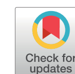
Marine Pollution Bulletin 137 (2018) 638–645

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Microplastic fiber uptake, ingestion, and egestion rates in the blue mussel (*Mytilus edulis*)



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ARTICLE INFO

Keywords:
Microplastic
Microfiber
Bivalve
Filtration rate
Seafood
Gulf of Maine

ABSTRACT

Microplastic fibers (MPF) are a ubiquitous marine contaminant, making up to 90% of global microplastic concentrations. Imaging flow cytometry was used to measure uptake and ingestion rates of MPF by blue mussels (*Mytilus edulis*). Mussels were fed a diet of *Rhodomonas salina* and MPF concentrations up to 30 MPF mL^{-1}, or 0.374% of available seston. Filtration rates were greatly reduced in mussels exposed to MPF. Uptake of MPF followed a Holling's Type II functional response with 95% of the maximum rate (5227 MPF h^{-1}) occurring at 13 MPF mL^{-1}. An average of 39 MPF (SE ± 15, n = 4) was found in feces (maximum of 70 MPF). Most MPF (71%) were quickly rejected as pseudofeces, with approximately 9% ingested and < 1% excreted in feces. Mussels may act as microplastic sinks in Gulf of Maine coastal waters, where MPF concentrations are near the order of magnitude as the experimental treatments herein.

1. Introduction

The ubiquity of small plastic particles called microplastics, < 5 mm in diameter (NOAA), is a major concern throughout the world's oceans (UNEP, 2016). An exponential increase in demand for plastic products over the last century has resulted in an estimated 4.8 to 15.11 million metric tons of plastic marine debris entering the ocean each year (Jambeck et al., 2015; Lebreton et al., 2017). Fragmentation of larger plastics over time has also increased the presence of microplastic fragments and fibers from the wind-driven surface layer down into deep ocean sediments (Browne et al., 2011; Van Cauwenberghe et al., 2013; Marathon and Hill, 2014; Lusher et al., 2015).

Estimates of microplastics abundance in the ocean currently range from 10^3 to 10^5 per m^3 (Noren, 2007; Andrady, 2017), or 0.001 to 0.1 mL^{-1}. However, recent evidence suggests that smaller plastic particles could be largely underestimated due to their ability to slip through the open mesh collection nets used in the majority of studies (Lozano and Mouat, 2009; Cole et al., 2013; Barrows et al., 2017). Microplastic fibers (MPF) specifically can constitute up to 91% of all plastics collected in global whole water grab samples (Barrows et al., 2018). This is in stark contrast to previous estimates of MPF contributions to total plastic abundances using net tows (Moore et al., 2001; Doyle et al., 2011; Uchida et al., 2016).

The small size and high availability of microplastics increase the chance for ingestion by marine organisms (Browne et al., 2008; Lusher et al., 2017). Over 220 marine organisms have been documented to ingest microplastics, including zooplankton (Cole et al., 2013; Setälä et al., 2014), fish (e.g., Neves et al., 2015; Nadal et al., 2016), seabirds (van Franeker et al., 2011; Tanaka et al., 2013), marine mammals (Lusher et al., 2015), and many benthic invertebrates (e.g., Murray and Cowie, 2011; Goldstein and Goodwin, 2013; Wright et al., 2013; Watts et al., 2015). Laboratory studies have documented effects of microplastics ingestion ranging from neurotoxicity and DNA damage in clams (Ribeiro et al., 2017), to delayed larval development in oysters (Sussarellu et al., 2016), and induced valve closure in mussels (Wegner et al., 2012), among others.

Filter-feeding bivalves have historically been used to monitor water quality in coastal areas due to their sessile lifestyle, ability to accumulate a wide range of particulate and dissolved pollutants, and their significant role in coastal ecosystems (Jørgensen, 1990; Farrington et al., 1995; Tanabea et al., 2000). It has been suggested that microplastics ingestion is highest in filter-feeding organisms (Setälä et al., 2016; Thushari et al., 2017), due to their efficient uptake and assimilation of particles from the water column (Ward and Shumway, 2004).

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https://doi.org/10.1016/j.marpolbul.2018.10.061
Received 24 July 2018; Received in revised form 28 October 2018; Accepted 29 October 2018
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As a consequence, mussels and oysters have begun to be used as indicators for environmental microplastics contamination as well, by measuring the average body load of microplastics (Avio et al., 2017; Thushari et al., 2017). However, an understanding is still lacking on how these organisms interact with microplastics in their environment. Many species naturally utilize a selective mechanism for processing filtered material, thereby eliminating unwanted particles as pseudofeces before ingestion (Ward and Shumway, 2004). Through an investigation of feces and pseudofeces produced by wild mussels in the laboratory with no laboratory microplastics exposure, Zhao et al. (2018) found that the length of microplastics was significantly longer in pseudofeces than in the digestive gland and feces. This was attributed to selective particle rejection by the mussels, which has sparked some debate on the reliability of mussels as indicators of microplastics contamination levels in the water column (Qu et al., 2018).

Few studies have addressed the uptake and ingestion of MPF by bivalves. The majority of published laboratory experiments expose bivalves to conditions very unlike those the animals might encounter in their natural habitat, typically using microplastic beads at concentrations up to 10^7 particles mL^{-1}, or eight orders of magnitude above the highest current environmental estimates (Brillant and MacDonald, 2002; Noren, 2007; Farrell and Nelson, 2013; Claessens et al., 2013). Recently, both Zhao et al. (2018) and Qu et al. (2018) suggest that microplastic beads are more likely to be ingested by mussels than fibers due to the beads smaller size that allows them to be transferred to and accumulated in the digestive system. However, Qu et al. (2018) report that mussels ingested 74–77% MPF when experimentally exposed to microbeads, fragments, and fibers at a 1:1:8 ratio. Based on natural microplastic abundance, bivalves are more likely to encounter microfibers in their environment rather than beads, and at much lower concentrations ($< 10^{-1}$ mL^{-1}, Noren, 2007; Andrady, 2017; Barrows et al., 2018; M.N. Woods, Shaw Institute, unpubl. data, 2014–2017).

In this study, we investigate the ingestion rate and fate of MPF taken up by the blue mussel (*Mytilus edulis*) from Gulf of Maine coastal waters. We used MPF concentrations ranging from 3 to 30 particles mL^{-1} to mimic natural concentrations and allow quantitative MPF detection. In addition, we present a new method to measure low concentrations of MPF in the water column, by using a FlowCam fluid imaging flow cytometer (FIT Maine, USA).

2. Materials and methods

2.1. Sample collection

Mytilus edulis (28.8 mm ± standard error (SE) 0.31 mm shell length) were collected from Salt Pond, Blue Hill, ME (44°22′ 26.1″N 68°33′ 36.5″W), and transferred to the laboratory where they were allowed to depurate for 7–10 days to reflect acclimation times of traditional mussel experiments (Riisgård and Randløv, 1981; Liutkus et al., 2012). During depuration, 25 mussels were placed in 4 L glass jars with 0.2 μm filtered seawater (FSW) changed daily, received continuous aeration under dark conditions at 13 °C and were fed a diet of *Rhodomonas salina* (8000 cells mL^{-1}; Riisgård et al., 2013). Cell concentrations were replenished daily to maintain actively feeding and healthy individuals.

2.2. Microplastics characterization and identification

MPF were obtained by carefully shaving fibers from a newly purchased neon pink polyethylene terephthalate (PET) fleece with sharp stainless steel scissors. This provided a highly visible MPF source that was easily distinguished from other natural or worn fibers. Neon pink was chosen since it is not typically observed in environmental or laboratory contamination; otherwise, MPF contamination was minimized by the use of covered glass or metal labware, white laboratory coats, and non-pink disposable gloves, and quantified with experimental controls described below. The MPF lengths were microscopically determined and the fibers averaged < 0.5 mm in length (459 ± SE 2.25 μm). This size distribution was selected to match the average MPF length from natural Gulf of Maine seawater samples near our sampling location (M.N. Woods, Shaw Institute, unpubl. data, 2014–2017). A stock solution of MPF in 0.2 μm FSW was used to inoculate the experimental glass jars. The MPF stock concentration was estimated by vigorously shaking the jar and taking 10 mL subsamples and diluting them with 90 mL FSW, of which 10 mL replicates were counted manually under a dissecting microscope. Fibers in the stock did not remain in suspension, however, subsamples were sufficiently homogenous after shaking to achieve a precision of < 3%. During experiments, aeration tubes were placed 1 in. above the bottom of the jars to keep MPF in suspension.

A VS series FlowCam (imaging flow cytometer -Fluid Imaging Technologies, Inc.) was used to identify and enumerate both MPF and algal cells in all uptake and egestion experiments. To our knowledge, this is the first time a FlowCam has been used to quantify microplastic *fibers* in either natural or experimental samples. Recently, FlowCam has been reported for quantification of microbeads (Davidson et al., 2015 unpubl.) and for the quantification of digestion efficiency towards the analysis of microplastics by other methods (Bergmann et al., 2017; Lorenz et al., 2017). Methodology was developed for optimizing the FlowCam to compensate for the intrinsic properties of the fibers, and a 98% autoimage retention efficiency (i.e., accuracy) was obtained via comparison to manual microscope counts (detailed protocol available in Supplemental information). All MPF FlowCam analyses utilized a Field of View 1000 Flowcell (FC1000FV), a corresponding coupler, and a 2× objective lens with a D12 thick wall. All MPF experimental samples were run with a 12.5 mL syringe at 10 mL min^{-1} and 18 frames per second (fps).

2.3. Laboratory exposure assays

2.3.1. Uptake experiments

The uptake of MPF was measured by placing a single mussel in 1 L glass jars filled with 0.2 μm FSW (n = 3 per each of 3 exposure levels), maintained at 13 °C in the dark. To ensure that the mussels maintained a constant filtration rate over the range of MPF concentrations, the feeding chambers were stocked with a background concentration of $8 * 10^3$ cells mL^{-1} of *R. salina* (Riisgård et al., 2013). Jars were inoculated with MPF at three tracer levels of exposure from 3 to 30 MPF mL^{-1}, or 0.0375 to 0.374% of available seston (i.e. algae + MPF). Subsamples of 10 mL and 1 mL were used to measure MPF and algal concentrations, respectively, at intervals of 0, 1, 3, 6, 12, 24, and 72 h. Two control types were carried out alongside uptake experiments: a system loss control (n = 3) which used the same experimental set up but did not contain a mussel; we used this control to ensure accurate recapture of MPF. The second type of control (n = 3) contained a mussel with only algae and no MPF. This allowed us to assess changes in the filtration rate in the presence of MPF. All specimens were sacrificed immediately following each experiment. Filtration rates were calculated using the clearance method described by Riisgård et al. (2013). Mussel soft tissue dry weights were calculated using the conversion factor described by Ricciardi and Bourget (1998), and the condition index (CI), defined by Riisgård et al. (2014) as $CI = W(mg) / L(cm)^3$, was used for comparison across groups, where W is the dry weight of soft tissue and L is shell length. Uptake rates of MPF were calculated by fitting our results to a functional response curve (Holling, 1959).

2.3.2. Pseudofeces and fecal production

Rejection of MPF by mussels was measured in three separate egestion experiments at a single exposure concentration of 30 MPF mL^{-1}. To measure the production of pseudofeces, three mussels were placed in a single jar using the same experimental set up as uptake experiments

(n = 3). Pseudofeces production rates are only accurate at initial seston concentrations due to the positive relationship of pseudofeces production to seston concentrations (Tenore and Dunstan, 1973; Ward and Shumway, 2004). The number of pseudofeces produced in each jar was counted after 3, 6, and 9 h and then divided by the number of mussels to calculate the total pseudofeces produced per animal. Therefore, the estimated number of MPF rejected as pseudofeces was calculated by combining the average MPF uptake at a concentration of 30 MPF mL^{-1} with the MPF ingestion average from mussels exposed to the same concentration for 3 h. The system loss of MPF from control jars and the average MPF ingested and excreted as feces by individual mussels were subtracted from the average MPF taken up after 3 h, assuming 100% efficiency (Møhlenberg and Riisgård, 1978). Concentrations of MPF in fecal pellets (n = 4) were averaged across treatments. Fecal production rates were defined as 0.167 pellet h^{-1} for all calculations (Hawkins et al., 1990).

The effect of depuration time on MPF rejection and retention was conducted via two experiments. The first consisted of six mussels in a single jar that were exposed to 30 MPF mL^{-1} for either 30 min, 1, 3, 6, or 9 h. Immediately following exposure, three of the six mussels were placed into clean, MPF-free FSW to depurate for 1 h (n = 3 per exposure), and the other three were immediately sacrificed (n = 3 per exposure). The second experiment was conducted in the reverse, where six mussels were exposed to 30 MPF mL^{-1} for a fixed period of 3 h, and then three of the six mussels were allowed to depurate for either 3, 6, or 9 h (n = 3 per exposure), and the other three of each experiment were immediately sacrificed after 3 h of MPF exposure (n = 9).

2.3.3. Ingestion rates

Sacrificed specimens were stored at −80 °C in individual 120 mL plastic bottles. Prior to analysis, animals were placed inside a laminar flow cabinet and thawed over ice for 2 h before dissection. The gills were removed first and the remaining tissue (i.e., everything but the gills) was rinsed with twice filtered DI water to remove any MPF that may have stuck to the surface. Gills, digestive gland, and the remaining soft tissue (i.e., the majority of the intestines, anus, muscles, mantle, kidneys, and heart) were digested separately, following modified methods outlined by Marathon and Hill (2014), using 120 mL of 32% H_2O_2 per gram tissue on magnetic hot plates at 60 °C and at 300 rpm for 3–5 h. This digestion did not bleach or otherwise visibly alter the experimental neon pink MPF. To quantify ingested MPF, the digested tissue samples were vacuum-filtered through 0.8 μm sterile, gridded MCE membrane filters (Sterlitech MCE0847100SG). The collected MPF were analyzed via Motic Images Plus 3.0 liveimage software. Measurements were used as snapshots of MPF ingested at the time of freezing. Individual ingestion rates were estimated across experiments, as MPF Ind^{-1} h^{-1} accumulated in whole mussel tissue.

2.4. Quality control

To prevent possible airborne microplastic contamination, all equipment was thoroughly rinsed with ultra-pure Milli Q water or twice filtered DI water and covered with tin foil at all times, including during digestions (Phuong et al., 2017). Mostly glass or metal labware, white lab coats and non-pink disposable gloves were used. Procedural blanks (i.e., all steps done for the samples except without the sample tissue), background laboratory airborne contamination controls (i.e., filters that captured deposition of ambient workspace microplastics) (Woodall et al., 2015), and no-MPF exposure mussel controls (i.e., mussel experiments with no MPF exposure) were used at every step of the experimental process. For all experiments, results were evaluated only after mitigation of airborne and/or processing microplastic contamination controls. Any fibers detected in these controls that matched characteristics of our manufactured neon pink microfibers were averaged and subtracted from our results (Vandermeersch et al., 2015).

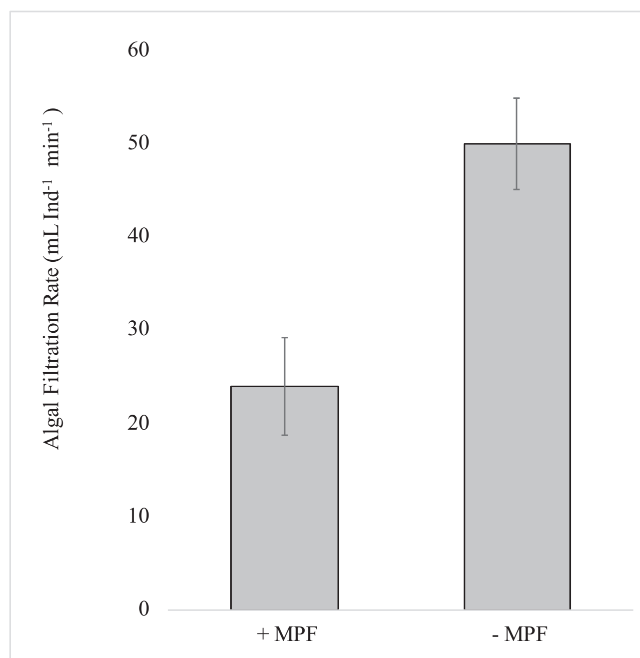

Fig. 1. Filtration rates of *Mytilus edulis* (average ± standard error) feeding on *R. salina* ($8 * 10^3$ cells mL^{-1}) with (n = 9) and without (n = 3) added MPF.

2.5. Statistical analysis

Data were analyzed using STATA software. All averages are given with their respective standard error (SE) and sample size (n). Statistical differences among treatments, exposure/depuration times, filtration/egestion rates, and tissue types were assessed using non-parametric tests (Kruskal-Wallis, followed by multiple comparisons). Statistical differences were considered significant when $p < 0.05$.

3. Results

3.1. Filtration rates and uptake of microplastic fibers by mussels

There was no significant difference in filtration rates (measured using the microalgal prey *R. salina*) across MPF exposure concentrations (3 to 30 MPF mL^{-1}); hence filtration rates for all MPF treatments were averaged. However, filtration rates were significantly different between mussels exposed and not exposed to MPF (Kruskal Wallis, $p < 0.05$). Mussels that were not exposed to MPF averaged filtration rates of 50.1 mL min^{-1} (SE ± 4.1), while mussels that were exposed averaged 23.9 mL min^{-1} (SE ± 5.3) (Fig. 1). There was also no significant difference in the condition index among mussels exposed to different MPF concentrations or between exposed versus non-exposed mussels.

The uptake rates of MPF by mussels increased with increasing MPF concentration (3–30 MPF mL^{-1}; Fig. 2). To determine the uptake rates (U) during the first 3 h as a function of MPF concentrations (Fig. 2), the data were fit to a functional response curve (2-parameter exponential rise to max) as $U = 5227 * (1 - e^{(-0.23*[MPF])})$ ($r^2 = 0.67$; ANOVA; $F_{1,5} = 11.01$; $p = 0.03$). A maximum uptake rate of 5227 MPF h^{-1} was reached at an exposure concentration of 13 MPF mL^{-1}. Mussels did not increase their uptake rate at concentrations above 13 MPF mL^{-1}.

3.2. Microplastic fibers in pseudofeces and feces

The average number of MPF found in feces was 39 ± SE 15 MPF (n = 4), with a maximum of 70 MPF in a single pellet. We estimate that, over a three-hour time frame, 71% of the available MPF (initial average count = 30,000 MPF) were rejected as pseudofeces by the mussels, with

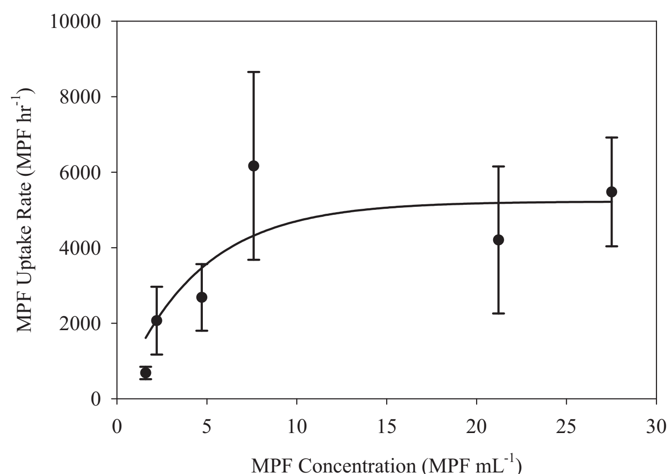

Fig. 2. Uptake rates of MPF per individual of *Mytilus edulis* (average ± standard error) with increasing MPF concentration. Background concentration of *R. salina* remained constant at $8 * 10^3$ cells mL^{-1}.

approximately 9% of MPF being ingested and < 1% excreted in feces.

3.3. Ingestion rates and biological partitioning of MPF

The accumulation of MPF in the tissue of whole mussels after 72 h showed a positive exponential correlation to MPF exposure concentration (Fig. 3a). Mussels exposed to 30 MPF mL^{-1} retained significantly more fibers (Kruskal-Wallis, $p < 0.05$; see Supplemental information for discrete data) than at the lower concentrations of 15 and 3 MPF mL^{-1} (Fig 3a). The average ingestion rate of MPF by mussels exposed to 30 MPF mL^{-1} across experiments was 352 MPF Ind^{-1} h^{-1} (SE ± 47) compared to an average uptake rate of 4205 MPF h^{-1} (SE ± 1948), or 8.35% assimilation.

The biological partitioning of ingested MPF inside mussel's tissue was significantly different across MPF exposure concentrations (Kruskal-Wallis, $p < 0.05$). At high concentrations (30 MPF mL^{-1}), 81.3% of ingested MPF accumulated in the digestive gland, with 4.3% and 14.4% found in the gill and remaining soft tissue, respectively (Fig. 3b). At the mid-concentration of MPF (15 MPF mL^{-1}), the ingested MPF were fairly evenly distributed among the digestive gland (44%), gill (23%), and remaining soft tissue (33%). At low MPF concentration (3 MPF mL^{-1}), more than half of the ingested MPF accumulated in the digestive gland (58%), with 23% and 20% found in the gill and remaining soft tissue, respectively (Fig. 3b).

The average length of ingested MPF was significantly different among tissue types (Kruskal-Wallis, $p < 0.05$). Across all experiments, the average MPF length retained was consistently highest in the gill (760 μm ± SE 22), followed by the digestive gland (521 μm ± SE 4), and the remaining soft tissue (442 μm ± SE 6).

3.4. Depuration of microplastic fibers

MPF accumulation within the tissue of *Mytilus edulis* increased with increasing exposure time. At a concentration of 30 MPF mL^{-1}, the maximum total body load of accumulated MPF in whole mussels occurred between 3 and 6 h of exposure (Fig. 4a), resulting in a body load of 451 MPF g^{-1} ± SE 100 and 494 MPF g^{-1} ± SE 28, respectively. At the highest MPF body loads, the animals expelled a considerable amount of MPF, depurating ~150 MPFs within 1 h of being placed in MPF free water (Fig. 4a). However, at low body loads (< 150 MPF g^{-1}), *M. edulis* did not eject particles, regardless of exposure times; this may be indicative of the maximum quantity of MPF the mussels can retain.

The accumulation of MPF in specific mussel tissues was evident within the first 30 min of exposure (Fig. 4). No significant difference was found in MPF accumulated in the digestive gland between the no depuration and 1 h depuration treatments, except at 30 min, (Fig. 4b), with accumulation increasing in both groups within the first 3 h of exposure and decreasing after 6 h of exposure. MPF accumulation in the gill was highly variable with 1382 MPF g^{-1} (SE ± 1345) and 1334 MPF g^{-1} (SE ± 820) at 0.5 h and 1 h exposure (Fig. 4c), respectively, decreasing to approximately 200 MPF g^{-1} thereafter. A 1 h depuration time greatly reduced the amount of MPF found within the gill. The remaining soft tissue had the lowest accumulation of MPF with a small peak at 0.5 h (366 ± SE 112 MPF g^{-1}) and a larger peak at 3 h of exposure (577 ± SE 95 MPF g^{-1}) (Fig. 4d); a 1 h depuration treatment did make a significant difference in the MPF load of the soft tissue (Kruskal-Wallis, $p < 0.05$).

The amount of MPF in whole mussels exposed to 30 MPF mL^{-1} for 3 h (Fig. 4a) decreased exponentially with increasing depuration times (Fig. 5). After at least 6 h in MPF-free water, mussels had removed an average of 63% (SE ± 20%) of their accumulated MPF, at an estimated

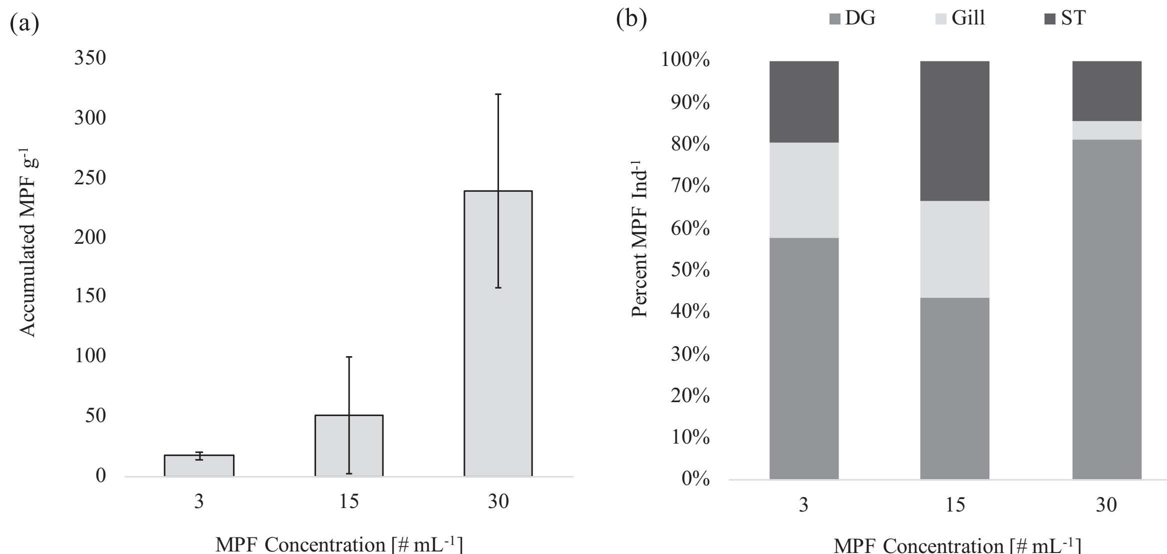

Fig. 3. (a) Accumulation of MPF g^{-1} (average ± standard error) in whole mussels after 72 h as a function of MPF concentration. (b) Biological partitioning of ingested MPF by tissue type (n = ~50). DG = digestive gland, ST = all other soft tissue.

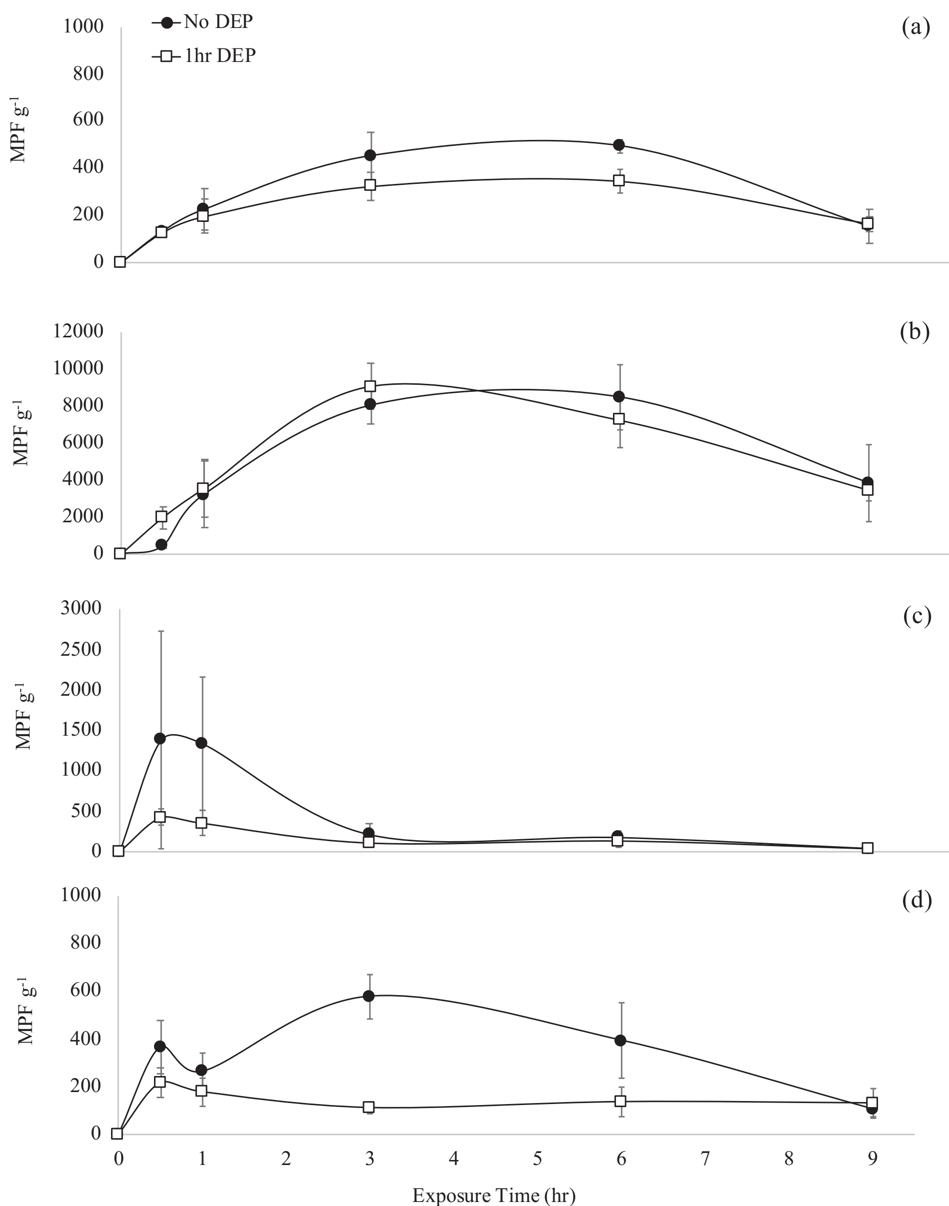

Fig. 4. Accumulation of MPF (average ± standard error) in whole mussel tissue (a) and in different tissue types (b-digestive gland; c-gill; d-other soft tissue; please note the different y-axis scales) of *Mytilus edulis* at increasing exposure times of 30 MPF mL^{-1}. Solid symbols show the amount of MPF accumulated at each time point. Open symbols show the remaining MPF after a 1-hour depuration (DEP) period (n = 3).

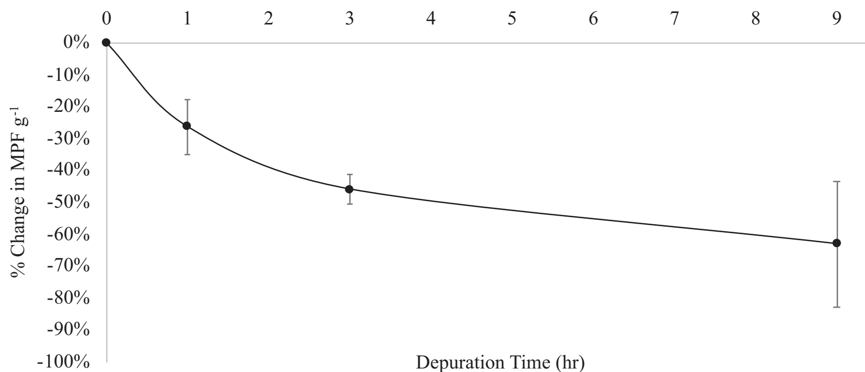

Fig. 5. Rate of MPF depuration (average ± standard error) by *Mytilus edulis* after a three-hour exposure period to 30 MPF mL^{-1} (n = 3). Initial body load of MPF shown in Fig. 4.

rate of 5–10% h^{-1}.

4. Discussion

This study confirms that *M. edulis* mussels accumulate MPF during filter-feeding. These results expand the findings of previous studies which show that the blue mussel and other closely related bivalves retain similarly sized microplastic spheres in experimental and natural conditions (Brillant and MacDonald, 2002; Browne et al., 2008; Von Moos et al., 2012; Wegner et al., 2012; Avio et al., 2015). Throughout all experiments, the initial algal concentration remained constant while the initial MPF concentrations ranged from 3 to 30 MPF mL^{-1}. In the absence of MPF, the measured filtration rates of *R. salina* prey by *M. edulis* are comparable to previously measured values feeding on similar algae and algal concentrations (Riisgård and Randløv, 1981). However, in the presence of MPF, mussels showed a decrease in filtration rates already at concentrations of 3 MPF mL^{-1}. This is similar to the findings of Xu et al. (2016) with smaller spherical microplastics (63 to 250 μm diameter) to the MPF used here (~450 μm length). At all our experimental MPF concentrations, filtration rates decreased precipitously to approximately 50% of the maximum value (Fig. 1).

Bivalves select for high-quality organic particles and reject inorganic material in pseudofeces (Ward and Shumway, 2004). However, particle selection is not 100% efficient (Urban and Kirchman, 1992; Bayne et al., 1993), and the physical and chemical criteria for selection are not well understood (Ward and Shumway, 2004). *M. edulis* reaches a maximum selection efficiency when the organic content is 40% of the total seston abundance, and decreases at higher organic ratios (Bayne et al., 1993; Hawkins et al., 1996). Although our changes in percent non-organic material were small (0.0375 to 0.374% of seston), our results appear to conflict with this trend, as mussel filtration rates were nearly two times higher at 100% organic particle concentrations (i.e., no MPF present).

At extremely high microplastic concentrations, mussels have been shown to close their valves and stop filtering (Wegner et al., 2012). Though valve closure was not observed during our experiments, large decreases in filtration rates are likely to have important consequences on the energy budgets of *M. edulis*, particularly at low food concentrations (Xu et al., 2016). At optimal food concentration of (5000 to 8000 cells mL^{-1}, Riisgård et al., 2013), *M. edulis* has a growth rate of 0.24 mm d^{-1} (Kiørboe et al., 1981). This rate is similar to the maximal growth of mussels in nature. At food concentrations below the incipient limiting level, a decrease in filtration rate can have negative consequences on growth and reproduction. It has been shown that once mussels are returned to microplastic-free water, filtration rates return to pre-exposure levels (Browne et al., 2008), suggesting that the effects of microplastics on mussel filtration rates are reversible; Fig. 5 shows a ~60% loss of accumulated MPF within 9 h of the onset of depuration, with a likely increase in filtration rate. More research into the effect of MPF on bivalve filtration and selection capacities (i.e., clogging of gills, etc.) is needed to understand whether the reduction in filtration rates seen here is a mechanical or a food quality effect by the MPF.

Organisms will accumulate microplastics when their uptake rates exceed the rate of egestion. Egestion can occur prior to ingestion through pseudofeces or post ingestion as a fecal pellet. *M. edulis* filtered more MPF as MPF concentrations increased, reaching a maximum of approx. 5000 MPF $indiv^{-1}$ h^{-1} at concentrations of 13 MPF mL^{-1} (Fig. 2). Furthermore, when mussels were exposed to MPF concentrations well in excess of reported values for coastal waters (Noren, 2007; Andrady, 2017), the organisms did not increase their uptake rate (Fig. 2) over the length of the experiments, suggesting a saturation level may have been reached for MPF. Acclimation of the experimental mussels to elevated MPF levels for longer periods of time, as might be encountered due to seasonal variability, were not tested; seasonal acclimation to changing prey levels has been shown for other filter feeders in temperate waters (e.g., Runge, 1980). Whereas under continued exposure to MPF, individual mussels might be expected to suffer physiological losses, such as body mass, the condition index did not show any significant difference among mussels exposed to increasing MPF concentrations, as intended (data not shown); i.e., any effect on filtration, uptake and ingestion rates was only due to the presence of MPF rather than previous feeding history. Such a result may also be due to the length of the feeding experiments described herein (up to 3 days) not being long enough to trigger a significant loss of body weight. Whether depuration during periods of low MPF concentration (Fig. 5) or further accumulation due to constant exposure to MPF even at low concentrations (Dris et al., 2016) is the ultimate process to control an equilibrium body load is still unknown.

Microplastics were observed in the gills, digestive gland and other soft tissue at all experimental times. However, the MPF retention times varied depending on tissue type. For example, the majority of MPF found in the gill were expelled faster than from the digestive gland (Figs. 3 and 4). This suggests that MPF remain inside the gut for at least 3 days, which supports findings by Xu et al. (2016), who measured a 15% gut retention of microplastic spheres in clams over 10 days, and Ward and Kach (2009) who identified florescent nanoplastics in the digestive gland of mussels 72 h after experiments. Further research is needed, however, to determine the average residency time of microplastics in filtering bivalves. Browne et al. (2008) found that microplastic spheres (< 10 μm) remained in the digestive glands of *M. edulis* for 3 days before translocating into the hemolymph, where the microplastics persisted for over 40 days. The present study was performed over a much shorter time frame, nonetheless MPF were found in the digestive glands in all experimental animals, where MPF may become lodged. Although no discernable difference in tissue health was observed, this study was not designed to quantify changes in animal health and thus cannot determine if individuals or populations of Gulf of Maine blue mussels are ultimately affected by MPF ingestion, as shown elsewhere (Sussarellu et al., 2016; Von Moos et al., 2012; Wegner et al., 2012; Ribeiro et al., 2017).

The capacity of mussels and other suspension-feeding invertebrates to reject undesirable particles either during or after capture by means of pseudofeces provides an alternative mechanism to maintain an organism's health. Indeed, the majority of the available MPF (71%) was found in pseudofeces (Fig. 6) at all experimental MPF concentrations, which were three orders of magnitude lower than published algal pseudofeces trigger concentrations (Riisgård et al., 2011). Because MPF abundances were at trace levels with respect to algal cell prey abundance, it is likely a MPF characteristic other than number triggers pseudofeces production; one possibility is the difference in size between MPF (~500 μm in length) and *R. salina* (ca. 8 × 12 μm). Experiments conducted by Newell and Jordan (1983) on particle selection by the oyster *C. virginica*, with mixtures of phytoplankton and silt, found that the diameter of particles was not a major factor in selection between 3 and 38 μm. As our MPF had diameters of roughly 20 μm, the elongated shape of the MPF could be the complicating factor. The expulsion of MPF via pseudofeces by the mussels as described above, especially at 30 MPF mL^{-1}, is further complemented by their ability to expel MPF (as indicated above) when exposed to seawater devoid of such particles (Fig. 5). Most of the loss by depuration occurred after 1 h of exposure to particle-free seawater, suggesting a high capacity for self-cleaning by the mussels, as shown for clams exposed to microplastic spheres (Xu et al., 2016).

In the natural environment, the filtration, rejection and/or ingestion of MPF by mussels may be influenced by many consumer and prey-related factors not captured under laboratory experiments (Valiela, 1995). Nonetheless, Gulf of Maine blue mussels clearly have the capacity to accumulate MPF, especially when MPF are persistently present, as they are in the natural environment. It should be noted that although the lowest experimental concentration of 3 MPF mL^{-1} is two orders of magnitude higher than ambient MPF concentrations measured in the Gulf of Maine (10.16 ± SE 0.54 MPF L^{-1} in summer and fall; M.

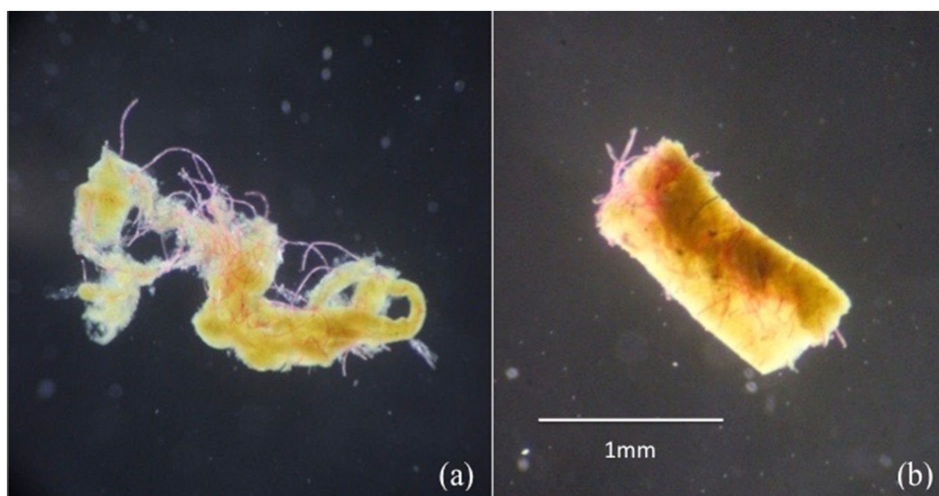

Fig. 6. Several MPF embedded in *Mytilus edulis* pseudofeces pre-ingestion (a) and a fecal pellet post-ingestion (b).

Woods, Shaw Institute, unpubl. data, 2014–2017), it is also well below the concentration that triggers the production of pseudofeces. Thus, feeding at a concentration of 3 MPF mL^{-1}, the mussels ingest and store MPF in their body and retain MPF in their tissue over periods of days.

There are > 5 trillion individual pieces of plastic floating on the surface of the world's oceans with an estimated collective weight of 2.7 k-tons (Eriksen et al., 2014). In spite of the alarming quantity of particulate plastic in the environment, actual MPF contamination is suspected to be even higher. The widespread and increasing presence of persistent micro- and nanoplastics in the marine environment indicate that MPF will increasingly affect the health, fecundity, ecology of and ecosystem services provided by these filter-feeding bivalves.

Acknowledgements

This work was supported by NSF award 1417517 to PAM, and NSF 1220068 and NSF 1460861 to DMF. MNW and MES were supported by the Shaw Institute. Thanks to Harry Nelson and Fluid Imaging Technologies, Inc. who graciously provided a VS series FlowCam imaging flow cytometer.

Declaration of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2018.10.061.

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