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# Field-Based Evidence for Microplastic in Marine Aggregates and Mussels: Implications for Trophic Transfer

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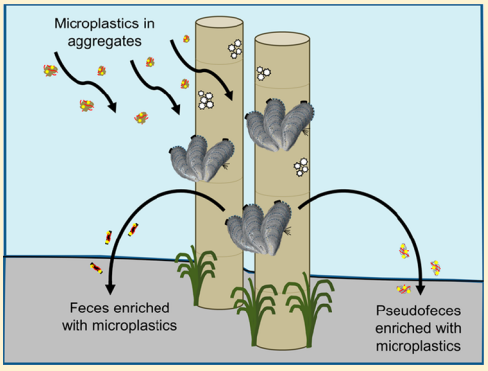
Supporting Information

**ABSTRACT:** Marine aggregates incorporate particles from the environment, including microplastic (MP). The characteristics of MP in aggregates and the role of aggregates in linking MP with marine organisms, however, are poorly understood. To address these issues, we collected aggregates and blue mussels, *Mytulis edulis,* at Avery Point, CT, and analyzed samples with microspectrometers. Results indicate that over 70% of aggregates sampled harbored MP (1290 ± 1510 particles/$m^3$). Fifteen polymer types were identified, with polypropylene, polyester and synthetic-cellulose accounting for 44.7%, 21.2% and 10.6%, respectively, of the total MP count. Over 90% of MP in aggregates were ≤1000 $\mu$m, suggesting that aggregations are a sink for this size fraction. Although size, shape, and chemical type of MP captured by mussels were representative of those found in aggregates, differences in the sizes of MP in pseudofeces, feces and digestive gland/gut were found, suggesting size-dependent particle ingestion. Over 40% of the MP particles were either rejected in pseudofeces or egested in feces. Our results are the first to identify a connection between field-collected marine aggregates and bivalves, and indicate that aggregates may play an important role in removing MP from the ocean surface and facilitating their transfer to marine food webs.




## INTRODUCTION

Plastic debris has been found in nearly every oceanic stratum, often starting as large floating items and drifting downward as they break into smaller pieces, with an estimated 4.8−12.7 million tons of plastic debris entering the oceans per year.[1] Microplastics (MP, <5 mm) originate from both fragmentation of large plastics and engineered micron-sized plastic production, and have been extensively studied since 2004.[2,3] The size range of MP is similar to that of the prey of many marine animals. Consequently, MP have been discovered in the digestive tracts of more than 100 different species,[4] which can pose physical,[5] chemical[6] and possible biological[7] harm to the exposed animals.

Recently, the global reservoir of buoyant plastic litter in the sea has been estimated to range from 7000 to 268 940 tons, based on the widespread data sets and oceanographic models.[8−10] However, these estimates account for only 1−10% of the amount of plastic entering the oceans from land in 2010.[1] Furthermore, a size-specific loss of floating plastic <1 mm was found.[8] Some plausible mechanisms have been hypothesized for the observed disparity, and marine aggregates are thought to be a pathway for the removal of small plastic particles from the ocean's upper layer.[11,12] Aggregation of living and nonliving particles is a natural and well documented process in the marine environment.[13,14] Aggregates are ubiquitous and abundant, and their high sinking rates serve as a mechanism for the transport of carbon, nutrients, and other materials to benthic ecosystems.[15,16] Incorporation of small plastic fragments into natural marine aggregates was first proposed in 2008,[17] but few studies have demonstrated that marine aggregates can incorporate MP. Long et al. (2015) showed that three model-aggregates formed by *Chaetoceros neogracile*, *Rhodomonas salina* and their mixture could incorporate and concentrate MP, as well as increase MP sinking rates.[18] Similarly, Porter et al. (2018) demonstrated

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that a range of plastic sizes, shapes, and polymer types could be incorporated into laboratory-generated marine snow with high incorporation values from 79% to 100%.[19] A field study by Zhao et al. (2017) identified MP in natural marine aggregates collected at Avery Point, CT USA, but the physical properties (e.g., size, shape) of the MP were not determined.[12] Hence, our knowledge concerning the characteristics of MP embedded in natural marine aggregates is surprisingly limited. Besides functioning as a pathway of vertical transport, aggregates have been shown to harbor and transmit pathogens,[20,21] and deliver petroleum residues[22] to marine animals. Additionally, several laboratory studies have shown that bioavailability of both MP[19,23] and nanoplastics[24,25] to several species of suspension feeders increased significantly when the plastic particles were incorporated in aggregates. The role of aggregates as a link between MP and marine animals in the environment, however, remains relatively unexplored.

One group of animals that might be particularly impacted by plastic-laden marine aggregates, is the suspension-feeding bivalve molluscs. Bivalves are an important benthic-community assemblage, and have been used to monitor MP pollution worldwide.[26−29] Many species are commercially important and are consumed whole by humans, especially in coastal communities. Bivalves capture a wide range of natural and synthetic particles. Capture efficiency increases asymptotically with increasing particle sizes above ca. 1 $\mu$m to a maximum efficiency of about 100% for particles larger than ca. 7 $\mu$m in diameter.[30] Although particles >7 $\mu$m are capture at a high efficiency, including those 100s of micrometers in size,[31] not all captured particles are ingested. Bivalves have well-developed mechanisms for particle discrimination and, thus, are selective particle feeders.[30] For example, as the size of individual particles increases above ca. 100 $\mu$m, ingestion efficiency declines, becoming less that 30% for some particle shapes (e.g., spheres).[32] In addition to size, other factors can mediate particle selection such as specific (e.g., carbohydrate-lectin)[33] and nonspecific (e.g., charge−charge, hydrophobe−hydrophobe)[34] interactions between particles and the feeding organs. For large, loosely bound marine aggregates, the matrix can be disaggregated by the ciliary action of the gill and labial palps.[35] Disaggregation releases the smaller constituent particles which then can be sorted individually and either ingested or rejected in pseudofeces. Material embedded within aggregates is thought to be an important source of food for some suspension feeders.[36,37] Therefore, marine aggregates could provide a vehicle for the transmission and accumulation of MP in bivalves.

The purpose of this study was to (1) determine the concentration and characteristics of MP embedded in marine aggregates and (2) explore the potential role of aggregates in both transporting MP and linking plastic particles with marine suspension feeders. Samples of marine aggregates and *Mytulis edulis* were collected in situ off a dock at Avery Point, CT, in April and September 2016. MP abundances and their characteristics were determined, and the types identified in mussels were compared to those from aggregates to infer a possible relationship. Data from our previous study[12] regarding MP within marine aggregates was compared to results of the current work. This study is the first to demonstrate, under field conditions, a connection between MP within marine aggregates and bivalves, and the results have implications for the fate of plastic particles in marine environments.

## MATERIALS AND METHODS

**Quality Control.** All liquids used for sample processing and analysis (e.g., Milli-Q water, seawater, hydrogen peroxide, methanol) were passed through glass-fiber filters prior to use (Whatman GF/F, 0.7 $\mu$m pore size, 47 mm diam.). Membrane filters were prechecked under the stereomicroscope for contaminating plastic particles. All glass-fiber filters and glassware (e.g., beakers, bowls, scintillation vials, Pasteur pipettes) were covered with aluminum foil, and combusted in a muffle furnace at 450 °C for 5 h. Imhoff cones were too large to combust and instead were thoroughly rinsed with Milli-Q water. MP extraction was conducted under a laminar-flow hood equipped with a 0.2 $\mu$m HEPA filter. Containers were kept covered whenever possible throughout the investigation to avoid airborne contamination. Procedural controls were performed in triplicate to verify the extraction process.

**Marine Aggregates.** Marine aggregates were collected off the Avery Point dock, Groton, CT in April and September 2016 with a modified method from Lyons et al. (2005)[20] and Zhao et al. (2017).[12] A plastic-free collecting system comprising glass and silicone tubing was developed in this study. Briefly, a silicone sampling tube was fixed at about 0.5 m from the bottom in about 3 m of water. By means of a large bore, hand-operated peristaltic pump, seawater was transferred into 4 Imhoff settling cones (1.0 L per cone) through the silicone tubing (ID: 10 mm). A glass serological test tube (15 mL) was attached to the bottom opening of each cone. Marine aggregates in each cone were allowed to settle into the test tube for 1 h. The cones were then drained, leaving water and aggregates in the test tubes, refilled with another 1 L of seawater, and the process was repeated. A total of 4 L of seawater were allowed to settle in each cone. After the final settling period, the test tubes were removed from each cone and stored.

**Mussels.** *Mytulis edulis* were collected from the Avery Point dock in April ($n$ = 18; 4.7−5.7 cm in shell length) and September ($n$ = 19; 3.7−5.4 cm in shell length) 2016. They were rinsed thoroughly with the filtered Milli-Q water to remove contaminating particles, placed into glass beakers, covered with aluminum foil and transported to the laboratory within 2 h.

**Pseudofeces/Feces.** In the laboratory, mussels were rinsed with Milli-Q water and placed into glass dishes. Dishes were filled with approximately 200 mL filtered seawater (0.7 $\mu$m, GF/F, Whatman) and then placed in an incubator for about 3.5 h at a temperature corresponding to in situ seawater temperatures (i.e., 10 °C in April, 23 °C in September). After this time period, pseudofeces and feces produced by each mussel were collected separately, transferred to glass test tubes, covered with aluminum foil and stored at 4 °C until further analysis.

**Tissues.** After collecting the biodeposits (pseudofeces and feces), the visceral mass of each mussel was isolated by dissection and the wet mass (g) determined. Tissues were rinsed with Milli-Q water three times, placed in 20 mL scintillation vials and stored at −20 °C until analyzed. Dissecting tools were rinsed with Milli-Q water after dissecting each mussel to prevent cross-contamination. The surface of the bench was wiped with three clean polycarbonate (PC) filters to quantify possible MP contamination.


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**Pretreatment of Hydrogen Peroxide.** Hydrogen peroxide ($H_2O_2$, Fisher Scientific) was used to eliminate the organic matter in marine aggregates, biodeposits and mussel tissues. For each marine aggregate and biodeposit sample, 30% $H_2O_2$ and samples were mixed in a 1:1 v/v ratio in a 30 mL centrifuge tube (Cortex). Each tissue sample was placed in a 200 mL beaker along with 30 mL $H_2O_2$ (30%). All samples were incubated in a sand bath (~75 °C) situated in a chemical hood for 24 h and then at room temperature for 36 to 48 h, depending on the degree of digestion. During digestion, 15% $H_2O_2$ (diluted with Milli-Q water) was added to replace evaporated liquid as needed. Three controls containing Milli-Q water and $H_2O_2$ were also prepared. The final volume of oxidative mixtures was then concentrated to about 1 mL for marine aggregates and biodeposit samples, and about 5 mL for tissue samples.

**Dual Density Extraction.** A previously described two-part density separation method was used to extract MP.[12] In brief, 10 and 20 mL of prefiltered sodium iodide solution (density: 1.6 g/cm$^3$; NaI, Fisher Scientific) was used to isolate MP particles from heavier matter for marine aggregate and biodeposits samples, respectively. After settling overnight, supernatants were passed through 0.8 $\mu$m PC filters (Isopore) to collect putative MP. The NaI-extraction was repeated three times. Each PC membrane then was rinsed with Milli-Q water, placed into a cortex tube with 20 mL of 100% methanol, and sonicated at 50−60 Hz for 10 min (bath sonicator, Fisher Scientific). Subsequently, tubes containing the methanol suspension were centrifuged at 5000 rpm for 5 min (J2−21M, Beckman), and the bottom contents (maximum of 1 mL) transferred onto a calcium fluoride ($CaF_2$) window (25 mm diameter, Edmund Optics). The sonication and centrifugation were performed three times for each filter. Samples on $CaF_2$ windows were kept in covered combusted glass Petri dishes until analyzed.

**Microscopy and Microspectrometer Analyses.** All particles on $CaF_2$ windows were imaged under a Zeiss Discovery V8 stereomicroscope (Zeiss, Oberkochen, Germany). The largest dimension ($\mu$m), 2D surface area, and perimeter of each plastic particle were measured by means of ImageJ (version 1.50a, U.S. National Institutes of Health, http://imagej.nih.gov/ij/). The shape factor (SF) of each particle was generated by the following formula:

$$SF = (4\pi \cdot A)/P^2$$

where $A$ and $P$ represent the 2D surface area and perimeter, respectively, of each particle. As with all suspension-feeding bivalves, particles must first be captured before they can be rejected in pseudofeces or ingested. Therefore, all microplastics found in the biodeposits (pseudofeces, feces) and digestive gland/gut are collectively referred to as captured particles.

Chemical identification of MP particles sampled in April 2016 was carried out as previously described using a Raman spectrometer (PeakSeeker Pro-785 with AmScope operated at 10−50 mW and; 5−20 s integration time; Raman Systems MII, Inc./Agiltron, Inc.,Woburn, MA).[12] The recorded spectra were corrected by means of a polynomial regression model using the R software package (version 3.4.3), and compared against a commercial Raman library (Bio-Rad Laboratories, Inc.). MP found in samples from September 2016 were analyzed under the ATR mode of FT-IR microscopy (LUMOS, Bruker, Germany). All data were measured at a resolution of 4 cm$^{-1}$ with 32 scans. Spectra were collected and compared with a spectral database from Bruker.

**Data Analysis.** The concentration of MP in samples collected in July 2014,[12] April 2016, and September 2016 were compared to allow for the examination of differences in the abundance and type of plastic particles in three different months. An analysis of variance (ANOVA, GLM) test was used to examine differences in abundance across months. If the model indicated significant differences, pairwise comparisons were conducted using Tukey's HSD. The Bray−Curtis similarity of polymer composition in the different samples also was calculated. Kernel density estimation (KDE) was utilized to approximate the population structure of particle size distribution (PSD) by estimating the probability density function of PSD. Based on size and shape factors, the *K-medoid* algorithm, a classical partitioning technique of clustering, was employed to group MP captured by mussels and those incorporated into marine aggregates. This algorithm was further used to group MP identified in the pseudofeces, feces and digestive glands/guts of mussels. Finally, differences in size and shape of MP in the biological compartments was examined using ANOVA General Linear Model (GLM) followed by Tukey's HSD posthoc tests. All statistical analyses were performed in R 3.4.3 or Systat 13. In all tests, an alpha level of 0.05 was used. Unless otherwise indicated, data are reported as mean ± SD.

## RESULTS

**Abundance and Characteristics of MP in Marine Aggregates.** No contamination was observed in the procedural controls. A summary of the characteristics of the MP identified in marine aggregate samples is provided in Supporting Information (SI) Table S1. MP were detected in 19 of the 26 (73.1%) samples of marine aggregates (Figure 1; SI Table S1), with a total of 85 MP particles confirmed by spectrometric analysis. The overall average plastic load was 1290 ± 1510 particles/m$^3$ (range 0−6000 particles/m$^3$, $n$ = 26; 1.3 ± 1.5 particles/L). No significant difference in abundance of plastic particles was found among samples collected on the

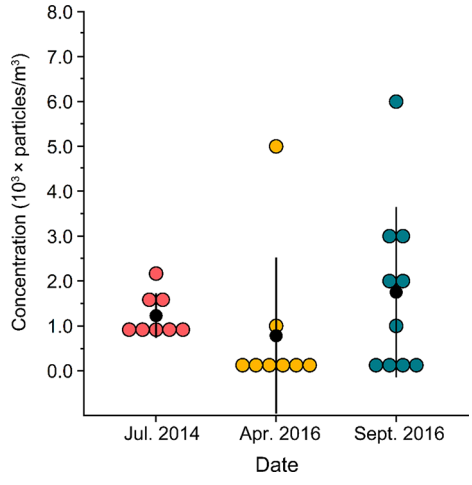


**Figure 1.** Concentration of MP in marine-aggregate samples collected in July 2014, April 2016, and September 2016. The same colored dots indicate the MP abundance of each sample at the same date. Black dots represent the mean MP concentrations of each season. No significant differences were found between means (ANOVA, $p$ > 0.05). Data are reported as mean ± standard deviation (SD).


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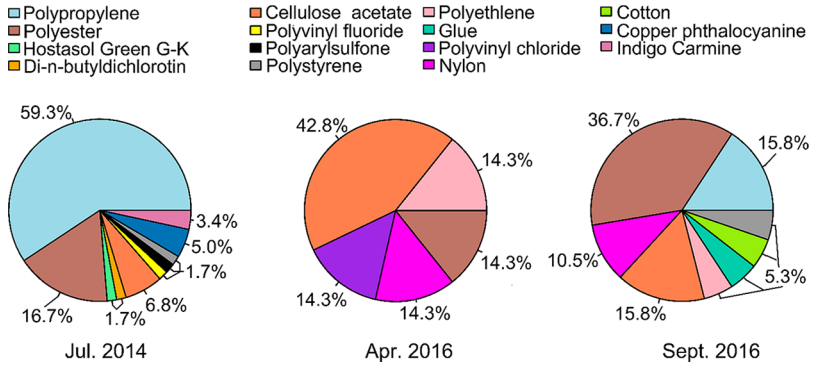


**Figure 2.** Chemical composition of particles identified in marine aggregates collected in three different months.

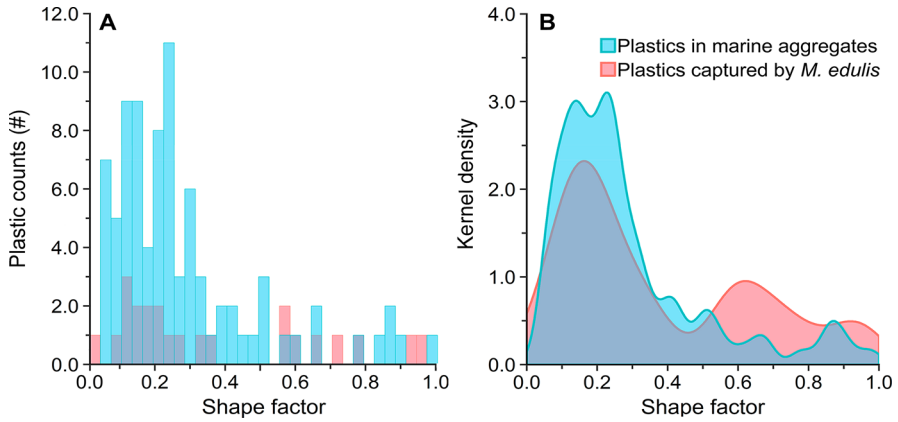


**Figure 3.** Distribution of plastic counts versus shape factor (A) and its kernel-density estimation (B) for MP in samples of marine aggregates and *M. edulis*.

three different dates (ANOVA, GLM, df = 2, 23, $p > 0.05$). The highest MP concentration was found in September 2016 (1750 ± 1800 particles/m$^3$, range 0−6000 particles/m$^3$, $n$ = 10; 1.8 ± 1.8 particles/L), followed by July 2014 (1230 ± 480 particles/m$^3$, range 830−2170 particles/m$^3$, $n$ = 8; 1.2 ± 0.5 particles/L; Zhao et al. (2017)[12]) and April 2016 (780 ± 1630 particles/m$^3$, range 0−5000 particles/m$^3$, $n$ = 8; 0.8 ± 1.6 particles/L).

Fifteen different polymer types were identified among the 85 particles in marine-aggregate samples, including plastic polymers (polypropylene, polyester, cellulose acetate, polyethylene, etc.), dyes (copper phthalocyanine and Hostasol Green G-K) and natural polymers (cotton) (Figure 2; SI Table S2). Generally, polypropylene (PP), polyester and cellulose acetate were the primary types, which accounted for 44.7%, 21.2% and 11.8% of the total counts of MP (SI Table S2). Polyester and cellulose acetate occurred in all samples. In contrast, only two polyethylene (PE) particles were found in April and September 2016. The chemical composition of particles ($n$ = 59) in July 2014 was dominated by PP and polyester, sharing 58.9% and only 16.6% similarity with that of samples in September ($n$ = 19) and April ($n$ = 7) 2016. Samples in the September 2016 predominantly contained polyester, PP, cellulose acetate and nylon, accounting for 36.8%, 15.8%, 15.8%, and 10.5% of all polymer types, respectively. In addition to polymers, colorants and stabilizers detected in this study are widely used in the plastics industry, such as copper phthalocyanine, Hostasol green G-K and Di-*n*-butyl-dichlorotin (Figure 2; SI Table S2). In the aggregates samples collected in July 2014, the pigment signal completely masked the polymer-type signal, making identification of the MP impossible. These colored particles have been reported as MP because of their man-made chemical nature and physical properties (e.g., shape, color, structure, and density).[12] When excluding these particles, The overall MP abundance in marine aggregates collected on the three different dates was 1278 ± 1548 particles/m3 (range 0−6000 particles/m$^3$; 1.3 ± 1.5 particles/L). Overall the average size of MP in marine aggregates was 500.8 ± 587.9 $\mu$m, ranging from 73.3 to 5180.7 $\mu$m. In total, 94.1% of particles (80 of the 85 particles identified) were less than 1000 $\mu$m in size (SI Figure S1). No significant differences in MP size were observed among the samples collected on the three dates (Kruskal−Wallis, $p$ = 0.99). Among the shape categories of plastic particles, fragments and fiber accounted for 65.9% ($n$ = 56) and 34.1% ($n$ = 29) of all particles, respectively (SI Table S1). The two-dimensional shape factors of plastic particles from all samples ranged from 0.06 to 0.98 (Figure 3; S3).

**Abundance and Characteristics of MP Captured by Mussels.** In total, 23 MP particles were identified in the pseudofeces, feces and digestive gland/gut of mussels (SI Table S3). Three MP particles were identified in 16 pseudofecal samples, whereas 7 MP particles were identified in 37 fecal samples (SI Table S3). Digestive-gland/gut samples contained on average 0.4 ± 0.7 particles/g (0.4 ± 0.7 particles/individual) in April 2016 and 0.8 ± 1.4 particles/g (0.3 ± 0.6 particles/individual) in September 2016, with an overall mean value of 0.6 ± 1.2 particles/g (0.4 ± 0.6 particles/individual). Overall, the abundance of MP varied from 0.0 to 5.1 particles/g and from 0.0 to 2.0 particles/individual for digestive-gland/


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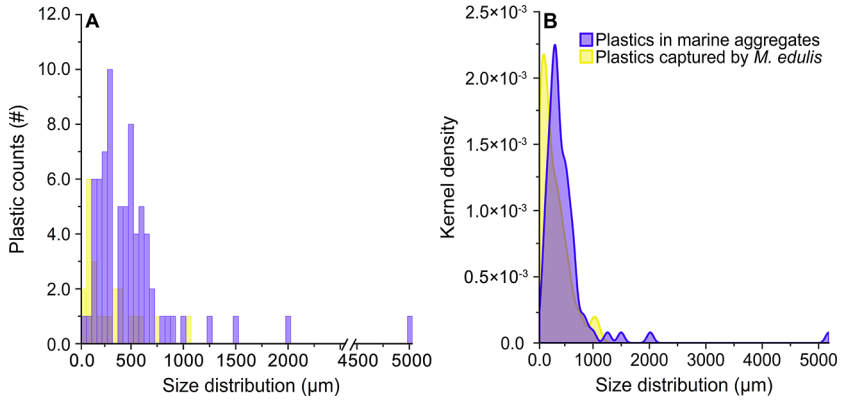


**Figure 4.** Distribution of plastic counts versus size (A) and its kernel-density estimation (B) for MP in samples of marine aggregates and *M. edulis*.

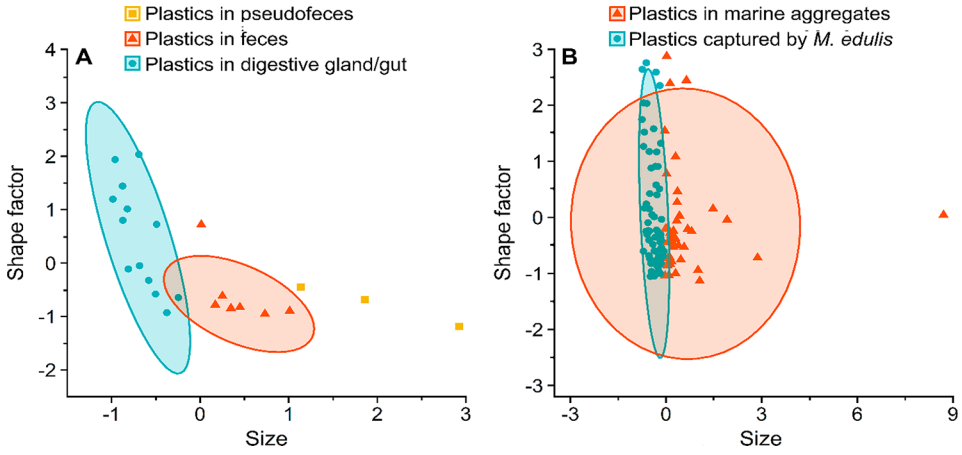


**Figure 5.** Clustering using a k-medoids algorithms depicting the relationships of MP, based on size and shape factors, in (A) pseudofeces, feces and digestive-gland/gut samples of *M. edulis*; and (B) marine aggregates and *M. edulis* samples.

gut samples. A high diversity of polymer types was observed in the MP captured by mussels, with a total 12 types identified, comparable to those found in aggregates (SI Table S4). The average size of particles captured by *M. edulis* was 295.5 ± 245.8 μm, ranging in size from the 47.9 to 1030.1 μm (median = 201.1 μm; Figure 4). Significant differences in particle size were found among plastic particles in the pseudofeces, feces and digestive glands/guts (ANOVA, GLM, df = 2, 15, $p$ = 0.0012; SI Figure S2). Particle size in the pseudofeces (780.9 ± 196.6 μm; median = 763.2 μm) was significantly larger than that in the feces (331.4 ± 167.8 μm; median = 338.4 μm; Tukey, $p$ = 0.047) and digestive gland/gut (164.1 ± 112.3 μm; median = 123.8 μm; Tukey, $p$ = 0.001). No significant differences in size of particles was found between samples of feces and digestive gland/gut (Tukey, $p$ > 0.05). Of these particles, 47.8% ($n$ = 11) were degraded fragments and 52.2% ($n$ = 12) were monofilament line (SI Table S5). The shape factor of MP captured by mussels was between 0.04 and 0.95. No significant differences in shape factors were found among the three biological compartments (ANOVA, GLM, df = 2, 15, $p$ = 0.192). Based on both size and shape factors, however, particles in the three compartments were clearly separated by the K-medoids algorithm into three clusters. Most of the MP retained in digestive glands/guts were smaller in size (47.9–408.2 μm) and had larger shape factors than plastic particles in the pseudofeces and feces (Figure 5A).

**Comparison of MP in Marine Aggregates and Mussels.** The size distribution of MP was consistent in marine aggregates and mussels, with 94.1% (80 of the 85 particles) and 95.7% (22 out of the 23 particles) of the particles being smaller than 1000 μm. The mean size in aggregates and mussels did not vary (Wilcoxon test, $p$ = 0.9983). Kernel density estimation for the size and shape factor revealed that plastic particles in marine aggregates and mussels shared similar physical properties (Figure 3 and 4). The K-medoids clustering plot showed that most particles in the samples of marine aggregates and mussels grouped together (Figure 5B). Chemical compositions of plastics in both marine aggregates and mussels were diverse, with 16 and 12 polymer types, respectively. The composition of plastic particles in both sample types was 52.5% similar.

## DISCUSSION

**MP in Marine Aggregates.** In the present study, ca. 73% of marine aggregates sampled contained plastic particles, providing further evidence for the widespread distribution of MP in the marine environment and implicating marine aggregates as an important bioavailability and water column transport mechanism for MP particles. The abundances of plastic particles in the marine aggregates were compared to values reported worldwide (Figure 6). In the absence of other field data on MP within aggregates, studies that examined particles in bulk water samples using sampling methods and analyses similar to those in the current research were considered. The average concentration of MP detected in marine aggregates (1291 ± 1510 particles/m$^3$, "three months"


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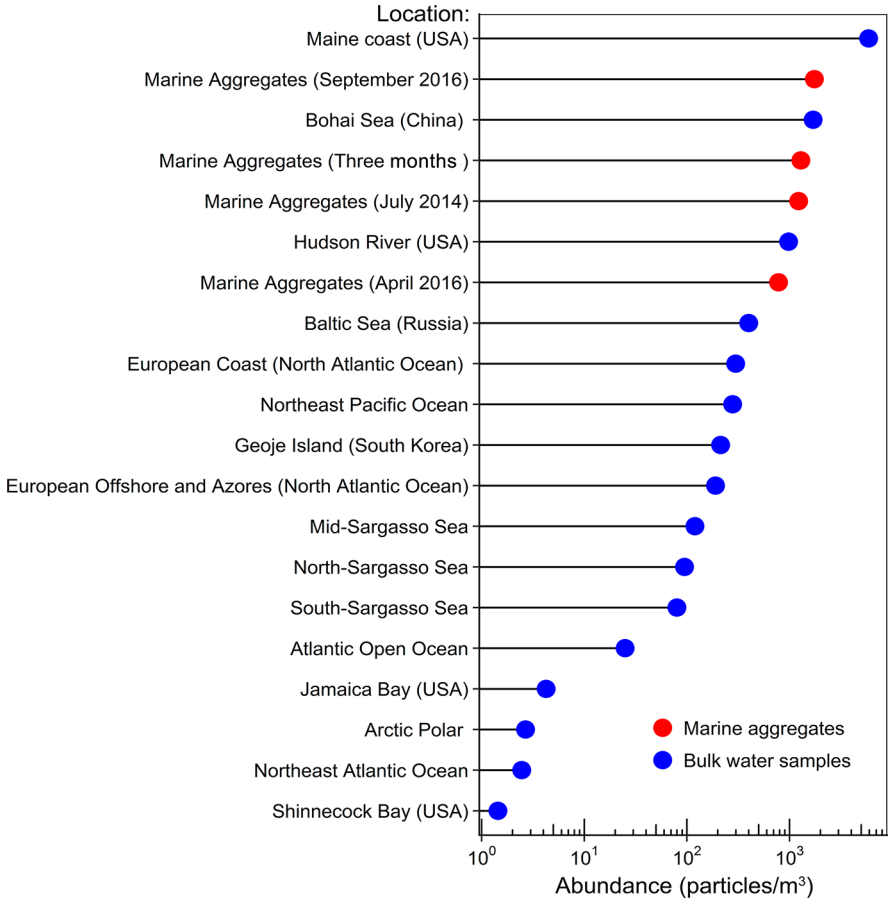


**Figure 6.** Comparison of MP abundance (mean value) in marine aggregates collected in the current study and in Zhao et al. (2017),[12] and in bulk water samples from Barrows et al. (2017),[38] Qu et al. (2018),[39] Miller et al. (2017),[40] Bagaev et al. (2017),[41] Enders et al. (2015),[42] Desforges et al. (2014),[43] Song et al. (2014),[44] Steve (2014),[45] Lusher et al. (2014),[46] and Lusher et al. (2015).[47]

in Figure 6) was in the upper range of concentrations measured in other studies (Figure 6), suggesting that marine aggregates scavenge and concentrate MP from the ambient environments. This value might be slightly higher, as two particles from July 2014 could only be identified by synthetic pigment additives, and illustrate some of the challenges in MP determination. The MP abundance observed in Shinnecock bay (1.4 particles/$m^3$) and in Jamaica bay (4.3 particles/$m^3$), which are geographically close to our sampling site, were about 3 orders of magnitude lower than that found in our study, however, these were sampled with a 200 $\mu$m trawl net.[45] Measurements of the bulk water samples in the North-Sargasso Sea revealed an average MP concentration of 95 particles/$m^3$.[42] This concentration is nearly 12 times lower than that found in aggregates off the dock at Avery Point, which is comparable to that in the coastal waters of Bohai, China.[39]

A lack of a cohesive approach by the scientific community to quantify MP is hindering spatial and temporal comparisons between and among regional studies.[44] For example, difference in the pore size of filters used to collect MP from bulk water samples (e.g., Figure 6), which range from 0.45 to 250 $\mu$m (SI Table S6), add to spurious differences in reported concentrations. Presorting of potential plastic particles under the microscope prior to spectroscopic (FTIR and Raman) confirmation relies heavily on the experience of the researcher and also could affect the reported quantities.[12] Presumptive techniques such as utilizing microscopic inspection alone, lead to false identification rates ranging from 20% to 70%.[48]

The process by which marine aggregates concentrate MP also has been documented in laboratory based studies. For example, Long et al. (2015) showed that the concentration of polystyrene microbeads (2 $\mu$m) in *Rhodomonas salina* aggregates ($3.6 \times 10^5$ beads/mL) was 160 times higher, and that in *Chaetoceros neogracile* aggregates ($1.9 \times 10^4$ beads/mL) was approximately 9 times higher than background concentrations (2200 beads/mL). Aggregates produced from a mix of the two algal species harbored a concentration of $4.3 \times 10^4$ beads/mL, 19 times higher than the background concentration.[18] This finding is not surprising given the process of aggregate formation. The sticky polymeric substances (i.e., extracellular polymeric substances, EPSs) produced by microbial, phytoplanktonic and benthic communities in natural waters greatly enhance the agglomeration of cells, detritus and mineral particles into rapidly sinking aggregates.[13,49−51] As these aggregates sink, they collide with and stick to smaller particles,[52] such as less dense, hydrophobic plastic particles, which are incorporated into the aggregation. In near-shore waters, resuspension of aggregates as a result of tidal forces and storm events[53] would allow more suspended MP particles to become incorporated over time. The peak abundance of MP within marine aggregates was recorded in September 2016, which could be a result of particle accumulation by summertime human activities (e.g., beach going, recreational boating, and fishing), meteorological events and changes in oceanographic conditions.[3]


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The chemical composition of MP identified from marine aggregates were highly diverse ($n$ = 15), with a broad range of domestic and industrial uses. Polypropylene (PP), polyester, and cellulose acetate made up nearly 76.3% of all plastic particles identified in sampled marine aggregates. The prevalence of PP and polyester are partly ascribed to their common usage in a wide range of domestic and industrial applications.[54] These two polymer types were often identified in Atlantic surface waters and sediments.[42,55,56] Of the particles collected from the coastal waters along the southeastern United States, 24% were identified as polyester fiber.[57] Polyester also made up the majority of polymers identified in Nisken-bottle samples of deep-sea waters at Rockall Trough, in North Atlantic Ocean.[56] Cellulose acetate is commonly used in cigarette filters, hygiene products, and clothing[55,58] and is mainly introduced into marine environments via municipal sewage discharge.[59] The semisynthetic rayon has been reported to have almost identical FT-IR spectra with cellulose,[46] and its presence has been widely reported in the marine environment. In Arctic waters particles identified with FT-IR analysis included man-made cellulose (30%), polyester (15%), polyamide (15%), polyethylene (5%), acrylic (10%), polyvinyl chloride (5%).[47] Man-made cellulose was also the dominate MP by abundance in other coastal[57] and deep-sea waters,[56] and sediments.[55] Marine aggregates sharing substantial primary polymer types with those detected in oceanic waters and sediments indicates that aggregates could redistribute microplastics. Of note is the rare presence of the polyethylene (PE) polymer, one of the most abundant pelagic microplastics in western North Atlantic Ocean.[60] The cause of this discrepancy is not clear, but it might be related to the UV stability of PE. High impact PE polymers fragment little in the water column,[61] resulting in a longer retention time of PE microplastics in the surface layer.

Models developed for MP fragmentation show a gradual increase toward small particle sizes as fragmentation proceeds over time. The observed size distribution of MP in the oceans, however, show a sharp decrease for plastic particles <1 mm.[8,9] This missing size fraction (<1 mm) is likely responsible for the gap in the estimated mass of buoyant plastics in the oceans and mass of plastic entering the oceans in 2010.[1,10] Some plausible mechanisms have been proposed for the loss of floating micron-size plastic from the ocean surface, including nanofragmentation, ingestion by biota, and density increases leading to sinking.[8,9] Particle aggregation is ubiquitous in the ocean and is a major pathway for the downward transportation of organic and inorganic material by gravitational settling,[62] and is plausibly one of the ways small plastic particles are removed from the ocean surface layer.[12,18,19] More than 90% of all MP identified in natural marine aggregates were less than 1 mm (SI Figure S1), suggesting that aggregates are important in the vertical transfer of small MP through the water column. Once incorporated, the dimensionality of MP could be modified. For example, some soft plastic particles (e.g., fiber and film) might adhere and be transformed into larger agglomerations.[25] The increase in particle size would enhance settling rates,[21] and result in increased encounter and bioavailability of MP to benthic suspension feeders.[24,25,63] Unexpectedly, fragmented plastics were the predominant type of synthetic particles identified in marine aggregates, accounting for 65.9% of all MP. This result contrasts with previously published data which indicate that microfibers are the dominant type of plastic particles in various environmental compartments, however, this difference could be explained by a different approach in environmental sampling.[42,55,56]

**MP Captured by Mussels.** MP were detected in the biodeposits or digestive gland/gut of nearly all the mussels collected from the Avery Point dock. Several blue and transparent fibers in the procedural controls whose physical characteristics were distinct from those identified in mussels, were omitted from analysis in this study on the off chance that they may have been inadvertently introduced during analysis (SI Figure S4). The average plastic load in digestive-gland/gut of *M. edulis* was 0.6 ± 1.2 particles/g (0.4 ± 0.6 particles/individual), which is comparable to that found in field and cultured *M. edulis* from Dutch coastal waters and a mussel farm in Germany (0.2 ± 0.3 and 0.4 ± 0.1 particles/g, respectively).[27,64] But the concentration of MP found in this study was nearly 3 times lower than that in wild mussels (2.7 particles/g) from the coastal waters of China.[65]

The lack of significant differences in the number of plastic particles in *M. edulis* across months is a consequence of the uniform load of MP in the environment and the consistency of particle-feeding processes of mussels (e.g., capture efficiency, preingestive and postingestive selection) during these specific dates. Of the 23 polymer particles captured by mussels (including MP in biodeposits and digestive gland/gut), 11 polymers and 2 pigments were identified (SI Table S4), which are common plastic types and pigments used in industry.[66] Moreover, these MP polymers included both higher and lower density plastics (SI Table S2). The high diversity suggests various origins of MP for the mussels. The size of plastic particles within the pseudofeces, digestive glands/guts, and feces differed considerably, which could be attributed to particle selection by *M. edulis*.

Particle selection is one strategy that bivalves use to enhance their diet quality and optimize energy intake. The consumption of indigestible plastic particles might lead to wasted energetic cost, which could affect the growth of mussels.[67,68] As a selective feeder, *M. edulis* sorts particles based on physical (e.g., size, shape, flexibility, and density), chemical, and nutritional properties.[69,70] Long particles detected in the pseudofeces were likely a result of the preferential rejection of these high-aspect ratio MP by *M. edulis*. In addition to preingestive selection, particles may also be selected in the stomach after ingestion.[71,72] Food passed directly through to the intestine will produce poorly digested intestinal feces, whereas food processed in the digestive diverticula will result in well-digested glandular feces. Particles directed to the digestive diverticula will have a longer gut retention time than those directed to the intestine.[30] Such separation of MP in the pseudofeces, feces and digestive gland/gut was shown by the K-medoids clustering on size and shape factors. Mussels in this study preferentially ingested particles of a smaller size (47.9 to 408.2 $\mu$m) and relatively larger shape factor (Figure 5A). To better understand the selection and ingestion of MP by mussels, the theoretical number of plastic particles that could have been captured by sampled mussels was calculated. The median length of mussels collect in April was 5.2 cm, and the median length in September was 4.5 cm. Using these lengths and the specific water temperature in each month, a median mussel in April could pump and clear 1.4 L/h, at 10−12 °C[73] and a median mussel in September could clear 2.8 L/h, at 20 °C.[74] Assuming ca. 100% retention efficiency of aggregates >10 $\mu$m[30] and a minimum pumping duration each day of 12 h,[75] sampled mussels could have captured 22 MP particles/day in April (1.4


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L/h × 12 h × 1.3 particles/L in aggregates), and 44 MP particles/day in September (2.8 L/h × 12 h × 1.3 particles/L in aggregates). In mussels, the amount of time required to transport captured particles to the labial palps for either ingestion or rejection is on the order of minutes.[76,77] In contrast, the residence time of plastic particles passed directly to the intestine is on the order of hours and that of particles passed to the digestive diverticula is on the order of days (10 μm plastic particles).[24] Given these data, we conclude that the majority of plastic particles encountered by mussels in marine aggregates were either rejected in pseudofeces or egested as intestinal feces. In contrast to what has been suggested recently,[39] our analysis suggests that mussels (*M. edulis*) are not a robust indicator of MPs in the marine environment as a result of their inherent ability to selectively feed.

As an important seafood, bivalves are eaten without removal of the gastrointestinal tract, and thus represent a pathway for MP to enter the human food chain. Eight of 13 plastic particles in the digestive-gland/gut samples were less than 150 μm, which is the upper size limit for particles translocating across the gut epithelium of humans and causing systemic exposure.[78] Our results indicate that particle selection by bivalves may be an important factor in determining the fate of MP in marine food webs, but more data must be collected and analyzed to validate this hypothesis. Additionally, the occurrence and characteristics of smaller sized particles (<150 μm) in seafood should be identified in future studies.

**Role of Aggregates in Facilitating MP Ingestion by Benthic Suspension Feeders.** Apart from being an important mechanism for vertical transport of organic and inorganic material in the ocean, marine aggregates also function as vectors for pathogens of bivalves and humans.[20,79,80] Marine aggregates have also been implicated in the transfer of surface oil to the seafloor and subsequent negative effects on benthic ecosystems.[81−83] Similarly, aggregates can plausibly provide a vehicle for enhanced transmission of MP to benthic suspension-feeders. Small micro- or nanoplastics, which cannot be efficiently captured by some aquatic fauna,[84] can be incorporated into aggregates. The results of kernel density estimation show that the sizes and shapes of ingested particles by mussels were representative of MP found in marine aggregates (Figures 3, 4, and 5B). Moreover, the high similarity in chemical composition of particles identified from aggregates and mussels also indicated the proposed link. Incorporation of nanoplastic (30 and 100 nm) and microplastic (0.5−1.0 μm) PS beads into aggregates has been documented to enhance uptake of these particles by suspension feeders compared to freely suspended beads.[23−25] Most recently, Porter et al. (2018) showed that the ingestion of micrometer-sized PS and PE spheres, and PP fibers by mussels was increased by several orders of magnitude when the particles were embedded in laboratory-made aggregates,.[19] Enhanced uptake was a result of increased delivery of MP-laden aggregates to the bottom of the container in which the mussels were held.

Results of this field study provides new insights into the role of marine aggregates in the fate of MP in the environment. Data demonstrate that high loads of small MP (<1 mm) can be concentrated in the downward flux of aggregates, and help explain the missing particle size class of MP in the surface layer of the world's oceans. Once incorporated into aggregates, several transformations of the MP can occur, including an increase in the effective particle size and change in surface topography and density as a result of the physical and biological processes in the aggregate microcosm. The characteristics of MP in aggregates and mussels were similar and imply that aggregates play an important role in the trophic transfer of plastic particles to benthic suspension-feeders.

The uniformity of MP sizes and shapes identified within mussels indicates that selective ingestion of certain types of plastic particles occurred. Current knowledge of the relationship between MP and marine aggregate microcosms is limited. Future studies should consider (1) detailing the characteristics of MP in marine aggregates on a global scale and estimating the downward flux of plastic particles carried by aggregates, (2) the changes of both MP and aggregates through physical process, and (3) the interaction between MP and microbes harbored in the organic aggregates. Such research would improve our understanding of the fate of MP in the world's oceans.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b03467.

Size distribution of plastic particles collected in each of the three months (Figure S1); Microplastic size found in the pseudofeces, feces and digestive gland/gut of mussels (Figure S2); Examples of the shape factor of microplastic particles collected in the current study (Figure S3); Contaminated fibers detected in the controls after dissection of mussels (Figure S3); Information regarding marine-aggregate samples and the plastic particles identified within (Table S1); Polymer types confirmed in the marine aggregates (Table S2); Detailed information regarding sampled mussels and plastic particles identified in their biodeposits and digestive gland/gut (Table S3); Plastic types captured by mussels and identified in their biodeposits and digestive gland/gut (Table S4); Shape composition of plastic particles captured by mussels (Table S5); Microplastic abundance reported for bulk water samples worldwide (Table S6) (PDF)

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### Notes

The authors declare no competing financial interest.

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