Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2019, 53, 8776−8784

pubs.acs.org/est

Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (*Mytilus edulis*) and Eastern Oyster (*Crassostrea virginica*): Implications for Using Bivalves as Bioindicators of Microplastic Pollution

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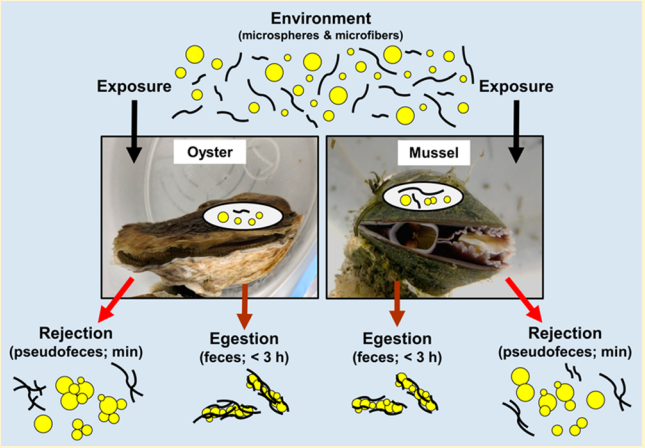
Supporting Information

ABSTRACT: Microplastics (MP; 1 μm to 1 mm) of various shapes and compositions are ingested by numerous marine animals. Recently, proposals have been made to adopt bivalve molluscs as bioindicators of MP pollution. To serve as indicators of MP pollution, however, the proposed organisms should ingest, without bias, the majority of plastic particles to which they are exposed. To test this premise, eastern oysters, *Crassostrea virginica*, and blue mussels, *Mytilus edulis*, were offered variously sized polystyrene microspheres (diameters 19−1000 μm) and nylon microfibers (lengths 75−1075 × diameter 30 μm), and the proportion of each rejected in pseudofeces and egested in feces was determined. For both species, the proportion of microspheres rejected increased from ca. 10−30% for the smallest spheres to 98% for the largest spheres. A higher proportion of the largest microsphere was rejected compared with the longest microfiber, but similar proportions of microfibers were ingested regardless of length. Differential egestion of MP also occurred. As a result of particle selection, the number and types of MP found in the bivalve gut will depend upon the physical characteristics of the particles. Thus, bivalves will be poor bioindicators of MP pollution in the environment, and it is advised that other marine species be explored.



INTRODUCTION

Plastic debris in the marine environment is a widespread pollutant interacting with and affecting a range of organisms from larvae to vertebrates.[1,2] Equally problematic are the myriad of microplastic (MP) particles (1 μm−1 mm)[3] that are manufactured for consumer products or are produced as a result of macroplastic degradation.[1,2,4] Marine waters globally are contaminated with a mixture of MP of various shapes (e.g., spherical, angular, and fibers) and compositions (e.g., polystyrene, polypropylene, nylon, and low- and high-density polyethylene). A large portion of MP particles are suspended in the water column, and are available for capture and ingestion by planktonic and benthic suspension feeders. Ingested MP can produce deleterious effects under certain laboratory conditions.[5−12]

Recently, many studies have focused on the uptake of MP by suspension-feeding bivalve molluscs because bivalves process large volumes of water per unit time, and capture particles as small as 3 μm with high efficiency (>50% depending on species[13−15]). Studies have shown that bivalves ingest MP under ambient environmental conditions,[16−21] and thus, it is assumed that these species will be one of the most impacted groups. Additionally, because bivalves are broadly distributed, abundant, easily accessible, and sessile organisms, they have been used to monitor numerous environmental contaminants worldwide (e.g., U.S. Mussel Watch; Assessment and Control of Pollution in the Mediterranean region [MEDPOL]; North East Atlantic Oslo and Paris Commission (OSPAR)).[22−25] Recently, several workers have proposed that bivalves could also be used to assess the load of MP in different environments.[18−20,26−32] These proposals are based largely upon correlations between the types and abundance of MP in the environment and those found in the soft tissues of several

Received: April 6, 2019
Revised: July 10, 2019
Accepted: July 15, 2019
Published: July 15, 2019

DOI: 10.1021/acs.est.9b02073
Environ. Sci. Technol. 2019, 53, 8776−8784

bivalve species. Criteria required for taxa to be indicators of environmental impacts have been outlined previously.[25] Upon the basis of these criteria, it is recommended that species proposed as bioindicators of MP pollution in the environment should have the following characteristics: (1) be ubiquitous and relatively easy to collect; (2) interact significantly with the surrounding environment through particle-feeding processes; and (3) ingest, without bias, the majority of plastic particles to which it is exposed. With respect to bivalves, a large body of research demonstrates that bivalves feed selectively on a range of particles, i.e., they do not simply ingest all particles that are captured by the gills.[13,15] Thus, bivalves would fail to meet the third criterion.

In this study, differently sized polystyrene microspheres and microfibers were delivered directly to the inhalant margin of the eastern oyster, *Crassostrea virginica*, and blue mussel, *Mytilus edulis*. Uptake and elimination of MP were assessed by determining the number of plastic particles rejected and egested in each size and shape category, and by examining the way in which particles were handled by the gill (in vivo) and eliminated at the pseudofeces-discharge site (aka, principal-discharge area[33]). These data were then used to test the following null hypotheses: (1) the number of MP particles rejected in each size class equals the number of particles ingested (spheres or fibers); (2) the proportion of MP rejected in pseudofeces and egested in <3 h is independent of size (spheres or fibers); (3) the proportion of large MP rejected is independent of shape (1000-μm spheres vs 1075-μm fibers).

METHODS

Collection and Maintenance of Animals. Oysters, *Crassostrea virginica*, and mussels, *Mytilus edulis*, were collected from natural populations in Long Island Sound and cleaned of fouling organisms. A strip of Velcro was secured to one shell of each animal using a two-part marine epoxy.[34] Bivalves were placed in lantern nets and suspended from a dock adjacent to the University of Connecticut at Avery Point. They were held in the natural environment for several days before use in the experiments. Approximately 24 h before the start of an experiment, oysters and mussels were secured to craft sticks by means of the attached Velcro, placed in a large holding tray filled with aerated, natural seawater (hereafter termed seawater), and transferred to an environmental chamber at 20 °C under a 12 h light, 12 h dark cycle. They were fed the microalga *Tetraselmis* sp.[35] and allowed to acclimate to experimental conditions.

Preparation of Plastic Particles. Fluorescent polystyrene microspheres with a median diameter of 19 μm, and nonfluorescent polystyrene microspheres with median diameters of 113, 287, 510, and 1000 μm (density = 1.04 g/cm^3, Table S1 of the Supporting Information, SI) were obtained from Polysciences, Inc. and Cospheric, Inc. The diameter of each microsphere size class was verified by light microscopy. Black nylon fibers (Nylon-6.6; ca. 30 μm diameter) were obtained from A.C. Moore, Inc., and cut to median lengths of 75, 587, and 1075 μm (density = 1.14 g/cm^3; Table S1). The 75 μm fibers were cut using a cryogenic microtome following previously published methods,[36] and the 587 and 1075 μm fibers were cut by hand with a razor blade under a stereomicroscope. The polymer compositions of microspheres and microfibers were verified with Raman (Renishaw System 2000, Renishaw plc) and FTIR (Nicolet Magna 560, Thermo Fisher Scientific) microspectroscopy. Recorded spectra were compared against commercial Raman and FTIR spectral libraries (KnowItAll Software, Bio-Rad Laboratories, Inc.; Figure S1).

Concentrated stock suspensions of each particle type were prepared in Milli-Q water. Working suspensions were prepared by diluting the stock suspensions with filtered seawater (GF/C filter, nominal pore size of 1.2 μm) and then aging the suspensions at ca. 20 °C for 3 days.[34,37] Aging MP in seawater better mimicked conditions in the natural environment. After aging, particles were used in experiments described below.

Selection Experiments. All experiments were conducted in an environmental chamber (20 °C, 12 h:12 h light/dark cycle) following the general procedures used in previous experiments.[38] Oysters (5.2−7.9 cm shell height) and mussels (4.6−7.2 cm shell length) were offered MP in round plastic containers filled with 700 mL of filtered seawater (cartridge filtered, nominal pore size = 0.2 μm; hereafter referred to as FSW). Containers were thoroughly cleaned and rinsed with deionized water prior to use. One bivalve was positioned in each container by securing the craft stick, to which it was attached, to the container rim by means of a wooden clip.[34] Each container was supplied with gentle aeration and an initial concentration of microalgal food (*Tetraselmis* sp.) at 5000 cells/mL. Three different groups of oysters and mussels were used in the experiments, with each group receiving one of three MP suspensions. In Experiment 1, bivalves were offered a mixed microsphere suspension (four different sizes, median diameters of 19, 113, 287, and 510 μm); in Experiment 2, oysters and mussels were offered a mixed microfiber suspension (two different sizes, median lengths of 75 and 587 μm); and in Experiment 3, bivalves were offered a mixture of spheres and fibers (median diameter of 1000 μm and median length of 1075 μm). The number of particles in each size class offered to bivalves decreased with increasing sphere diameter or fiber length (Table S1).

Bivalves were offered MP by slowly delivering a small volume of one of the working suspensions near the inhalant aperture of an actively feeding animal using a micropipette.[39,40] Three, 200-μL aliquots were offered sequentially to each animal over 5 to 10 min during a single dosing period, with delivery of doses separated by 20 min. With each dose, bivalves were offered (nominal number) 735 microspheres (Experiment 1, all sizes), 495 microfibers (Experiment 2, all sizes), or 34 spheres and fibers (Experiment 3, both sizes). Not all MP particles offered to the bivalves entered the mantle cavity as a result of the minute and instantaneous adjustments bivalves made in the position of the inhalant mantle margin and in pumping rate. Those that were drawn into the mantle cavity were captured and represent the actual number of plastic particles to which the bivalves were exposed. In total, animals were offered six doses over a 2-h time period. After the first, third, and fifth dose, microalgal food (*Tetraselmis* sp.) was added to each container (concentration ca. 5000 cells/mL). The total concentration of particles to which bivalves were exposed (microalgal cells, MP) was below the threshold that stimulates excessive production of pseudofeces.[41−43] During the 2-h selection experiments, bivalves were continuously monitored and visible pseudofeces produced by the animals were collected. Any bivalve that closed before receiving at least five doses of MP was not used in the final analyses.

At the end of the 2-h exposure period, bivalves were held for an additional 1 h in their original containers so that they could purge residual pseudofeces (total of 3 h after initial exposure).

DOI: 10.1021/acs.est.9b02073
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Microalgal food was delivered at the same intervals as during the exposure period. Bivalves were then transferred to clean, aerated containers filled with filtered seawater and microalgal food (*Tetraselmis* sp.) at a concentration of 10 000 cells/mL and allowed to depurate MP. All discernible pseudofeces and feces in the original containers were identified under a stereomicroscope and collected in separate centrifuge tubes (15 mL). Importantly, identifying pseudofeces with the aid of a microscope was essential for two reasons: (1) at the low particle concentrations used, MP were often rejected as individual particles or clumps containing several particles (verified by endoscopic examination, see below) which were not visible with the unaided eye; and (2) some MP particles were not captured by the bivalves and instead settled to the bottom of the container. Therefore, to distinguish between particles rejected as pseudofeces and those that settled to the bottom before entering the mantle cavity and being captured, only particles with a mucus corona (Figure S2) were collected as pseudofeces. This approach ensured that estimates of the number of particles rejected were conservative values. Feces that were produced during the first 3 h were considered intestinal in origin, and were analyzed separately from glandular feces produced later in time.[44] After 24 h, animals were again transferred to clean containers with seawater and microalgal food, and biodeposits collected as described above. After 48 h, bivalves were removed from the containers, and final biodeposits collected. Twice each day during the depuration period, animals were delivered a volume of microalgal food (*Tetraselmis* sp.) to bring the final concentration in the containers to ca. 10 000 cells/mL. Previous studies have demonstrated that >90% of anthropogenic particles are egested by oysters and mussels within the first 48 h postexposure.[37,45−49] Thus, the quantity of MP found in feces is representative of the quantity of plastic particles ingested.

To release microspheres and microfibers from collected biodeposits for numeration, samples were subjected to a digestion protocol. Each sample was first centrifuged for 5 min at 1500 rcf (g). The seawater supernatant was decanted, the pellet resuspended in 5 mL of DI water, spun for another 5 min, and again decanted. This washing process was repeated two additional times to remove salts which react with sodium hydroxide (NaOH) to form a precipitate. After preparation, 2 mL of 1 N NaOH were added to each centrifuge tube.[34] Samples were then resuspended by means of a Vortex Genie and allowed to digest for at least 3 days. After digestion, samples were diluted with 2 mL of DI water to bring the total volume of each to ca. 4.0 mL. Subsamples (1 mL) were added to a rafter cell and the number of microspheres and fibers in each size class counted under a stereo or compound microscope (depending upon size). For the 19-μm spheres, counts were performed by means of fluorescent microscopy. Three to four replicate counts were performed for each sample. The number of particles per mL was then multiplied by the volume of sample to obtain the total number of plastic particles of each size class that were rejected or ingested. When analyzing samples of pseudofeces and feces from bivalves exposed to 75- and 587-μm fibers, tightly bound agglomerates often were observed. As there was no way to determine when the agglomerates formed (i.e., during production of biodeposits, prior to, or after treatment with NaOH), individual particles in agglomerates with five or more fibers were not counted. Instead the agglomerates were quantified. No significant differences were found between the number of agglomerates in pseudofeces and feces produced by either oysters or mussels ($p > 0.1$, paired t test).

Data Analysis. Separate tests were conducted for each species of bivalve. Two-way mixed model analysis of variance (ANOVA, GLM) for repeated measures procedures were used to compare the number of particles rejected (pseudofeces) to that ingested (total in all feces) using particle size and biodeposit type (pseudofeces, feces) as fixed effects and individual bivalves as the random effect. Separate models were run for Experiment 1 (mixed microspheres) and Experiment 2 (mixed microfibers). For microsphere data, both oyster and mussel models demonstrated a significant interaction effect between size and biodeposit type ($p < 0.001$). Therefore, each model was divided, and paired t tests used to examine differences in the number of particles rejected versus ingested in each size class. For microfiber data, only the model for mussels showed significant treatment effects. Differences in the mean number of particles rejected versus ingested for each size class were determined using a multicomparison test (Tukey's HSD). Paired t tests were also used to compare the number of 1075-μm fibers and 1000-μm beads rejected and ingested by oysters and mussels (Experiment 3).

One-way mixed-model ANOVA (GLM) for repeated measures procedures were used to compare the proportion of particles rejected and proportion of particles egested in <3 h using particle size as the fixed effect and individual bivalves (oysters or mussels) as the random effect. The proportion of microplastics rejected (spheres or fibers) was calculated as number rejected ÷ total number of captured particles (number in pseudofeces, intestinal feces, glandular feces). The proportion of microplastics egested in <3 h was calculated as number in intestinal feces ÷ total number in both intestinal and glandular feces. Separate models were run for Experiment 1 (microspheres) and Experiment 2 (microfibers). If significant differences were found, then a multicomparison test (Tukey's HSD) was used to determine differences between means. Paired t tests were used to compare the proportion of the largest microspheres (1000 μm) and microfibers (1075 μm) rejected in pseudofeces (same group of oysters or mussels, experiment 3). Two-sample t tests were used to compare the proportion of 1000-μm and 510-μm spheres, and proportion of 1075-μm and 587-μm fibers rejected in pseudofeces (two different groups of oysters, or two different groups of mussels, comparison of selected data from Experiments 1, 2 and 3). Prior to analyses, data were tested for normality and homoscedasticity, and transformed (square root) if required. Statistical analyses were performed using Systat 13, and for all tests an alpha level of 0.05 was used.

Endoscopic Examination. Detailed observations of the production of pseudofeces and the handling of plastic particles on the gills and labial palps of oysters and mussels were accomplished by means of video endoscopy (SI).[50,51] The endoscope, optical adapter, and attached CCD camera (Cohu, Inc.) were mounted onto a micromanipulator to enable fine positioning around the pseudofeces-discharge site and within the mantle cavity. This site is the region of the mantle at which pseudofeces are rejected and varies with species of bivalve. For oysters, the site is located at the anteroventral region of the mantle, adjacent to the labial palps. In contrast, for mussels, the site is located at the most posterior region of the mantle, near the junction between the inhalant aperture and exhalant siphon. Digital video was recorded onto 8 mm videocassettes

DOI: 10.1021/acs.est.9b02073
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Table 1. Number of Microspheres (A) and Microfibers (B) Rejected and Ingested by Oysters and Mussels[a]

A. microspheres			
species median diameter, μm	rejected (mean ± SD)	ingested (mean ± SD)	significance
oyster			
19	171.1 (164.1)	550.1 (377.1)	**
113	402.1 (276.1)	315.5 (151.8)	ns
287	215.1 (100.9)	55.2 (35.0)	**
510	6.7 (3.3)	2.6 (3.6)	**
1000	15.4 (8.6)	0	**
mussel			
19	143.7 (170.8)	1073.1 (319.5)	**
113	268.9 (205.3)	1065.2 (327.9)	**
287	113.4 (105.4)	198.3 (96.1)	ns
510	5.6 (3.9)	4.0 (4.1)	ns
1000	14.2 (8.4)	0	**
B. microfibers			
species median length, μm	rejected (mean ± SD)	ingested (mean ± SD)	significance
oyster			
75	232.4 (79.9)	241.3 (193.6)	ns
587	156.7 (108.1)	51.4 (57.4)	ns
1075	48.8 (44.6)	26.1 (26.6)	ns
mussel			
75	607.2 (789.1)	1302.5 (485.1)	ns
587	67.7 (50.8)	220.2 (77.3)	*
1075	23.6 (13.0)	92.7 (36.3)	**

[a]Outcomes of statistical comparisons are also shown. Note that not all particles offered to the bivalves were actually drawn into the mantle cavity and captured as a result of minute and instantaneous adjustments in the position of the inhalant mantle margin and pumping rate. Data are means ± standard deviation in parentheses; n = 11 oysters and 8 mussels for mixed spheres (19−510 μm), 7 oysters and 8 mussels for mixed fibers (75 and 587 μm), and 8 oysters and 10 mussels for the largest spheres (1000 μm) and fibers (1075 μm); * = $P < 0.05$, ** = $P < 0.01$, ns = not significant.

(Hi-8, Sony) for archival purposes (SI). Representative video sequences were captured and saved to a computer hard drive using Movie Maker (Microsoft). Still images were captured from video segments using VideoPad Editor (NCH Software), and minor adjustments to brightness and contrast were made to improve clarity.

Oysters (7.4−11.5 cm shell height) and mussels (6.8−8.0 cm shell length) were acclimated to laboratory conditions in a 38-L aquarium filled with aerated, filtered seawater (20−22 °C). Animals were delivered microalgal food ad libitum, consisting of a mixture of the microalga *Tetraselmis* sp. and Shellfish Diet (Reed Mariculture), and 50% of the water in the aquarium was changed daily. Prior to internal observations, a small portion of the ventral region of the shell of each oyster and mussel was trimmed to accommodate the optical insertion tube (OIT) of the endoscope and prevent damage to the tube when the animal adducted its valves. Shell material was carefully removed without damaging the underlying mantle, and animals were allowed to recover for 1 day before being examined.[52−54] Prior to endoscopic observation, each bivalve was placed in a 1-L aerated chamber filled with filtered seawater (ca. 21 °C), delivered several mL of microalgal food (*Tetraselmis* sp.), and allowed to acclimate to experimental conditions. Observations were made after the animal opened its valves and showed signs of feeding (i.e., shells open, mantles extended).

Two different observational assays were performed. In the first, the endoscope was oriented near the pseudofeces-discharge site, and the relative form and amount of pseudofeces produced was assessed (individual particles, small particle clumps, large particle bolus). In the second assay, the OIT was inserted between the valves of the bivalve and observations made of the capture and transport of plastic particles on the gills and labial palps. As in the selection experiments, mixed microspheres, mixed microfibers, and a mixture of large microspheres and microfibers were offered to the bivalves. Three, 200-μL aliquots were offered sequentially to each animal over 5 to 10 min during a single dosing period, with delivery of doses separated by 20 min. For the second assay, occasionally it was necessary to deliver near the inhalant aperture more than three aliquots of MP suspension to observe particle capture in the small area of the gill that was being examined.

RESULTS

Selection Experiments. The number of microspheres rejected versus ingested by oysters and mussels depended upon particle size (Table 1). For oysters, a significantly lower number of 19-μm spheres was rejected in pseudofeces compared to that ingested, whereas for the larger diameter spheres (287, 510, 1000 μm), significantly higher numbers were rejected ($P < 0.01$). Equal numbers of 113-μm spheres were rejected and ingested. Mussels showed a similar trend, but rejected significantly lower numbers of 19- and 113-μm spheres and rejected a significantly higher number of 1000-μm spheres compared to that ingested ($P < 0.01$; Table 1). Equal numbers of 287- and 510-μm spheres were rejected and ingested. Notably, no 1000-μm spheres were ingested by either oysters or mussels. The rejection and ingestion of microfibers by the bivalves showed a different trend (Table 1). Oysters rejected and ingested equal numbers of fibers regardless of size.

DOI: 10.1021/acs.est.9b02073
Environ. Sci. Technol. 2019, 53, 8776−8784

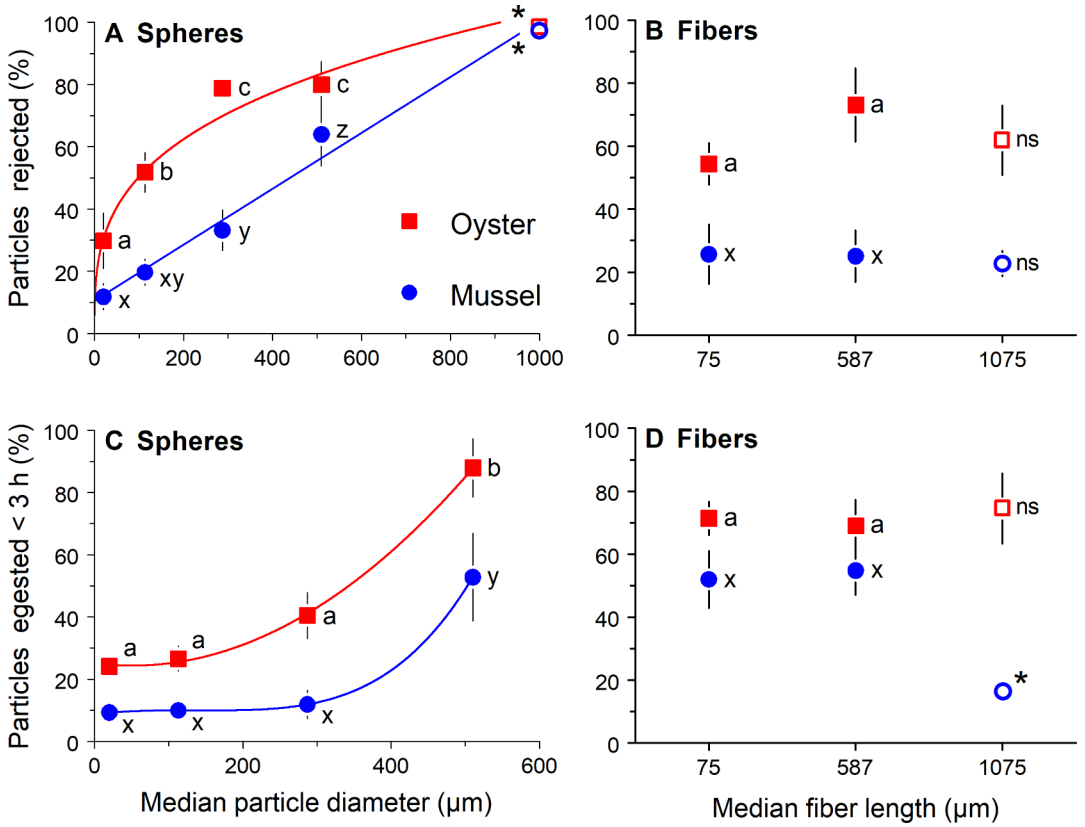

Figure 1. Proportion (%) of microspheres and microfibers rejected in pseudofeces and egested in feces in <3 h (see text for determination of proportions). Closed symbols indicate data from bivalves that were delivered a mixture of microspheres of different diameters (A, C) or microfibers of different lengths (B, D). Open symbols indicate data from a separate group of bivalves delivered a mixture of large microspheres (1000-μm diameter) and microfibers (1075-μm length; A, B, D). For each species (oyster, mussel), means that are significantly different are designated by different letters (repeated-measures tests; P at least <0.05). Trends based on lines of best fit (regression) are provided for data that show a relationship with particle size. Asterisks and ns indicate significant and nonsignificant differences, respectively, between means of largest and second largest size classes (two-sample t tests; $P < 0.05$). Data are means ± standard error of the mean; n = 7−11 (oysters) and 8−10 (mussels).

In contrast, mussels rejected a significantly lower number of 587- and 1075-μm fibers ($P < 0.05$ and $P < 0.01$, respectively), and rejected and ingested equal numbers of 75-μm fibers.

For both oysters and mussels, the proportion of microspheres rejected in pseudofeces increased with sphere size, whereas rejection of fibers was variable and showed no trend with size (Figure 1A, B). Significantly different proportions of spheres were rejected by oysters across the 19-, 113-, 287-, and 510-μm size classes ($P < 0.01$). No difference was found in the proportions of 287- and 510-μm spheres rejected. Mussels also rejected significantly different proportions of spheres across the four size classes ($P < 0.05$), but no differences were found between 113-μm spheres and the 19- and 287-μm spheres (Figure 1A). For both species, a significantly higher proportion of 1000-μm spheres was rejected compared to the proportion of 510-μm spheres rejected ($P < 0.05$). In contrast, there was no significant difference in the proportion of 75- and 587-μm fibers, or between the proportion of 587- and 1075-μm fibers rejected by either species (Figure 1B).

Additionally, for both oysters and mussels, the proportions of ingested 510-μm spheres that were egested in <3 h was significantly higher than those of the other three size classes ($P < 0.01$; Figure 1C). No differences were found between the 19-, 113-, and 287-μm size classes for either species. The proportions of 75- and 587-μm fibers egested by oysters in <3 h were not significantly different, nor were the proportions of egested 587- and 1075-μm fibers (Figure 1D). In contrast, although the proportions of 75- and 587-μm fibers egested by mussels in <3 h were not significantly different, there was a significant difference in the proportions of egested 587- and 1075-μm fibers ($P < 0.01$). A lower proportion of the longer fibers was egested by mussels in <3 h (Figure 1D).

When microspheres and microfibers were delivered simultaneously, both oysters and mussels rejected a significantly higher proportion of 1000-μm diameter spheres than 1075-μm long fibers (oysters $P < 0.05$, mussels $P < 0.01$; Figure 2).

Endoscopic Examination. Examinations in vivo indicated that the gills of oysters and mussels could capture and transport all sizes of microspheres and microfibers (Figure 3A, B). The heterorhabdic gills of oysters generally carried larger spheres (diameter >19 μm) and fibers (length >75 μm) to the ventral (aka, marginal) grooves, and smaller particles to the dorsal (aka, basal) tracts. Upon entering the grooves and tracts, MP were transported anteriorly toward the labial palps. The homorhabdic gills of mussels carried all MP to the ventral grooves. In both species, large spheres (e.g., 510 μm) rotated on the frontal surface during ciliary transport, and large fibers (587 and 1075 μm) were oriented parallel to the anterior-posterior axis before entering the ventral grooves. Examination of the pseudofeces-discharge sites on the mantle provided information on the process by which plastic particles are rejected. Oysters accumulated MP destined for rejection in mucous boluses of various sizes. Periodically, oysters adducted

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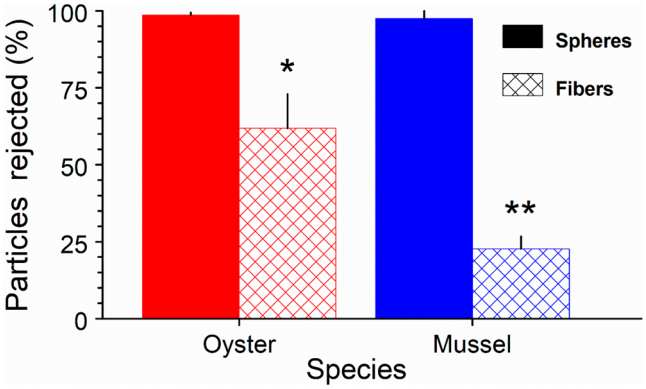

Figure 2. Proportion of large microspheres (1000-μm diameter) and microfibers (1075-μm length) rejected by oysters and mussels (see text for determination of proportions). Asterisks indicate significant differences between rejection of spheres and fibers for oysters ($P < 0.05$) and mussels ($P < 0.01$). Data are means ± standard error of the mean; $n = 8$ (oysters) and 10 (mussels).

their valves and ejected the material from the mantle cavity which often caused the boluses to fragment into smaller masses. Plastic particles of all sizes and shapes were rejected (Figure 3C, E), and the process of accumulation and ejection often took 20 min or longer. Mussels also rejected MP of all sizes and shapes. Generally, microspheres were ejected as singlets, doublets, or in small boluses (Figure 3D). Large fibers (587 μm) were released individually or in mucous boluses containing smaller fibers (75 μm; Figure 3F). Typically, spheres and fibers began to be rejected within 20 min of exposure. Importantly, most of the pseudofeces rejected by oysters and mussels, including the small boluses, were too small to be seen by the unaided eye.

DISCUSSION

The quantitative data provided here falsify all three null hypotheses. Oysters and mussels did not ingest all encountered MP indiscriminately. Rather, they rejected a higher proportion of large spheres and ingested a higher proportion of small spheres. Although there were no similar relationships with fibers, on average oysters rejected >50% and mussels >20% of all fibers to which they were exposed. Differences between the two species may reflect the more complex heterorhabdic gill structure of oysters, which perform bidirectional transport and particle selection.[55−58] The homorhabdic gill structure of mussels perform predominately unidirectional transport and cannot carry out particle selection.[51,59] As a result, oysters have two sites for particles selection (gills and labial palps), whereas mussels have only one (labial palps). The rejection of all microspheres with a diameter of 1000 μm by both species of bivalves demonstrates that there is an upper limit to the size of plastic particles that can be handled and ingested. In this study, the limit for ingestion was 1000 μm for low aspect-ratio particles (e.g., spheres, fragments). For particles with a high aspect ratio, such as fibers, handling and ingestion is less constrained provided that one dimension is within the size that can be ingested. Although the current study did not test the selection of MP < 19 μm in size, previous studies have demonstrated that synthetic particles with a diameter of 10 μm (e.g., alumina, silica, polystyrene) can be preferentially ingested or rejected based on their surface properties (i.e., surface charge, wettability, organic coating)[45,60] Therefore, even

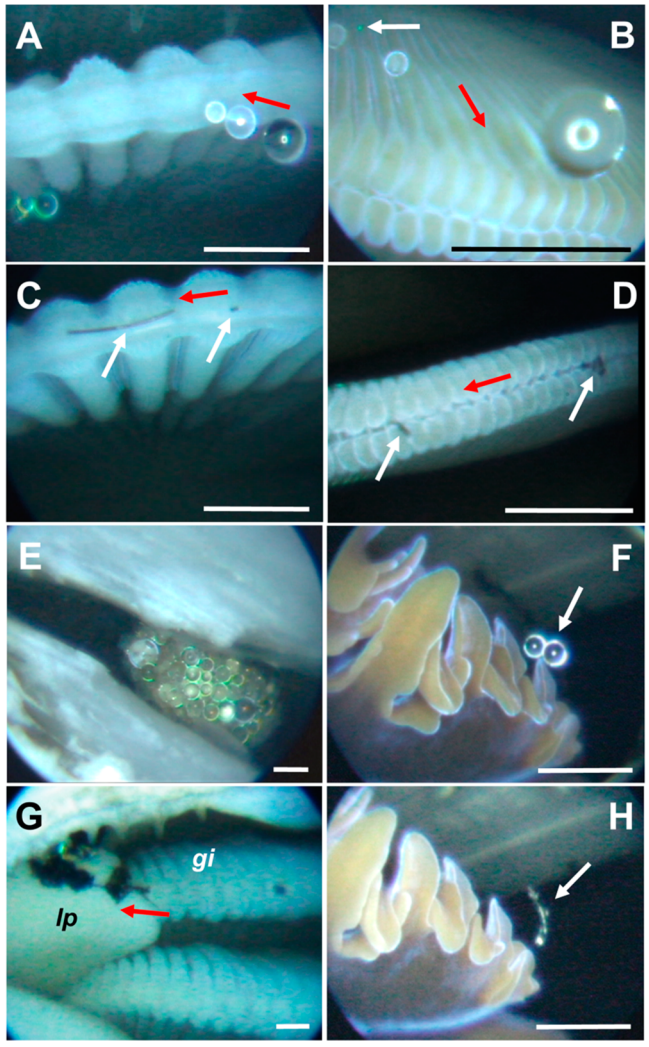

Figure 3. Endoscopic examination of feeding structures of oysters and mussels at low particle concentrations. After capture, microspheres of all sizes were transported to the ventral margin of the gill of oysters (A) and mussels (B). Note the transport of a 19-μm sphere (white arrow in B) alongside larger spheres. Fibers of different sizes (white arrows) were also transported anteriorly in the ventral groove of the gill of oysters (C) and mussels (D). At the pseudofeces-discharge site of the oyster, spheres (E) and fibers accumulated in mucus boluses and were rejected. At the pseudofeces-discharge site of the mussel, one or two spheres (F) or fibers (H, white arrows) at a time were often rejected. Within the mantle cavity of the oyster (G), small fiber boluses were transported from the gills (*gi*) to the smooth side of the labial palps (*lp*), and then to the pseudofeces-discharge site for rejection. In many instances, the rejected microplastics could not be seen by the unaided eye. Magnification ca. 150×; Red arrows indicate direction of movement of material on the gills and palps. Scale bars ca. 500 μm for foreground images.

plastic particles smaller than 19 μm can be subjected to the selection process and potentially be rejected or ingested depending on their surface characteristics. Results of the current study are congruent with those of previous research that has examined the selection of plastic particles by bivalves.[46,60−62] For example, using different diameters of glass and polystyrene microspheres (10, 40, 150, 275, 370, 410 μm), Tamburri and Zimmer-Faust[46] found that oysters (*C. virginica*) rejected 30−40% of the smallest spheres and ca. 100% of the largest spheres, regardless of sphere type. Woods

DOI: 10.1021/acs.est.9b02073
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et al.[62] examined the rejection and ingestion of polyethylene terephthalate fibers (ca. 460 μm in length) by mussels (*M. edulis*). They found that at a concentration of 30×10^3 microfibers/L, mussels rejected 71% of the fibers in pseudofeces, with only ca. 9% of the particles being ingested.

The residence time of MP within the gut of bivalves also can be affected by microsphere size, with particles <500 μm being retained longer. For oysters, no relationship was found between length of microfiber and proportion egested in <3 h; however, ca. 70% of all fibers were egested within this time period. For mussels, a lower proportion of the longest microfibers were egested in <3 h compared to the shorter fibers, suggesting that the gut residence time for these fibers is higher. Postingestive selection of MP by bivalves has been demonstrated previously.[63,64] In a study on the sea scallop, *Placopecten magellanicus*, Brillant and MacDonald[64] found that 20-μm polystyrene spheres were retained in the gut longer than 5-μm spheres. They also reported that residence time of 9-μm polystyrene spheres was longer than that of similar-sized glass spheres (8 μm) with a higher density. None of the spheres, however, were observed in histological sections of the digestive gland, suggesting that the differential treatment of spheres occurred in the stomach. Taken together, these data demonstrate that the selection of plastic particles in the gut of bivalves occurs, and the time course over which MP are egested will depend on particle size and shape.

Qualitative results from in vivo examinations demonstrate that MP of different sizes and shapes are captured and handled by the feeding organs in the same manner as natural particles.[53,65−67] Additionally, examination of the pseudofeces-discharge sites of oysters and mussels provided information that has implications for previous and future studies on interactions between MP and bivalves. The observations presented here demonstrate that at low concentrations, bivalves can reject individual plastic particles or small particle masses that cannot be seen with the unaided eye. This fact has not been appreciated by many previous workers who have collected biodeposits without the aid of a microscope. By doing so, they have likely underestimated the number of plastic particles rejected, because not all of the rejected pseudofecal material was collected, and overestimated the number of plastic particles that were ingested, because the feces were contaminated with pseudofeces.[12,62,68] Such errors have led some researchers to suggest, incorrectly, that the quantity and types of MP ingested by bivalves accurately represent those suspended in the natural environment. Future studies that aim to examine selection of MP by bivalves under environmentally relevant concentrations should differentiate and collect biodeposits with the aid of a microscope.

The presented data clearly demonstrate that MP size and shape affect the rejection, ingestion, and egestion of plastic particles by oysters and mussels. These results are congruent with previous laboratory studies on particle feeding in bivalves, and support the results of field studies that examined uptake of MP by mussels.[21,49] For example, in a recent study Zhao et al.[21] quantified the number and type of MP in mussels (*M. edulis*) and suspended marine aggregates in samples collected during two different months of the year. Calculations of the number of plastic particles that mussels encountered per day, based on known clearance rates and the measured abundance of microplastics in aggregates, demonstrated that mussels contained only ca. 1% of the available MP in their digestive gland and gut. Therefore, a large portion of plastic particles were likely rejected or rapidly egested in feces. Although MP abundances in marine aggregates varied significantly over time, no temporal differences in the abundance of plastic particles ingested by mussels were observed. These data demonstrate the consistency of particle-feeding processes of mussels (e.g., capture efficiency, particle selection).

Results of the current study and the rich body of literature on particle-selection capabilities of many bivalve species[13,15,69] clearly demonstrate that bivalves are not robust indicators of MP pollution, and explain why the number of MP identified in bivalves is typically low compared to that in the environment.[21,29,49] The quantity and quality of MP identified in bivalves collected in situ will not be a good proxy for the concentration and type suspended in the water, and will be biased toward small, low aspect-ratio particles (e.g., spheres) and high aspect-ratio particles (e.g., fibers). If the loads of MP to which bivalves are exposed in the environment are episodic rather than constant (e.g., higher concentrations after a wind-induced resuspension event), then the time course over which plastics of different size and shape are egested will further complicate attempts to extract environmental information. It is strongly advised that other marine species be explored as sentinel organisms of MP pollution.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b02073.

Size and number of microspheres and microfibers offered to oysters and mussels in the three experimental treatments (Table S1); Raman and FTIR spectra of microspheres and microfibers showing concordance with polystyrene and nylon-66 polymers, respectively (Figure S1); examples of microplastics in mussel pseudofeces and feces (Figure S2); and descriptions of video sequences from endoscopic observations corresponding to uploaded videos 1−7 (PDF)

Video sequence 1, Mussel frontal-ventral fibers (AVI)

Video sequence 2, Mussel frontal-ventral spheres (AVI)

Video sequence 3, Mussel pseudofeces small and large spheres (AVI)

Video sequence 4, Mussel pseudofeces small fibers (AVI)

Video sequence 5, Oyster frontal-ventral-dorsal fibers (AVI)

Video sequence 6, Oyster frontal-ventral-dorsal spheres (AVI)

Video sequence 7, Oyster pseudofeces spheres (AVI)

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The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Norm Bloom and Son, LLC for assistance in collecting oysters. This work was supported by grants from the

DOI: 10.1021/acs.est.9b02073
Environ. Sci. Technol. 2019, 53, 8776−8784

United States Department of Agriculture, NIFA program 2016-67017-24427 (to J.E.W.), and from the National Oceanic and Atmospheric Administration, Marine Debris program NA17NOS9990121 (to J.E.W., S.E.S.).

REFERENCES

(1) Secretariat of the Convention on Biological Diversity [SCBD] and the Scientific and Technical Advisory Panel-GEF. *Impacts of Marine Debris on Biodiversity: Current Status and Potential Solutions*; SCBD: Montreal, 2012, Technical Series No. 67; 61 pages.

(2) UNEP. *Marine Plastic Debris and Microplastics: Global Lessons and Research to Inspire Action and Guide Policy Change*; United Nations Environment Programme: Nairobi, 2016.

(3) Hartmann, N. B.; Hüffer, T.; Thompson, R. C.; Hassellöv, M.; Verschoor, A.; Daugaard, A. E.; Rist, S.; Karlsson, T.; Brennholt, N.; Cole, M.; Herrling, M. P.; Hess, M. C.; Ivleva, N. P.; Lusher, A. L.; Wagner, M. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. *Environ. Sci. Technol.* **2019**, *53*, 1039−1047.

(4) Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T. S. Microplastics as contaminants in the marine environment: a review. *Mar. Pollut. Bull.* **2011**, *62* (12), 2588−2597.

(5) Von Moos, N.; Burkhardt-Holm, P.; Köhler, A. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environ. Sci. Technol.* **2012**, *46* (20), 11327−11335.

(6) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T. S. Microplastic ingestion by zooplankton. *Environ. Sci. Technol.* **2013**, *47* (12), 6646−6655.

(7) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Galloway, T. S. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. *Environ. Sci. Technol.* **2015**, *49* (2), 1130−1137.

(8) Wright, S. L.; Thompson, R. C.; Galloway, T. S. The physical impacts of microplastics on marine organisms: a review. *Environ. Pollut.* **2013**, *178*, 483−492.

(9) Foley, C. J.; Feiner, Z. S.; Malinich, T. D.; Höök, T. O. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Sci. Total Environ.* **2018**, *631*, 550−559.

(10) Paul-Pont, I.; Lacroix, C.; Gonzalez Fernandez, C.; Hegaret, H.; Lambert, C.; Le Goic, N.; Frere, L.; Cassone, A.-L.; Sussarellu, R.; Fabioux, C.; et al. Exposure of marine mussels *Mytilus* spp. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. *Environ. Pollut.* **2016**, *216*, 724−737.

(11) Sussarellu, R.; Suquet, M.; Thomas, Y.; Lambert, C.; Fabioux, C.; Pernet, M. E. J.; Le Goïc, N.; Quillien, V.; Mingant, C.; Epelboin, Y.; et al. Oyster reproduction is affected by exposure to polystyrene microplastics. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (9), 2430−2435.

(12) Xu, X.; Lee, W.; Chan, A.; Lo, H.; Shin, P.; Cheung, S. Microplastic ingestion reduces energy intake in the clam *Atactodea striata*. *Mar. Pollut. Bull.* **2017**, *124* (2), 798−802.

(13) Ward, J. E.; Shumway, S. E. Separating the grain from the chaff: particle selection in suspension-and deposit-feeding bivalves. *Journal of Experimental Marine Biology and Ecology* **2004**, *300* (1−2), 83−130.

(14) Cranford, P. J.; Ward, J. E.; Shumway, S. E. Bivalve filter feeding: variability and limits of the aquaculture biofilter. In *Shellfish Aquaculture and the Environment*; Shumway, S. E., Ed.; John Wiley & Sons: Ames, IA, 2011; pp 81−124.

(15) Rosa, M.; Ward, J. E.; Shumway, S. E. Selective capture and ingestion of particles by suspension-feeding bivalve Molluscs: A review. *J. Shellfish Res.* **2018**, *37* (4), 727−747.

(16) Li, J.; Qu, X.; Su, L.; Zhang, W.; Yang, D.; Kolandhasamy, P.; Li, D.; Shi, H. Microplastics in mussels along the coastal waters of China. *Environ. Pollut.* **2016**, *214*, 177−184.

(17) Setälä, O.; Norkko, J.; Lehtiniemi, M. Feeding type affects microplastic ingestion in a coastal invertebrate community. *Mar. Pollut. Bull.* **2016**, *102* (1), 95−101.

(18) Li, J.; Green, C.; Reynolds, A.; Shi, H.; Rotchell, J. M. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. *Environ. Pollut.* **2018**, *241*, 35−44.

(19) Qu, X.; Su, L.; Li, H.; Liang, M.; Shi, H. Assessing the relationship between the abundance and properties of microplastics in water and in mussels. *Sci. Total Environ.* **2018**, *621*, 679−686.

(20) Su, L.; Cai, H.; Kolandhasamy, P.; Wu, C.; Rochman, C. M.; Shi, H. Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems. *Environ. Pollut.* **2018**, *234*, 347−355.

(21) Zhao, S.; Ward, J. E.; Danley, M.; Mincer, T. J. Field-based evidence for microplastic in marine aggregates and mussels: implications for trophic transfer. *Environ. Sci. Technol.* **2018**, *52* (19), 11038−11048.

(22) Zorita, I.; Apraiz, I.; Ortiz-Zarragoitia, M.; Orbea, A.; Cancio, I.; Soto, M.; Marigómez, I.; Cajaraville, M. P. Assessment of biological effects of environmental pollution along the NW Mediterranean Sea using mussels as sentinel organisms. *Environ. Pollut.* **2007**, *148* (1), 236−250.

(23) O'Connor, T. P. Mussel Watch results from 1986 to 1996. *Mar. Pollut. Bull.* **1998**, *37* (1−2), 14−19.

(24) Goldberg, E. D. The mussel watch: a first step in global marine monitoring. *Mar. Pollut. Bull.* **1975**, *6*, 111−114.

(25) Goodsell, P.; Underwood, A.; Chapman, M. Evidence necessary for taxa to be reliable indicators of environmental conditions or impacts. *Mar. Pollut. Bull.* **2009**, *58* (3), 323−331.

(26) Avio, C. G.; Gorbi, S.; Regoli, F. Plastics and microplastics in the oceans: from emerging pollutants to emerged threat. *Mar. Environ. Res.* **2017**, *128*, 2−11.

(27) Wesch, C.; Bredimus, K.; Paulus, M.; Klein, R. Towards the suitable monitoring of ingestion of microplastics by marine biota: A review. *Environ. Pollut.* **2016**, *218*, 1200−1208.

(28) Bonanno, G.; Orlando-Bonaca, M. Perspectives on using marine species as bioindicators of plastic pollution. *Mar. Pollut. Bull.* **2018**, *137*, 209−221.

(29) Bråte, I. L. N.; Hurley, R.; Iversen, K.; Beyer, J.; Thomas, K. V.; Steindal, C. C.; Green, N. W.; Olsen, M.; Lusher, A. *Mytilus* spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: A qualitative and quantitative study. *Environ. Pollut.* **2018**, *243*, 383−393.

(30) Li, H.-X.; Ma, L.-S.; Lin, L.; Ni, Z.-X.; Xu, X.-R.; Shi, H.-H.; Yan, Y.; Zheng, G.-M.; Rittschof, D. Microplastics in oysters *Saccostrea cucullata* along the pearl river estuary, china. *Environ. Pollut.* **2018**, *236*, 619−625.

(31) Li, J.; Lusher, A. L.; Rotchell, J. M.; Deudero, S.; Turra, A.; Brate, I. L. N.; Sun, C.; Shahadat Hossain, M.; Li, Q.; Kolandhasamy, P.; Shi, H. Using mussel as a global bioindicator of coastal microplastic pollution. *Environ. Pollut.* **2019**, *244*, 522−533.

(32) Phuong, N. N.; Poirier, L.; Pham, Q. T.; Lagarde, F.; Zalouk-Vergnoux, A. Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: location, season and/or mode of life? *Mar. Pollut. Bull.* **2018**, *129* (2), 664−674.

(33) Galtsoff, P. S. The American Oyster, Crassostrea virginica (Gmelin). U.S. Fish. Wildl. Serv. *Fish. Bull.* **1964**, *64*, 1−457.

(34) Ward, J. E.; Kach, D. J. Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. *Mar. Environ. Res.* **2009**, *68* (3), 137−142.

(35) Wikfors, G. H.; Patterson, G. W.; Ghosh, P.; Lewin, R. A.; Smith, B. C.; Alix, J. H. Growth of post-set oysters, *Crassostrea virginica*, on high-lipid strains of algal flagellates *Tetraselmis* spp. *Aquaculture* **1996**, *143* (3−4), 411−419.

(36) Cole, M. A novel method for preparing microplastic fibers. *Sci. Rep.* **2016**, *6*, 34519.

(37) Kach, D. J.; Ward, J. E. The role of marine aggregates in the ingestion of picoplankton-size particles by suspension-feeding molluscs. *Mar. Biol.* **2008**, *153*, 797−805.

(38) Milke, L. M.; Ward, J. E. Influence of diet on pre-ingestive particle processing in bivalves: II. Residence time in the pallial cavity and handling time on the labial palps. *J. Exp. Mar. Biol. Ecol.* **2003**, *293* (2), 151−172.

DOI: 10.1021/acs.est.9b02073
Environ. Sci. Technol. 2019, 53, 8776−8784

(39) Rosa, M.; Ward, J. E.; Holohan, B. A.; Shumway, S. E.; Wikfors, G. H. Physicochemical surface properties of microalgae and their combined effects on particle selection by suspension-feeding bivalve molluscs. *J. Exp. Mar. Biol. Ecol.* **2017**, *486*, 59−68.
(40) Rosa, M.; Ward, J. E.; Ouvrard, M.; Holohan, B. A.; Pales Espinosa, E.; Shumway, S. E.; Allam, B. Examining the physiological plasticity of particle capture by the blue mussel, *Mytilus edulis* (L.): Confounding factors and potential artifacts with studies utilizing natural seston. *Journal of Experimental Marine Biology and Ecology* **2015**, *473*, 207−217.
(41) Bayne, B.; Iglesias, J.; Hawkins, A.; Navarro, E.; Heral, M.; Deslous-Paoli, J. Feeding behaviour of the mussel, *Mytilus edulis*: responses to variations in quantity and organic content of the seston. *J. Mar. Biol. Assoc. U. K.* **1993**, *73* (4), 813−829.
(42) Kiørboe, T.; Mølenberg, F.; Nøhr, O. Feeding, particle selection and carbon absorption in *Mytilus edulis* in different mixtures of algae and resuspended bottom material. *Ophelia* **1980**, *19* (2), 193−205.
(43) Bayne, B.; Newell, R. Physiological energetics of marine mollusks. In *The Mollusca. Physiology Part 1*; Salevddin, A. S. M., Wilbur, K. M., Eds.; Academic Press: New York, 1983; pp 407−515.
(44) van Weel, P. B. The comparative physiology of digestion in mussels. *Am. Zool.* **1961**, *1*, 245−252.
(45) Ward, J. E.; Targett, N. Influence of marine microalgal metabolites on the feeding behavior of the blue mussel *Mytilus edulis*. *Mar. Biol.* **1989**, *101* (3), 313−321.
(46) Tamburri, M. N.; Zimmer-Faust, R. K. Suspension feeding: basic mechanisms controlling recognition and ingestion of larvae. *Limnol. Oceanogr.* **1996**, *41* (6), 1188−1197.
(47) Doyle, J. J.; Ward, J. E.; Mason, R. An examination of the ingestion, bioaccumulation, and depuration of titanium dioxide nanoparticles by the blue mussel (*Mytilus edulis*) and the eastern oyster (*Crassostrea virginica*). *Mar. Environ. Res.* **2015**, *110*, 45−52.
(48) Doyle, J. J.; Ward, J. E.; Mason, R. Exposure of bivalve shellfish to titania nanoparticles under an environmental-spill scenario: Encounter, ingestion and egestion. *J. Mar. Biol. Assoc. U. K.* **2016**, *96* (1), 137−149.
(49) Van Cauwenberghe, L.; Claessens, M.; Vandegehuchte, M. B.; Janssen, C. R. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environ. Pollut.* **2015**, *199*, 10−17.
(50) Ward, J. E.; Beninger, P.; MacDonald, B.; Thompson, R. Direct observations of feeding structures and mechanisms in bivalve molluscs using endoscopic examination and video image analysis. *Mar. Biol.* **1991**, *111* (2), 287−291.
(51) Ward, J. E.; MacDonald, B.; Thompson, R.; Beninger, P. Mechanisms of suspension feeding in bivalves: resolution of current controversies by means of endoscopy. *Limnol. Oceanogr.* **1993**, *38* (2), 265−272.
(52) Ward, J. E.; Levinton, J. S.; Shumway, S. E. Influence of diet on pre-ingestive particle processing in bivalves: I: transport velocities on the ctenidium. *J. Exp. Mar. Biol. Ecol.* **2003**, *293* (2), 129−149.
(53) Mafra, L. L., Jr; Bricelj, V. M.; Ward, J. E. Mechanisms contributing to low domoic acid uptake by oysters feeding on *Pseudo-nitzschia* cells. II. Selective rejection. *Aquat. Biol.* **2009**, *6*, 213−226.
(54) Robbins, H. M.; Bricelj, V. M.; Ward, J. E. In vivo effects of brown tide on the feeding function of the gill of the northern quahog *Mercenaria mercenaria* (Bivalvia: Veneridae). *Biol. Bull.* **2010**, *219* (1), 61−71.
(55) Ribelin, B. W.; Collier, A. Studies on the gill ciliation of the American oyster *Crassostrea virginica* (Gmelin). *J. Morphol.* **1977**, *151* (3), 439−449.
(56) Ward, J. E.; Newell, R. I.; Thompson, R. J.; MacDonald, B. A. In vivo studies of suspension-feeding processes in the eastern oyster, *Crassostrea virginica* (Gmelin). *Biol. Bull.* **1994**, *186* (2), 221−240.
(57) Ward, J. E.; Levinton, J. S.; Shumway, S. E.; Cucci, T. Site of particle selection in a bivalve mollusc. *Nature* **1997**, *390* (6656), 131.
(58) Ward, J. E.; Levinton, J.; Shumway, S.; Cucci, T. Particle sorting in bivalves: in vivo determination of the pallial organs of selection. *Mar. Biol.* **1998**, *131* (2), 283−292.
(59) Atkins, D. On the ciliary mechanisms and interrelationships of lamellibranchs. Part III: Types of lamellibranch gills and their food currents. *Quart. J. micr. Sci.* **1937**, *79*, 375−421.
(60) Rosa, M.; Ward, J. E.; Shumway, S. E.; Wikfors, G. H.; Pales-Espinosa, E.; Allam, B. Effects of particle surface properties on feeding selectivity in the eastern oyster *Crassostrea virginica* and the blue mussel *Mytilus edulis*. *J. Exp. Mar. Biol. Ecol.* **2013**, *446*, 320−327.
(61) Chaparro, O. R.; Thompson, R. J.; Ward, J. E. *In vivo* observations of larval brooding in the Chilean oyster, *Ostrea chilensis* Philippi, 1845. *Biol. Bull.* **1993**, *185* (3), 365−372.
(62) Woods, M. N.; Stack, M. E.; Fields, D. M.; Shaw, S. D.; Matrai, P. A. Microplastic fiber uptake, ingestion, and egestion rates in the blue mussel (*Mytilus edulis*). *Mar. Pollut. Bull.* **2018**, *137*, 638−645.
(63) Cranford, P. J.; Emerson, C. W.; Hargrave, B. T.; Milligan, T. G. *In situ* feeding and absorption responses of sea scallops *Placopecten magellanicus* (Gmelin) to storm-induced changes in the quantity and composition of the seston. *J. Exp. Mar. Biol. Ecol.* **1998**, *219*, 45−70.
(64) Brillant, M. G. S.; MacDonald, B. A. Postingestive selection in the sea scallop, *Placopecten magellanicus* (Gmelin): the role of particle size and density. *J. Exp. Mar. Biol. Ecol.* **2000**, *253*, 211−227.
(65) Ward, J. E.; Sanford, L.; Newell, R.; MacDonald, B. A new explanation of particle capture in suspension-feeding bivalve molluscs. *Limnol. Oceanogr.* **1998**, *43* (5), 741−752.
(66) Cognie, B.; Barillé, L.; Massé, G.; Beninger, P. G. Selection and processing of large suspended algae in the oyster *Crassostrea gigas*. *Mar. Ecol.: Prog. Ser.* **2003**, *250*, 145−152.
(67) Defossez, J.-M.; Hawkins, A. Selective feeding in shellfish: size-dependent rejection of large particles within pseudofaeces from *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes decussatus*. *Mar. Biol.* **1997**, *129* (1), 139−147.
(68) Browne, M. A.; Dissanayake, A.; Galloway, T. S.; Lowe, D. M.; Thompson, R. C. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ. Sci. Technol.* **2008**, *42* (13), 5026−5031.
(69) Ward, J. E.; Rosa, M.; Shumway, S. E. Capture, ingestion and egestion of microplastics by suspension-feeding bivalves: a 40-year history. *Anthropocene Coasts* **2019**, *2*, 39−49.

DOI: 10.1021/acs.est.9b02073
Environ. Sci. Technol. 2019, 53, 8776−8784