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SHORT COMMUNICATION

IDENTIFYING MICRODEBRIS IN BIODEPOSITS OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*§

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INTRODUCTION

Microdebris, which is typically comprised of microplastics and other anthropogenic particles <5 mm in diameter, is a pervasive pollutant of growing global concern, particularly in shellfish (e.g., Rochman et al. 2015, Littman et al. 2020, Alfred et al. 2022). Microplastics are often the major component of microdebris and may be directly produced for use in products such as abrasive cleaners or derived from degradation of larger particles (Browne et al. 2009). Microdebris enters freshwater and marine environments through wastewater and terrestrial run—off, posing a risk to aquatic species (Browne et al. 2009, Tang et al. 2021). Filter and suspension feeders, such as bivalves, may be at particular risk of microdebris ingestion as they uptake particulate matter in the water column (Guzzetti et al. 2018, Thomas et al. 2020). Microdebris alone can have direct effects on bivalves by altering filtration, reproduction, immune response, and mortality, as well as indirect impacts on associated benthic communities and ecological services (von Moos et al. 2012, Sussarellu et al. 2016, Green et al 2017, Thomas et al. 2020). Microdebris ingested by bivalves also has the potential to convey contaminants as either leachates or associates that affect physiological processes and immune function (e.g., Sendra et al. 2021). Furthermore, some microdebris in bivalves may bioaccumulate to higher trophic levels, including through human consumption (e.g., Van Cauwenberghe and Janssen 2014). These factors make bivalves an important candidate species for biomonitoring of microdebris in aquatic environments (Goncalves et al. 2019, Littman et al. 2020).

To better understand the direct and indirect effects of microdebris on bivalves and assess the effectiveness of these species as bioindicators of microdebris pollution, it is important to first understand their respective uptake, ingestion, and expulsion of microdebris. Upon uptake, bivalves selectively eject particles as pseudofeces or transport them to the mouth for ingestion followed by excretion as feces (Craig et al. 2022). Pseudofeces and feces, collectively known as biodeposits, can be visually discriminated, allowing for study of particle selection (Fila et al. 2001, Dalrymple and Carmichael 2015). To date, most studies of microdebris (often generically referred to as microplastics) in bivalves have examined whole tissues without examining biodeposits, yielding information only on microdebris uptake but not expulsion or ingestion (Li et al. 2021). These studies, therefore, are limited in assessing important factors such as the duration that bivalves are exposed to microdebris, the mechanisms of microdebris retention, and the potential for bioaccumulation and trophic transfer.

In this study, we quantified microdebris in the biodeposits of the Eastern oyster (*Crassostrea virginica*), an ecologically important and commercially harvested species that is distributed from the Gulf of St. Lawrence in eastern Canada to the Gulf of Mexico (GOM) in the southeastern United States (Comeau et al. 2012). We determined the number and types (fibers, fragments, films) of microdebris particles in pseudofeces and in feces separately. We then compared particle number to oyster shell height to determine if oyster size affected microdebris abundance in biodeposits and normalized data by particle type on a per oyster basis. Because harvest regulations for oysters are based on size, this information is important to understand how oysters of various sizes process or retain microdebris following uptake from the environment. Our results have implications for future microdebris biomonitoring and bioaccumulation studies and informing management regarding safe harvest of bivalve species.

MATERIALS AND METHODS

Ten clusters of live, wild—stock *C. virginica* were hand—collected from The Nature Conservancy (TNC) restored reef at Bayfront Park, Alabama, USA on 11 March 2020. The reef is located in about 1.0 ± 0.5 m deep water on the southwestern shore of the Mobile Bay estuary, a freshwater dominated system with among the highest freshwater discharge of watersheds in the USA (Alarcon et al. 2009, United States Census Bureau 2019). Microplastics have been documented in sediments at various sites throughout Mobile Bay and adjacent coastal waters (Wessel et al. 2016), indicating the potential for microplastic exposure and uptake by shellfish in this system. The external surface of each oyster cluster was cleaned with filtered (0.2μm) reverse osmosis (RO) water and gentle rubbing by hand to remove mud, debris, and epibionts. To collect biodeposits, each

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cluster (1 — 3 oysters per cluster, n = 18 total oysters; Table 1) was immediately placed in a 2 L glass beaker of RO water overnight (mean time = 17 hr 40 min ± 2 min) with an oxygen bubbler suspended ~6 cm above the oysters. Beakers were pre—cleaned in 10% hydrochloric acid for 24 h and thoroughly rinsed with RO water to remove microdebris particles before the addition of oysters. Following depuration, oysters were carefully removed from beakers, ensuring loose biodeposits settled to the bottom of the beaker. Shell height (longest length) of each oyster was measured to the nearest 0.1 mm using Vernier calipers. The RO water remaining in beakers was decanted to concentrate biodeposits in a final volume of ~20 ml before gently decanting to 100 mm pre—cleaned glass petri dishes to quantify microdebris in biodeposits under a Discovery V12 stereoscope (Carl Zeiss Microscopy), equipped with an AxioCam imaging system. Pseudofeces and feces were distinguished visually according to established methods (Fila et al. 2001, Dalrymple and Carmichael 2015) and minimally manipulated with forceps to reveal microdebris among the biodeposits.

Microdebris particles were categorized by shape including fibers, fragments, and films (Rochman et al. 2019, Wessel 2019). Fibers were further categorized by color. Additional non—natural particles (colored glass, chips of unknown composition) were also counted but not included in subsequent analyses. We did not attempt to classify particles < 20 µm in the longest dimension. To test the relationship between microdebris counts in pseudofeces or feces and oyster size, we used linear regression to compare the total number of microdebris particles per sample to the mean shell height of oysters in the cluster contributing to each sample (Table 1). To standardize particle counts per oyster, we divided the total number of particles per sample from a given cluster by the total number of oysters in the cluster. We then compared microdebris particle counts by type between pseudofeces and feces by calculating the mean number of each particle type found per oyster. Statistical analyses were performed in StatPlus:mac Pro 8.0.4.0.

TABLE 1. *The number of oysters and the mean and range of shell height comprising the 10 natural oyster clusters sampled for this study. Standard error is reported for the single cluster with >2 oysters.*

Cluster	Number of oysters	Shell height (mm) Mean	Range
1	1	66.5	–
2	3	92.6 ± 10.9	77.0 – 113.6
3	2	67.3	46.5 – 88.1
4	2	93.0	83.9 – 102.1
5	2	58.7	47.7 – 69.7
6	1	86.6	–
7	2	53.9	32.7 – 75.1
8	1	80.4	–
9	2	55.7	52.5 – 58.9
10	2	57.8	51.2 – 64.3

Results

A total of 635 microdebris particles were found in biodeposits from the 18 oysters (10 clusters) analyzed for this study, with 631 microdebris particles found loose or entangled in pseudofeces and 4 found embedded in fecal pellets. The quantity of microdebris particles detected varied with oyster size, with smaller oysters having higher numbers of particles (Figure 1A). The number of particles in pseudofeces decreased as shell height increased ($y = -1.16x + 145.83$, $F_{1,8} = 6.19$, $p < 0.05$, $r^2 = 0.44$). In contrast, the number of particles in feces did not vary continuously with shell height, and microdebris was only found in samples from clusters with mean shell height <70 mm and individual oysters sized <76 mm (Figure 1A, Table 1).

The microdebris found in oyster biodeposits represented a variety of fibers, fragments, films, and other non—natural particles (Figure 2A). The majority (99%) of particles found were fibers (Figures 1B, C), including 4 distinct colors – blue, black,

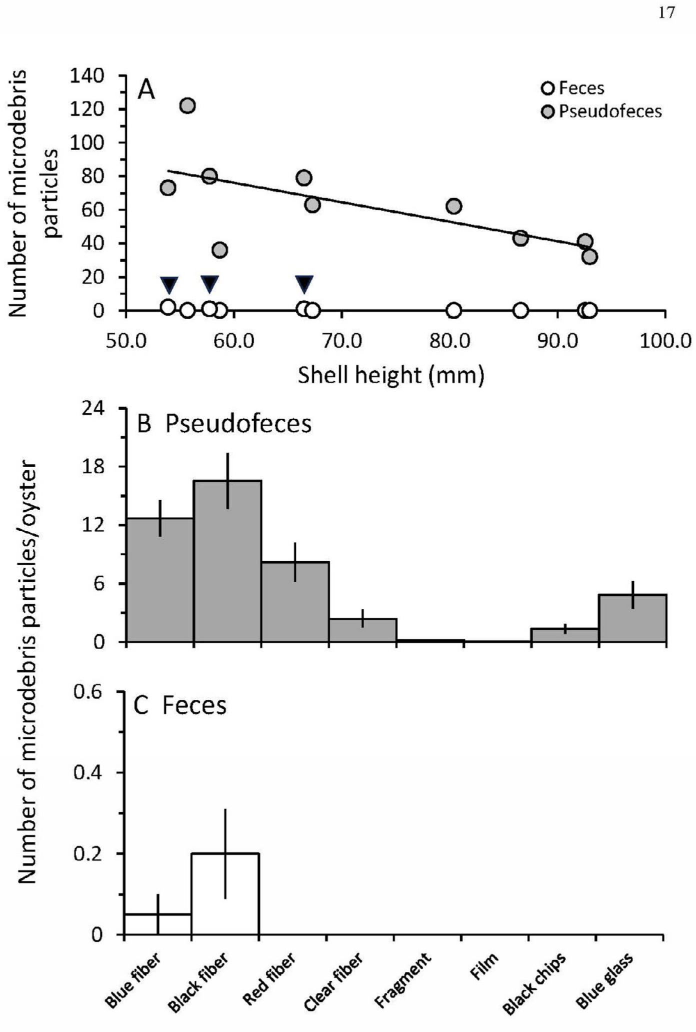

FIGURE 1. *Microdebris particles in pseudofeces and feces in Eastern oysters collected in Alabama. A. Number of particles compared to mean shell height per sample. Black arrows indicate feces samples in which microdebris was found. B. Mean (± se) number of microdebris particles of each type detected per oyster in pseudofeces. C. Mean (± se) number of microdebris particles of each type detected per oyster in feces.*

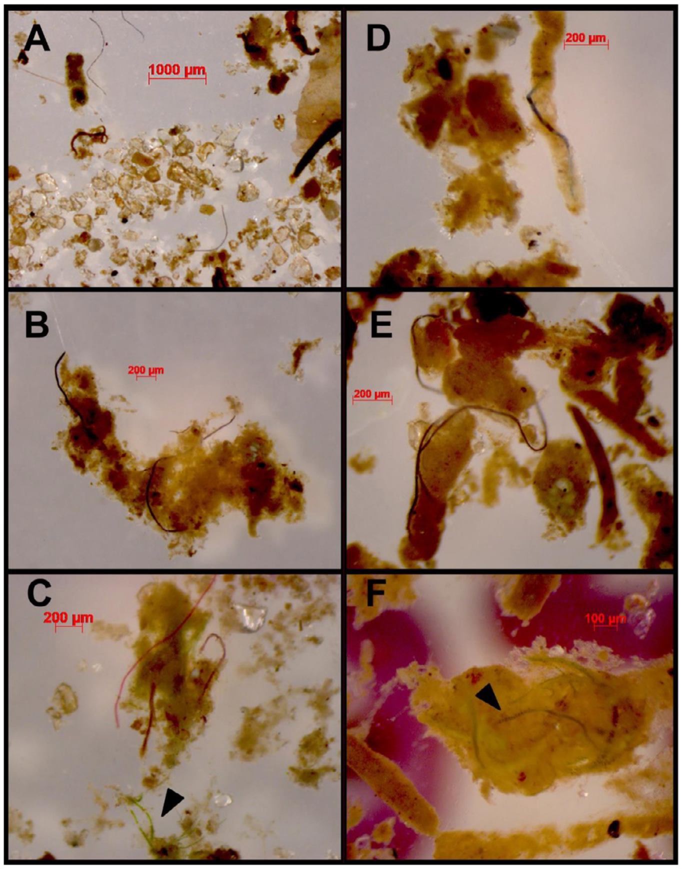

FIGURE 2. *Examples of microdebris in oyster biodeposits. A. A typical sample of primarily pseudofeces with multiple particles. B. Black fibers in pseudofeces. C. Red fibers in pseudofeces. Black arrow indicates filamentous algae D. Blue fibers embedded in feces. E. Black fibers embedded in feces. F. Filamentous algae in feces (black arrow).*

red, and clear (Figure 2). All microdebris types and colors were present in pseudofeces (Figures 2B, C), while only blue and black fibers were found in feces (Figures 1B, C; Figures 2D, E). Black fibers made up the highest percentage of fibers in pseudofeces and feces combined (42%), followed by blue (33%), red (19%), and clear (6%). Overall, the mean number of microdebris particles per oyster was 40.1 ± 6.9 in pseudofeces and 0.4 ± 0.2 in feces, and the mean number of the dominant microdebris type, black fibers, per oyster was 16.53 ± 2.82 in pseudofeces and 0.20 ± 0.11 in feces. In addition to microdebris fiber, filamentous algae, a natural food source that appears similar to fibers, was also seen in both pseudofeces and feces (Figures 2C and 2F, respectively).

Discussion

We detected a variety of microdebris particles in biodeposits from *C. virginica* collected in coastal Alabama waters in the northern GOM, a region with documented microdebris in estuarine, coastal, and open water habitats (Wessel et al. 2016, Lestrade and Hernandez 2023). The number and type of particles detected per oyster was 1 to 2 orders of magnitude higher in pseudofeces than feces, indicating that oysters selectively rejected the majority of microdebris particles. The detection of only fibers in feces suggests that, at least within the size range considered for this study, oysters may primarily ingest this form of microdebris, but further study using larger sample sizes is warranted. Our results align with previous studies that found selective rejection of microplastics in oysters and mussels (Graham et al. 2019, Ward et al. 2019a, Choi et al. 2022, Mladinich et al. 2022), with larger particles more likely to be rejected as pseudofeces at least partly due to size constraints of the mouth (Graham et al. 2019, Ward et al. 2019a, Ward et al. 2019b, Choi et al. 2022, Mladinich et al. 2022).

Similarly, microplastic shape has been shown to affect ingestion, with fibers accounting for the majority of microplastics found in both bivalve tissues and biodeposits (Craig et al. 2022, Wootton et al. 2022). Fibers are the most prevalent type of microplastics in the environment, making up 91% of microplastics found in surface waters globally (Barrows et al. 2018), and in a previous study microplastics were documented as the dominant form of microdebris in northern GOM waters (Lestrade and Hernandez 2023). Hence, the high uptake of fibers by oysters in this study and others may simply be due to their greater availability. The long and narrow aspect ratio of fibers, however, may make them easier to ingest through the mouth (Ward et al. 2019b, Craig et al. 2022). It has been suggested that smaller fibers (<500 µm), in particular, may mimic phytoplankton food sources, making them more likely to be ingested than rejected as pseudofeces (Graham et al. 2019, Ward et al. 2019b). Of note, in this study we documented microdebris fibers >500 µm long, resembling filamentous microalgae, embedded in feces, confirming ingestion of larger microdebris and supporting the latter potential mechanism of selective uptake for fibers. These findings may be conservative if some particles became dislodged from feces during deposition or handling. Further study of microdebris types in biodeposits and their occurrence relative to rates of feces and pseudofeces production could help better define the mechanisms ultimately driving ejection versus ingestion.

While microdebris properties influence particle ejection versus ingestion, overall size of bivalves may also affect interactions with microdebris. We detected fewer microdebris particles in the biodeposits (particularly pseudofeces) of larger oysters, and the lack of a relationship between shell height and particle content in feces was likely due to the overall small number of particles found in feces. Microplastic expulsion efficiency in *C. virginica* has been shown to increase with shell height (Craig et al. 2022); therefore, we may expect less overall retention of microdebris in larger oysters, a finding which seems to be supported by our data. In contrast, a study on Indian oysters (*Magallana bilineata*) found that microplastic abundance and concentration in tissues increased with oyster size (Patterson et al. 2019). Larger—sized bivalves may also preferentially retain smaller sized microdebris (<20 µm; Van Cauwenberghe and Janssen 2014), a finding not tested in our study. The complexity of these findings suggests that in addition to oyster size, other location— or species—specific factors may affect microdebris uptake and retention.

Our study highlights the value of examining biodeposits (rather than tissue alone) for understanding the uptake and retention of microdebris in bivalves. Studies that focus only on whole tissues do not yield information on microdebris types and quantities that may be ingested, and therefore retained longer as part of the body—burden of microdebris in oysters or other bivalves, including the potential for bioaccumulation and movement through or up food webs. Many of the studies that have examined the fate of microdebris, particularly microplastics, in bivalves have found the mollusks are effective at eliminating most microplastics whether selectively in pseudofeces or ultimately in feces, when given sufficient time to depurate (Woods et al. 2018, Graham et al. 2019, Craig et al. 2022, Liu et al. 2023). Ingested microdebris has increased potential to translocate to other organs or leach hazardous substances that are derived from or absorbed to them (Sendra et al. 2021), and our finding of microdebris embedded in oyster feces indicates some potential for this type of contaminant retention and trophic transfer. Pseudofeces are expelled more quickly than feces without passing through the digestive tract, potentially reducing the time that bivalves are exposed to microdebris and reducing potential for negative effects to them or their consumers (Mladinich et al. 2022). Our findings further indicate that variation in microdebris ingestion and retention may be influenced by a combination of oyster and particle size and type, meriting additional study. While our study focused on *C. virginica*, our methods could be broadly used among bivalve species (and coupled to advanced particle identification techniques) to inform microdebris biomonitoring, effects on bivalve physiology, and future research and management applications for seafood safety.

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