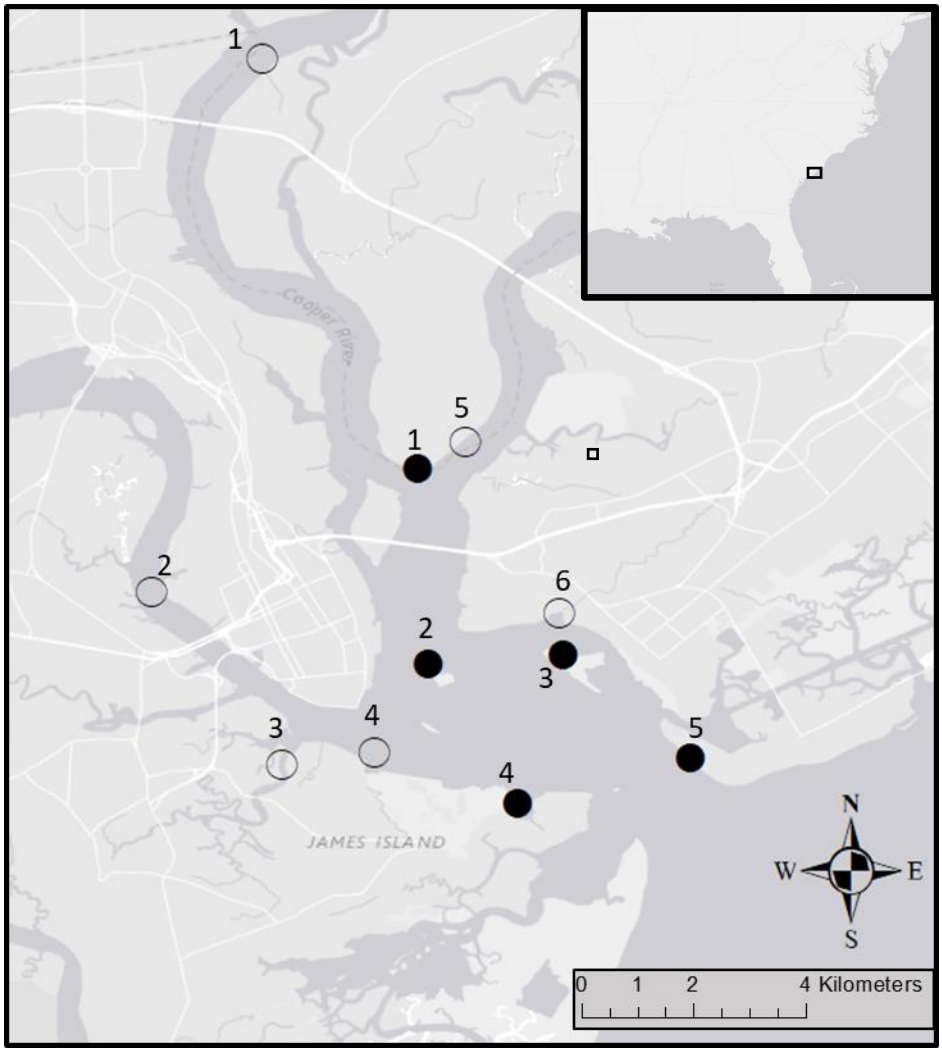
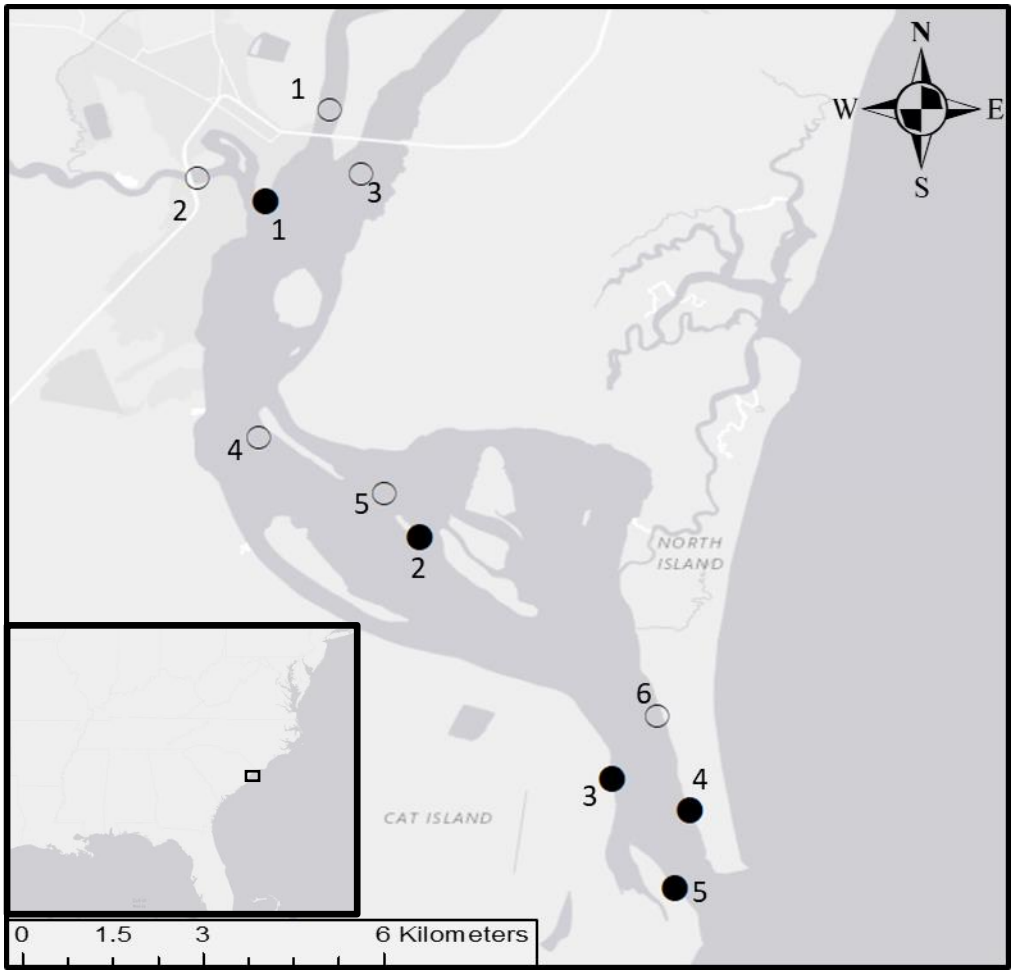


Figure 2.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6 Kilometers

Figure 3.

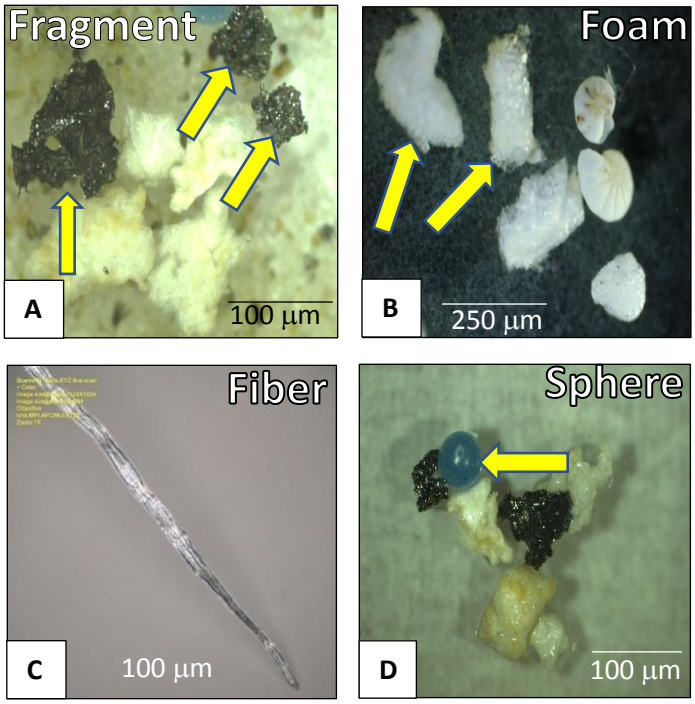


Figure 4.

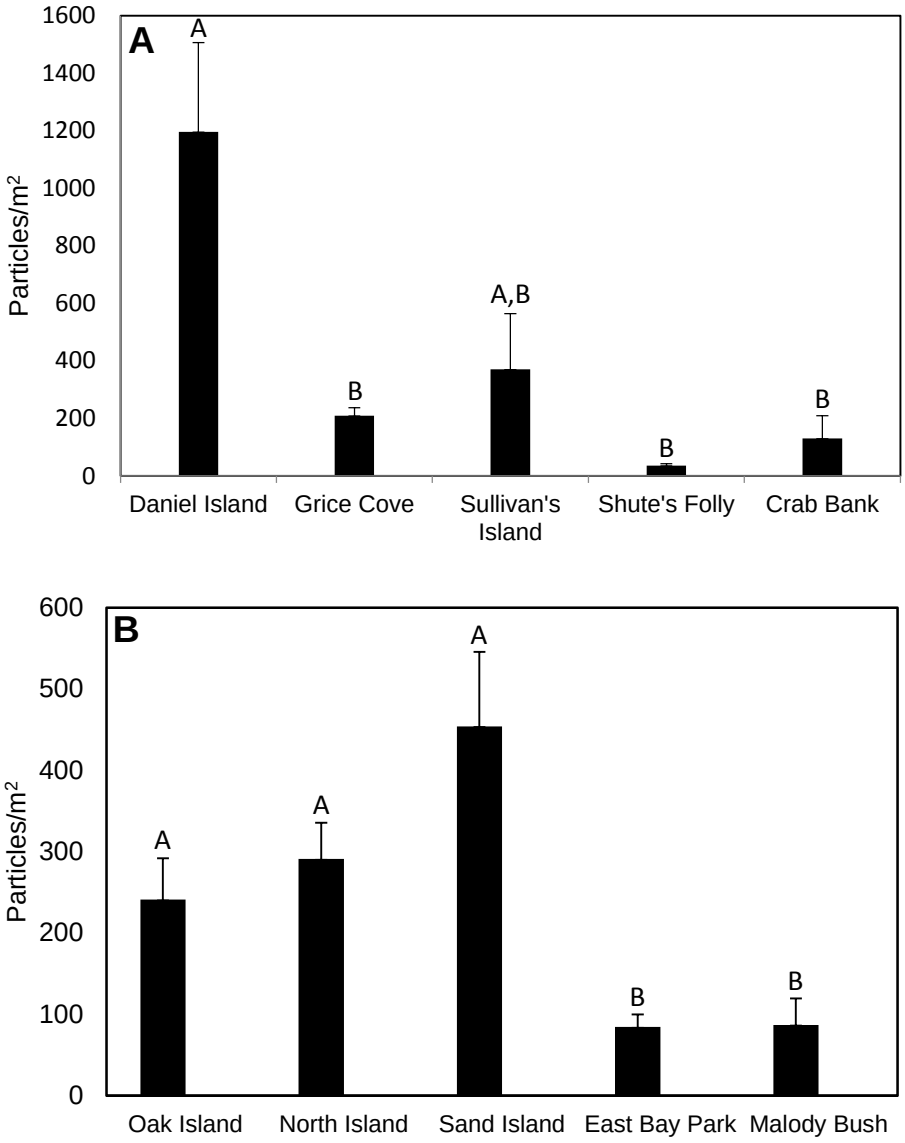

A
Particles/m²
1600
1400
1200
1000
800
600
400
200
0
A
B
A,B
B
B
Daniel Island
Grice Cove
Sullivan's Island
Shute's Folly
Crab Bank
B
Particles/m²
600
500
400
300
200
100
0
A
A
A
B
B
Oak Island
North Island
Sand Island
East Bay Park
Malody Bush

Figure 5.

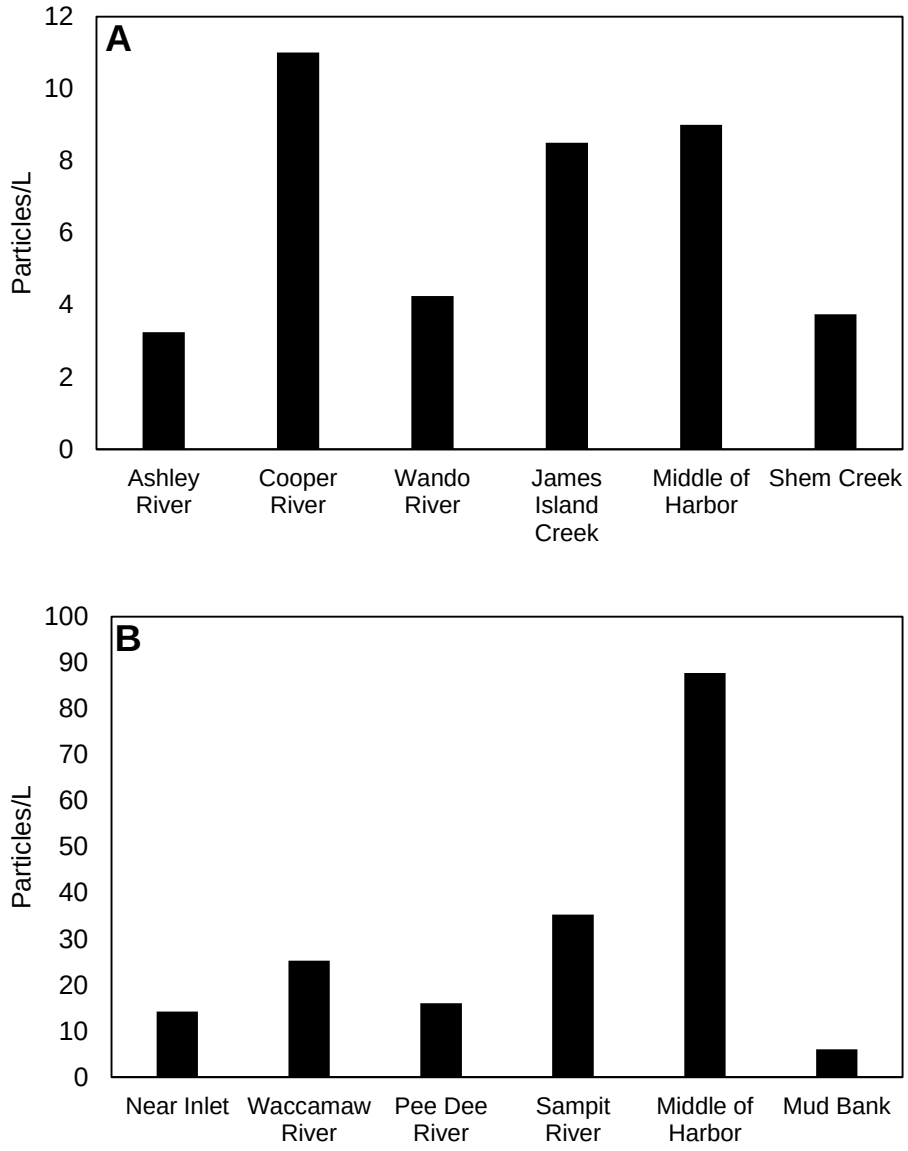

A
Particles/L
12
10
8
6
4
2
0
Ashley River
Cooper River
Wando River
James Island Creek
Middle of Harbor
Shem Creek
B
Particles/L
100
90
80
70
60
50
40
30
20
10
0
Near Inlet
Waccamaw River
Pee Dee River
Sampit River
Middle of Harbor
Mud Bank

Figure 6.

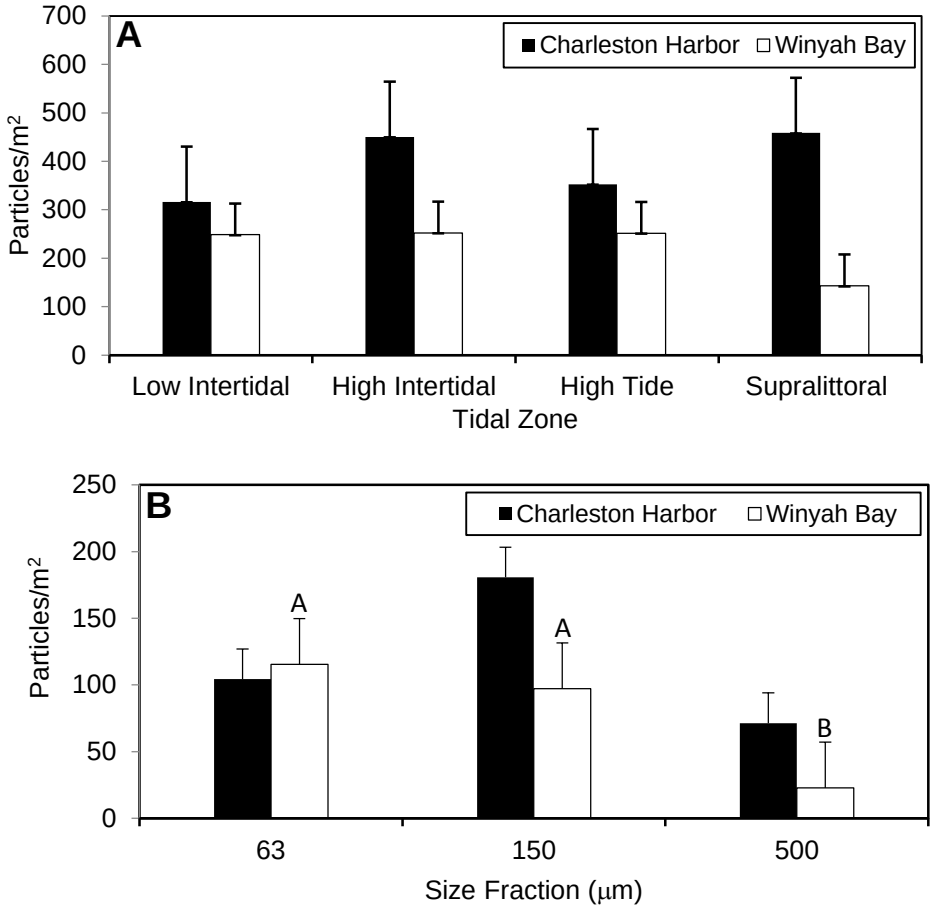

A
Charleston Harbor
Winyah Bay
Particles/m²
700
600
500
400
300
200
100
0
Low Intertidal
High Intertidal
High Tide
Supralittoral
Tidal Zone
B
Charleston Harbor
Winyah Bay
Particles/m²
250
200
150
100
50
0
A
A
B
63
150
500
Size Fraction (μm)

Figure 7.

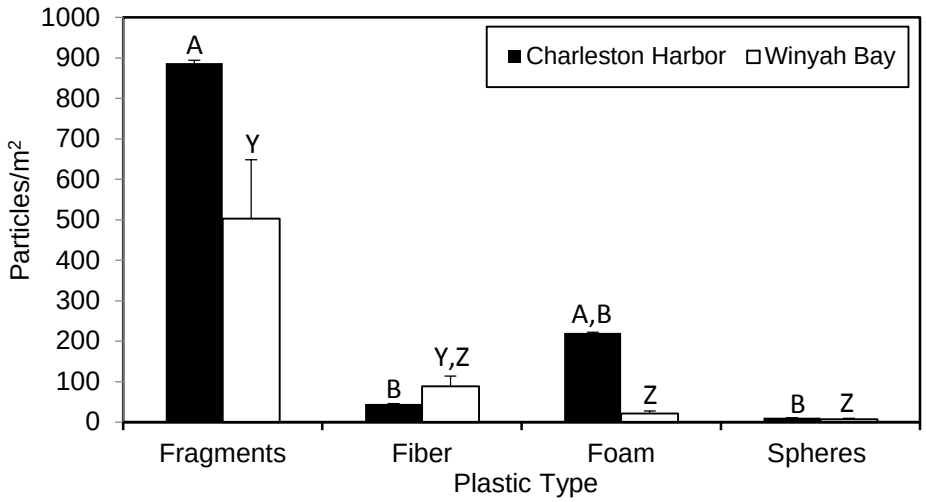

1000
900
800
700
600
500
400
300
200
100
0
Particles/m²
Charleston Harbor
Winyah Bay
A
Y
B
Y,Z
A,B
Z
B
Z
Fragments
Fiber
Foam
Spheres
Plastic Type

Figure 8.

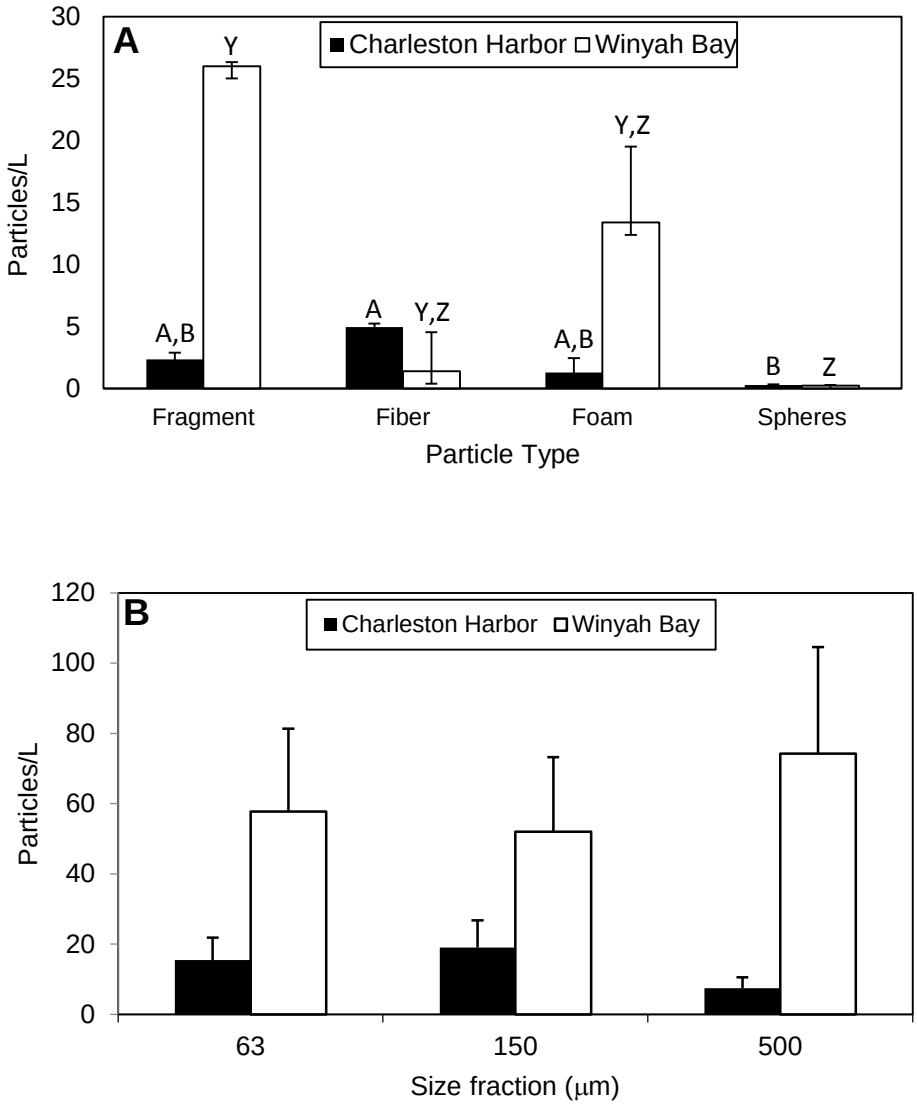
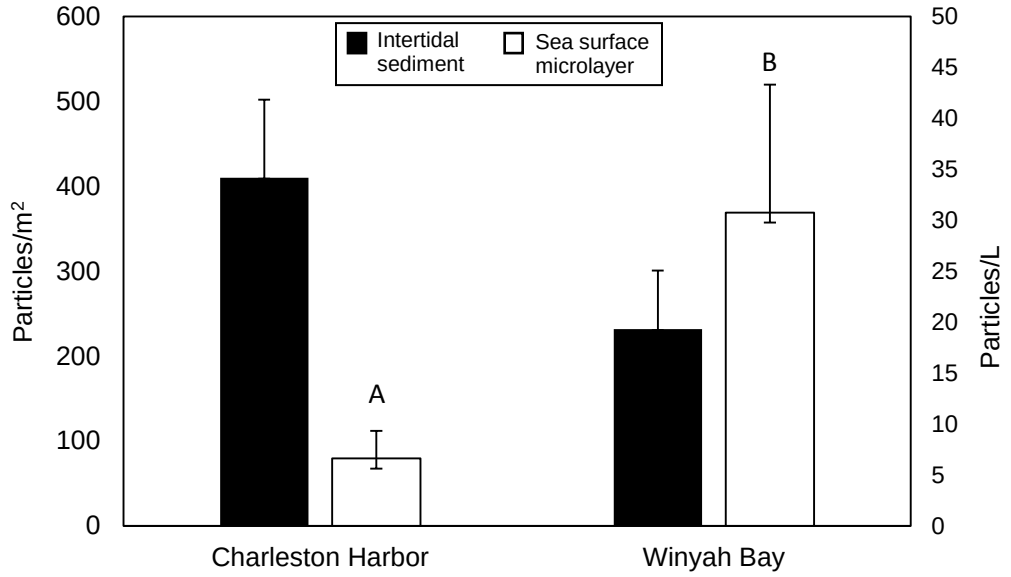
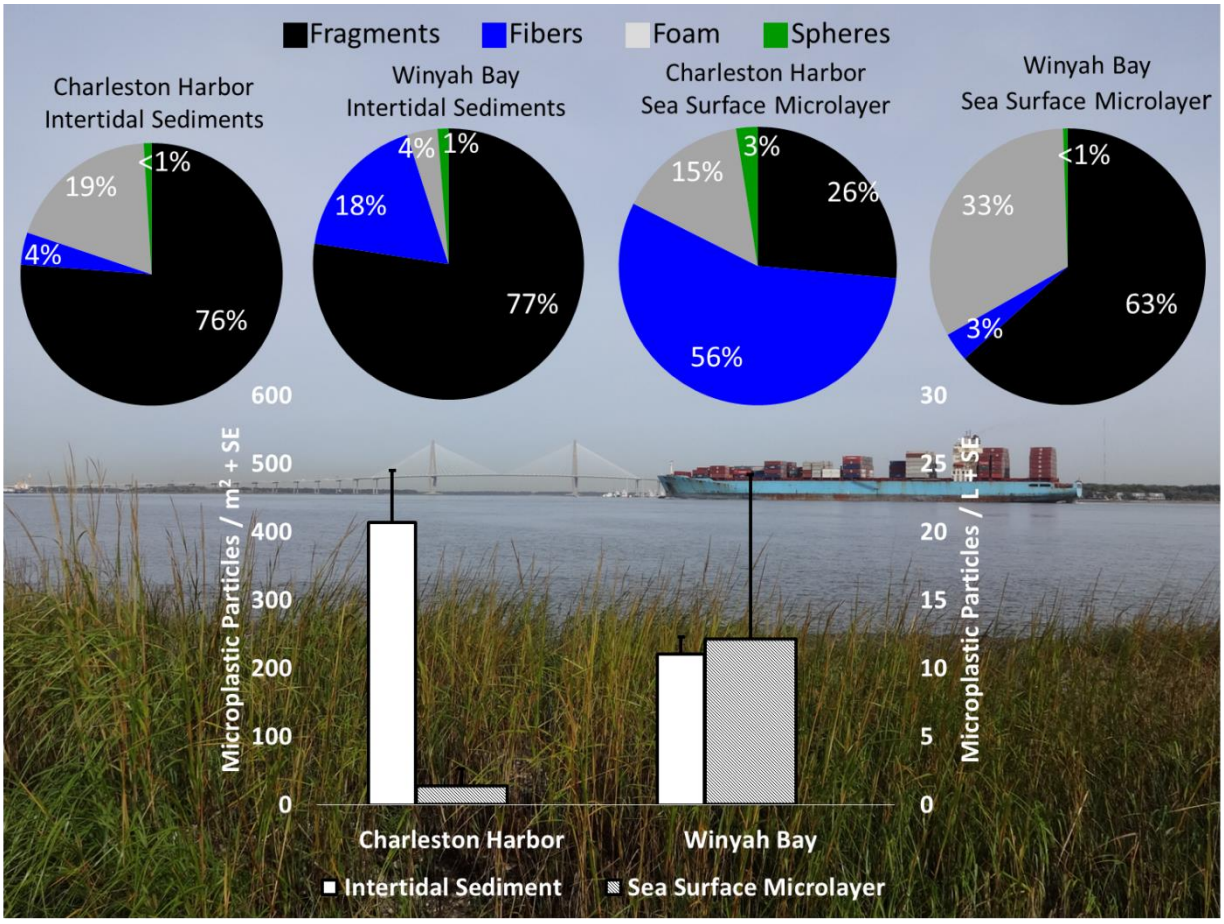

A
Charleston Harbor
Winyah Bay
Particles/L
30
25
20
15
10
5
0
Y
A,B
A
Y,Z
A,B
Y,Z
B
Z
Fragment
Fiber
Foam
Spheres
Particle Type
B
Charleston Harbor
Winyah Bay
Particles/L
120
100
80
60
40
20
0
63
150
500
Size fraction (μm)

Figure 9.


Intertidal sediment
Sea surface microlayer
Particles/m²
Particles/L
600
500
400
300
200
100
0
50
45
40
35
30
25
20
15
10
5
0
A
B
Charleston Harbor
Winyah Bay


Fragments
Fibers
Foam
Spheres
Charleston Harbor
Intertidal Sediments
<1%
19%
4%
76%
Winyah Bay
Intertidal Sediments
4%
1%
18%
77%
Charleston Harbor
Sea Surface Microlayer
3%
15%
26%
56%
Winyah Bay
Sea Surface Microlayer
<1%
33%
3%
63%
Microplastic Particles / m2 + SE
600
500
400
300
200
100
0
Microplastic Particles / L + SE
30
25
20
15
10
5
0
Charleston Harbor
Winyah Bay
Intertidal Sediment
Sea Surface Microlayer