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Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia

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ABSTRACT

Humans continue to increase the use and disposal of plastics by producing over 240 million tonnes per year, polluting the oceans with persistent waste. The majority of plastic in the oceans are microplastics (<5 mm). In this study, the contamination of microplastic fibers was quantified in sediments from the intertidal zones of one exposed beach and two protected beaches along Nova Scotia's Eastern Shore. From the two protected beaches, polychaete worm fecal casts and live blue mussels (*Mytilus edulis*) were analyzed for microplastic content. Store-bought mussels from an aquaculture site were also analyzed. The average microplastic abundance observed from 10 g sediment subsamples was between 20 and 80 fibers, with higher concentrations at the high tide line from the exposed beach and at the low tide line from the protected beaches. Microplastic concentrations from polychaete fecal casts resembled concentrations quantified from low tide sediments. In two separate mussel analyses, significantly more microplastics were enumerated in farmed mussels compared to wild ones.

1. Introduction

1.1. Origin and distribution of microplastics

Humans have been mass-producing plastics since the early 1940s, and production has increased extensively in subsequent years. Approximately 240–280 million tonnes of plastic have been produced annually since 2008, compared to an annual production rate of 1.5 million tonnes in 1950 (Cole et al., 2011; Wright et al., 2013). About 50% of plastic produced is disposed after one use, with packaging materials as the main contributor. Another 20–25% of plastics entering the natural environment have intermediate life spans and come from durable consumer products, such as electronics and vehicles (Hopewell et al., 2009). Most plastics are extremely durable and can persist from decades to millennia in their polymer forms (Hopewell et al., 2009; Thompson et al., 2004). Their durability causes plastics to persist and contaminate environments worldwide. Marine habitats are particularly affected (Lithner et al., 2011).

Microplastics constitute plastics that are <5 mm, as classified by the National Oceanic and Atmospheric Administration (NOAA), and they are present in a heterogeneous array of shapes and sizes (Betts, 2008; Hidalgo-Ruz et al., 2012; Wright et al., 2013). Some authors classify microplastics with an upper size limit of 1 mm (e.g. Browne et al., 2008); and, upper size limits of 1 mm and 5 mm are currently acceptable to describe microplastics in the literature. The most prominent microplastic forms contaminating the marine environment are spheres, pellets, irregular fragments, and fibers (Wright et al., 2013). They are ubiquitous throughout the global oceans, and microplastics (<1 mm) in the water column and seabed have been observed to weigh 100 times and 400 times more than macroplastic debris, respectively (Van Cauwenberghe et al., 2013). Microplastics are distributed throughout the water column, sediments, and the deep sea, with highest concentrations along populated coastlines and within mid-ocean gyres (Cole et al., 2011; Wright et al., 2013). A study conducted on the spatial distribution of microplastics revealed that accumulation is higher at downwind sites and in areas with decreased water flow. A relationship has yet to be observed between microplastic concentrations and grain size distribution (Browne et al., 2010, 2011). Although microplastics have been observed throughout the oceans globally, the extent of microplastic contamination to the marine environment is still largely unknown (Browne et al., 2009, 2011).

Plastics are synthetic organic polymers, created by polymerization of monomers extracted from crude oil and gas (Cole et al., 2011). Some of the most prominent plastic polymers found in the environment include polystyrene (most commonly used in packaging and industrial insulation), acrylic, polyethylene (used

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in facial scrubs), polypropylene (commonly used in fishing gear), polyamide (nylon), polyvinyl chloride (PVC), and polyester fragments (Browne et al., 2008, 2011). Primary microplastics are produced at a microscopic size, and are integrated into a variety of facial exfoliating cleansers, air-blasting boat cleaning media, and are increasingly used in medicine as vectors for drugs (Cole et al., 2011). Secondary microplastics form when macroplastics undergo mechanical, photolytic, and/or chemical degradation, resulting in fragmented microplastic pieces and fibers. There is evidence that a primary source of microplastics is synthetic fibers from garments. A study quantifying microplastic concentrations at 18 sites worldwide showed that a single synthetic clothing garment can release >1900 microplastic fibers per wash. These microfibers enter the marine environment via wastewater discharge. Marine habitats in close proximity to sewage discharge sites contain proportions of polyester and acrylic microplastic fibers resembling proportions used in synthetic clothing (Browne et al., 2011).

1.2. Potential harms

Harmful components of plastics reside in the monomer constituents, in the additives and plasticizers, and in hydrophobic Persistent Organic Pollutants (POPs) and metals that absorb in plastics in the marine environment (Koelmans et al., 2013). Contaminants can be transferred to organisms most commonly by ingestion, inhalation, and dermal sorption (Teuten et al., 2009). The danger lies in the fact that microplastics are ingested by a variety of marine biota, and therefore have the potential to translocate these harmful constituents to organisms. However, the toxicological effects of many of the plastic components are not yet well known (Hidalgo-Ruz et al., 2012). Over 180 species have been documented to ingest plastic debris (Teuten et al., 2009), and as plastics fragment into smaller pieces, the potential for ingestion and accumulation in animal tissues increases (Browne et al., 2008; Wright et al., 2013). It has been discovered in previous studies that amphipods (detrivores), lugworms (deposit feeders), barnacles (filter feeders), and mussels (suspension feeders) all ingest microplastics when present in their environments (Thompson et al., 2004; Browne et al., 2008).

Microplastics, especially in fiber form, pose threats to organisms that consume them as they can cause blockages in the digestive tract, become translocated to different tissues within the organism, and undergo accumulation (Wright et al., 2013). Once microplastics enter the marine environment, they can be subjected to density changes through biofouling, which increases microplastic density (Wright et al., 2013). As microplastic density changes, they become available to organisms at different depths in the water column and in the sediments. This indicates that marine life occupying surface water all the way down to the benthos are vulnerable to microplastic interactions and contamination.

Many organic contaminants have been shown to accumulate on and inside plastics. Some of the contaminants previously observed in microplastics include polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons, organochlorine pesticides, polybrominated diphenylethers, alkylphenols, and bisphenol A (BPA). BPA is also commonly used as a monomer in plastic polymerization (Teuten et al., 2009). Concentrations of PCBs on polypropylene pellets have been observed 10^6 times higher than in surrounding seawater (Mato et al., 2001).

Enhanced leaching of organic contaminants from microplastics has been demonstrated in the presence of organic matter. If organic matter contents are higher in an organism's gastric environment compared to the surrounding seawater, this could imply enhanced desorption of POPs within the organism (Betts, 2008). As well, a feeding experiment conducted with Shearwater chicks demonstrated that chicks consuming PCB concentrated polyethylene pellets undergo tissue contamination from the PCBs (Betts, 2008). Polyethylene, one of the most concentrated microplastics in the marine environment, has a relatively high capacity to uptake and release organic contaminants, making it more efficient at translocating contaminants than other plastics (Teuten et al., 2009). On the other hand, the study conducted by Koelmans et al. (2013) suggested that plastics may reduce bioaccumulation of POPs in organisms, as equilibrium partitioning between plastics and POPs can dilute free aqueous concentrations. This would decrease bioavailability and bioaccumulation of POPs. As well, if ingested plastic had lower concentrations of POPs than an organism's body tissue, plastic would absorb POPs from the organism tissue, thereby decreasing the concentration of POPs in an organism once the plastic is egested.

Some of the organic contaminants associated with plastics interfere with hormone regulation in animals. BPA monomers and alkylphenol additives have estrogenic effects, while phthalates (a primary plasticizer) have been associated with reducing testosterone production (Teuten et al., 2009). Both BPA and phthalates can act as endocrine disruptors by competing with or disrupting endogenous hormones (Fossi et al., 2012). Each plastic polymer has a different capacity to adhere to different organic contaminants in the water column, therefore each type of plastic and organic contaminant must be analyzed individually, to determine absorption capacities. It would also be relevant to analyze desorption mechanisms of various organic contaminants from plastics in a gastric environment, in order to help quantify the dangers of contaminated plastics.

1.3. Purpose of this study

Urban, intertidal environments are exposed to heightened risk of microplastic contamination because of proximity to microplastic sources. In addition, there are heightened risks of chemical contamination within microplastics, as chemical concentrations are high in urban intertidal environments as well. The goal of this study is to assess microplastic contamination in the intertidal environment of Halifax Harbor, which is an urban estuary on the Atlantic coast of Canada. Various intertidal organisms may be negatively impacted by microplastics, and indirectly, microplastics have the potential to impact humans through the food chain. The results from this study add to the growing body of literature on microplastic contamination around the world, and they are especially pertinent to urban, coastal environments.

The objectives of this research are as follows: the enumeration of microplastics in intertidal sediments as a function of beach location, elevation on the shoreline and grain size distribution; the enumeration of microplastics in the fecal casts of deposit feeders, which in this study are polychaete worm species; and the enumeration of microplastics in wild and farmed blue mussels (*Mytilus edulis*), which are suspension feeders. From 2 protected beach sites, polychaete worm fecal casts and live mussels were collected, processed, and analyzed for microplastic fiber content. In addition, live mussels from an aquaculture site off of Newfoundland and Labrador were purchased from a local grocery store to analyze and compare the microplastic content of wild and farmed mussels.

Cultured mussels are grown in coastal waters that are separated from population centers where adjacent human pollution could threaten the quality of the mussels. Given the relatively pristine nature of mussel culture sites compared to an urban harbor, one can hypothesize that the microplastic load should be lower in farmed mussels. Alternatively, because cultured mussels are grown in plastic sock nets that are suspended on polypropylene long lines (Mussel Farmer, 2013, personal communication), farmed mussels may be exposed to microplastic contamination. The aquaculture site is about 800 km from the wild sites. *M. edulis* is an important organism in the benthic community assemblage, and has been

previously used as a model organism for observing microplastic content and digestive pathways due to its selective suspension feeding mechanisms (Browne et al., 2008). As well, it is one of the most popular shellfish species sold into the seafood industry, and is consumed by humans worldwide, especially in coastal populations (see Fig. 1).

2. Materials and methods

2.1. Sample collection and site description

Sediment samples were taken on July 16th, 2012 at McCormack's Beach (N44.609°, W63.493°), Rainbow Haven Beach's exposed side, and Rainbow Haven Beach back lagoon (N44.648°, W63.417°). These 3 sites are all located within 10 km of one another, in the Eastern Passage of Nova Scotia, Canada. The Eastern Passage is located on an actively eroding drowned coastline, surrounded with abundant drumlin islands made of deep glacial tills. Long inlets create interactive islands and headlands, forming beaches. McCormack's Beach is a sandy beach located at the mouth of the inlet to Halifax Harbor, and is protected from intense wave action by McNabs Island and Lawlor Island. Rainbow Haven Beach lies between the mouth of the inlet to the Cole Harbor estuary and Halifax Harbor. It includes an exposed sandy beach, subject to intense wave action, and a protected back lagoon mudflat. Both McCormack's Beach and Rainbow Haven Beach have a mean tidal range of 1.5 m.

Replicate samples of 225 cm^2 quadrats, ~3 to 4 cm deep, were obtained using a trowel at low tide. Samples were taken at the high, mid, and low tide lines at each beach. Samples were placed in previously unused, sealed freezer bags, and stored in the freezer for preservation upon returning to the lab that day.

Replicate polychaete worm fecal cast samples of 25 small casts were obtained on August 8th, 2012 at low tide at McCormack's Beach and Rainbow Haven Beach back lagoon respectively. Three polychaetes were collected for identification, which were found in the depressions adjacent to their fecal mounds. Two of the polychaetes were identified as *Clymenella torquata* and the remaining polychaete was identified as *Alitta virens* (Jon Grant, 2012, personal communication).

Ten live mussels were obtained on August 8th, 2012 at low tide from McCormack's Beach and Rainbow Haven Beach back lagoon respectively. As well, 10 live mussels were purchased at the local grocery store on September 21, 2012. The mussels came from an aquaculture site off the west coast of Newfoundland and Labrador, approximately 800 km away from the wild sites. These mussels were used in the first of two analyses quantifying microplastic

Fig. 1. Location map of the 3 sample collection sites in Nova Scotia.

fibers in mussels. For the second mussel analysis, fifteen mussels were collected from McCormack's Beach at low tide on February 23rd, 2013, and from Rainbow Haven Beach back lagoon at low tide on May 20th, 2013 respectively. As well, 15 live mussels were purchased from the local grocery store on January 22, 2013, which came from the same aquaculture site as in the first mussel analysis.

Both the cast samples and mussel samples were placed in previously unused, sealed freezer bags, and into the freezer for preservation the same day they were collected or purchased (Dawson et al., 1998). The preservation of mussel tissues was not required, as this study was only interested in the inorganic materials within the mussel, therefore a freezer of −5 °C was sufficient.

2.2. Grain size analysis

Sandy samples were obtained from McCormack's Beach and Rainbow Haven Beach's exposed side. Wet weights of 500 g were measured from the first replicate of each sandy sample from each tide line. Samples were dried in an oven at 65.5 °C. Grain size distributions for these samples were analyzed by sieving. The sieve stack consisted of sieves with mesh diameters of: 500 μm, 355 μm, 250 μm, 150 μm, 106 μm, 63 μm, and <63 μm. From each sample, 40 g of dried sediment was weighed and placed in the sieve stack. The stack was placed in the sieve shaker for 15 min. Sediment remaining in each sieve was weighed. The resulting discrete size distribution of mass was used to obtain the median grain size from each sample. All sediment analyzed in the grain size analysis was discarded after being sieved and weighed. Wet weights of 500 g from the second replicates collected from each tidal elevation at each beach were dried for later use in the microplastic analysis, along with the remaining sediment from the first replicates.

Muddy samples were obtained from Rainbow Haven Beach back lagoon. Frozen wet weights of 50 g from each muddy sample were collected and transferred to beakers. The beakers were covered with porous tin foil, and placed in a Christ, model Alpha 1-4 LD freeze drier at −60 °C for 5.5 days. Dry weights of ~0.08 to 0.1 g of sediment from replicate samples from each tidal elevation were treated with 3 applications of 5 ml of 30% hydrogen peroxide (H_2O_2) to destroy any organic matter present. A Beckman–Coulter Multisizer 3 was used to analyze the grain size distributions. Different sized apertures were used to detect and determine the distribution of particles of different sizes. Unscreened sediment 100 μm was analyzed with the 400-μm-aperture tube, sediment screened at 100 μm was analyzed with the 200-μm-aperture tube, and sediment screened at 25 μm was analyzed with the 30-μm-aperture tube. The three size distributions were merged to produce a complete particle size distribution (Law et al., 2013).

2.3. Hydrogen peroxide treatment

Three subsamples of 10 g each were taken from the two sediment samples collected at each position on the shore, as well as from the two polychaete worm fecal cast samples, respectively. The resulting 6 samples were treated as replicates on the justification that disturbance and mixing of each sample during collection is similar to the disturbance and mixing that occurs with the rise and fall of the tides. In the fume hood, ~150 ml of 30% H_2O_2 (multiple applications) was added to each 10 g subsample to remove organic matter present. Subsamples were placed on hotplates heated to ~55 to 65 °C, in order to catalyze the evaporation of the H_2O_2 (Liebezeit and Dubaish, 2012). Large concentrations of organic matter in some samples caused sediment to be suspended above the bubbling fluid, and stick to the sides of the beaker. This effect may have reduced the number of microplastics recovered.

In the first mussel analysis, two subsamples of 5 mussels from each location were subjected to H_2O_2 treatment. The shelled length of each mussel was first recorded, as well as the cumulative shelled weights from each mussel subsample. All mussels from the subsamples were dissected, and inner contents were emptied into a large beaker. In the fume hood, ~150 to 200 ml of 30% H_2O_2 was added to each subsample in order to digest the majority of the organic matter. Beakers were placed on hot plates at ~55 to 65 °C until all H_2O_2 was evaporated. It was challenging to dissolve all of the organic matter from 5 mussels at once, and large particles of mussel matter remained in some of the samples after the H_2O_2 treatment. Due to challenges with dissolving all the organic matter of 5 mussels at once, the mussel analysis was redone. In the second analysis, fifteen individual mussels from each location were subjected to H_2O_2 treatment. The shelled length and shelled weight of each mussel were recorded (Table 1). All mussels were dissected, and inner contents were emptied into large beakers containing one mussel each. The hydrogen peroxide treatment was applied to each beaker. Most of the organic matter was removed using this method, but some mussel samples had flakes of debris remaining after all the H_2O_2 was dissolved.

2.4. Saline (NaCl) solution floatation and filtration

A concentrated saline solution (250 g NaCl/L H_2O) was prepared to separate microplastics from sediments, fecal casts, and mussel matter via floatation. After all the H_2O_2 was evaporated from each 10 g sediment and cast subsample, 100 ml of NaCl solution was added. Using a magnetic stirrer, each subsample was stirred at high intensity for 1–2 min, followed by a settling time of 3–6 min, depending on the observed clearance rate of the sediment/fecal matter from suspension (Thompson et al., 2004). Using a 10 ml pipette, the supernatant was transferred onto 0.8-μm pore size, 47 mm gridded nitrocellulose filters using a vacuum system. After all the supernatant was extracted, an additional 100 ml of NaCl solution was added to each sample, and the same procedure was repeated to extract any remaining microplastics. Samples with higher concentrations of suspended sediment in the supernatant required multiple filters for efficient extraction. When reaching the bottom of the supernatant, it was challenging to extract every drop of remaining solution, which could have contributed to a reduction in total microplastics recovered. Subsequent to filtration, there were occurrences where some sediment residue remained on the vacuum filter piece, which could have contained microplastics that were unaccounted for. After 2 NaCl solution extractions, filters were placed in unused, previously sealed petri dishes and were dried at room temperature.

For the mussel samples, the same procedure was conducted as the sediment and cast samples, except an additional 100 ml of NaCl solution was added to perform a total of 3 microplastic extractions. As well, because of the lack of suspended sediment, the settling time was reduced to 1–2 min after stirring.

2.5. Microplastic extraction efficiency

To test the efficiency of the double and triple extractions, 5 NaCl solution extractions and filtrations of supernatant were performed on a sediment sample from the exposed side of Rainbow Haven

Table 1
Average and median shelled lengths (cm) and weights (g) of 15 mussels from McCormack's Beach, Rainbow Haven Beach back lagoon, and an aquaculture site respectively.

Location	Shelled length (cm)		Shelled weight (g)	
	Average	Median	Average	Median
McCormack's Beach	7	7	42	39
Rainbow Haven Beach	7	7	38	38
Aquaculture site	7	7	24	22

Beach, in order to characterize the proportion of total microplastics recovered after 2–3 extractions. As well, 3 control samples of distilled water were placed on hot plates for the same duration as it took for all the H_2O_2 to evaporate from the samples used in this study. Three NaCl solution extractions were applied to each control sample to determine if microplastics circulating throughout the lab were a possible source of contamination.

The sediment sample used in the control analysis was subjected to the same collection procedure and drying mechanism as rest of the sediment samples used in this study. This assessment of contamination was focused on the H_2O_2 treatment and filtration process, as these were the steps in the procedure where the samples were predominantly left uncovered, and therefore exposed to potential microplastic contamination.

2.6. Microplastic enumeration

Microplastics in the form of microfibers were enumerated from filters corresponding to each subsample. Spherical microplastics, because they resemble sediment grains, were too difficult to identify visually. Once filters were dry, microplastic fibers on each filter were enumerated under a Motic Dissecting Microscope using 2× magnification. One of the most commonly used methods for identifying microplastics is through visual sorting (Hidalgo-Ruz et al., 2012). Solid, lightweight strands of a variety of lengths and colors were identified as microplastic fibers (Wright et al., 2013). A visual assessment was applied to help distinguish microplastics originating from field samples to microplastics originating from laboratory contamination. In the study conducted by Davidson and Asch (2011), small-diameter, brightly colored microplastic fibers were considered to be originating from exterior contamination, as they observed similar looking fibers in empty petri dishes. A similar visual assessment was applied to the samples in this study. Fig. 2 contains magnified photographs of microplastic fibers extracted from a sediment sample, a polychaete worm fecal cast sample, and a farmed mussel.

On certain filters it was challenging to identify clear microplastic fibers and short fibers, which most likely resulted in undercounting. Microfibers were observed on the exterior of the petri dish perimeters as well as on the filters. The source of microplastics on the petri dish perimeters is not clear, but two likely scenarios are that they derive from microplastics located on the filters themselves, or they derive from microplastics circulating in the air throughout the lab.

2.7. Contamination assessment

Microplastic fibers were the predominant microplastic form that appeared in the intertidal samples. Thompson et al. (2004) also found brightly colored microfibers as the predominant microplastic form in coastal environments. As for monitoring contamination, the freezer bags and petri dishes used for sample collection and storage were new and unopened. Therefore, it was presumed that the interiors of the freezer bags and petri dishes were not contaminated. To avoid contamination, all containers and beakers were rinsed with distilled water prior contacting samples. Samples were covered when they were not in use, during the duration of drying in the oven and freeze drier, and after the filtration process. The only times the samples were predominantly uncovered was during the H_2O_2 treatment, filtration, and during counting. Therefore, control samples were tested for that portion of the procedure. For the second mussel analysis, all mussel samples were covered with aluminum foil during the H_2O_2 treatment.

2.8. Statistical analyses

All statistical analyses were conducted using Matlab (2009b, The MathWorks, Natick, MA). Kolmogorov–Smirnov tests for normality were applied to each beach site and tidal elevation pair; polychaete worm fecal casts and low tide sediments from each site; and mussels from each site for each mussel analysis. ANOVA tests were applied once all necessary preconditions for normality

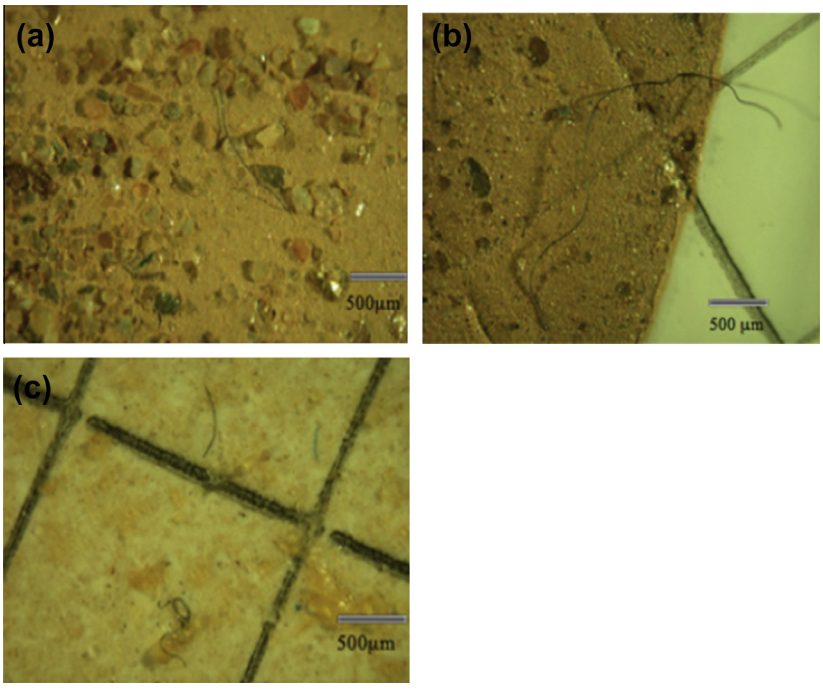

Fig. 2. (a) Microplastic fiber extracted from a low tide sediment sample from McCormack's Beach. (b) Microplastic fiber extracted from a polychaete fecal cast sample. (c) Microfibers extracted from a farmed mussel.

were justified. A 2-way ANOVA analysis was applied to compare microplastic fiber concentrations in the sediments at the different beaches and tidal heights. As well, a 2-way ANOVA analysis was performed to compare microplastic concentrations in low tide sediments from McCormack's Beach and Rainbow Haven Beach back lagoon to concentrations found in polychaete worm fecal casts. A one way analysis of variance was used to compare microplastic concentrations from mussels collected at McCormack's Beach, Rainbow Haven Beach back lagoon, and from an aquaculture site for both the first and second mussel analyses respectively. If these tests indicated significant differences, then pairwise *t*-tests were used to determine the significantly different groups through multiple comparisons. Last, a linear regression analysis was conducted to determine if correlation existed between grain size and microplastic concentrations. Significance levels for all statistical analyses were 0.05.

3. Results

Microplastic fibers were enumerated and compared within different reservoirs in the intertidal zone from 3 beaches on the outskirts of Halifax Harbor in addition to an aquaculture site. These reservoirs are the sediments of different tidal elevations, fecal matter of polychaete worms, and internal organs and tissues of wild and farmed mussels.

3.1. Beach and tide sediments

Overall, the 10 g sediment subsamples from all 3 beaches and tide lines contained microplastics, with each tide line having an average microplastic concentration ranging from ~20 to ~80 microplastics/10 g sediment (Fig. 3). Specific concentrations should be regarded with caution, as there is evidence samples suffered microplastic fiber contamination from within the lab.

Kolmogorov–Smirnov tests indicated that the null hypothesis that the data are normally distributed could not be rejected for all sites and tidal elevation pairs ($p > 0.45$ for all datasets).

The 2-way ANOVA showed that beach site or tidal elevation did not have a significant effect on microplastic concentrations when no interaction term was considered ($p = 0.213$, 0.084 respectively). When an interaction term was considered, there was a significant interaction between beach site and tidal elevation ($p = 0.022$). Due to the significant interaction term, three group ANOVAs were carried out to see if elevation was a significant factor at each site. Mean abundances were not significantly different at the different elevations at Rainbow Haven Beach's exposed beach ($p = 0.120$) or at McCormack's Beach ($p = 0.318$). Mean abundances were significantly different at different elevations at Rainbow Haven Beach back lagoon ($p = 0.004$). Pairwise *t*-tests were carried out for the Rainbow Haven Beach back lagoon site. Microplastic abundances were significantly higher at the low tidal elevation than they were at the mid and high tidal elevations ($p = 0.005$ and 0.003 respectively). They were not significantly different between the mid and high tidal elevations ($p = 0.765$).

Positions and accumulation of stranding may depend on the energy level in the environment, and more research is necessary in testing the main effects under when there is significant interaction between beach location and tidal elevation.

3.2. Low tide sediments and polychaete worm fecal casts

Kolmogorov–Smirnov tests indicated that the null hypothesis that the data are normally distributed could not be rejected for all polychaete worm fecal casts and low tide sediment pairs at each site ($p > 0.86$ for all datasets). The 2-way ANOVA showed that means did not differ among sites ($p = 0.096$) or among sediment versus fecal samples ($p = 0.361$). There was no significant interaction term ($p = 0.645$).

3.3. Wild mussels and farmed mussels

Abundances of microfibers in farmed mussels were higher than in wild mussels. In the first mussel analysis, the 5-mussel subsamples had high concentrations of microplastics, with the average number in wild mussels observed to be ~170/5 mussels and the average number in farmed mussels observed to be ~375/5 mussels (Fig. 5).

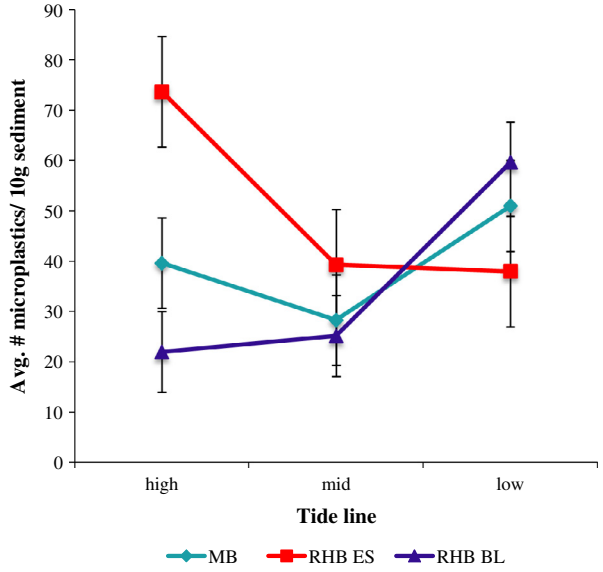

Fig. 3. Average number of microplastic fibers enumerated from six 10 g subsamples from the high, mid, and low tide lines at MB = McCormack's Beach, RHB ES = Rainbow Haven Beach's exposed side, and RHB BL = Rainbow Haven Beach back lagoon respectively. Error bars on each averaged microplastic concentration represent the average standard deviation from the samples at each site.

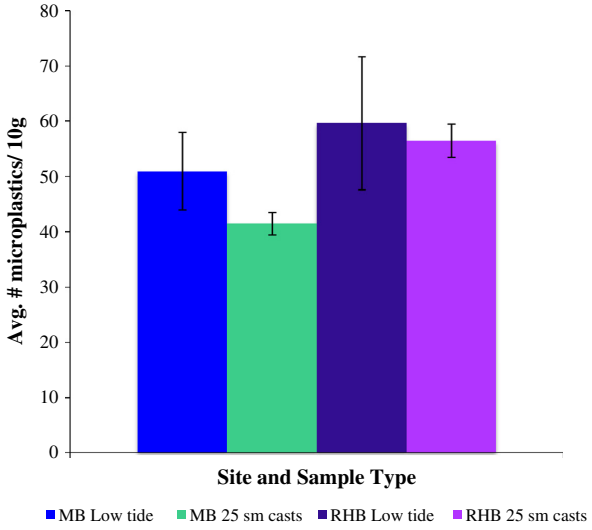

Fig. 4. Average number of microplastic fibers enumerated from six 10 g subsamples of low tide sediments and two 10 g subsamples of 25 small polychaete worm fecal casts from McCormack's Beach and Rainbow Haven Beach back lagoon, respectively. Error bars on each averaged microplastic concentration represent the standard deviation of each sample type at each site.

Kolmogorov–Smirnov tests indicated that the null hypothesis that data are normally distributed could not be rejected for any of the sites ($p = 0.999$ for Rainbow Haven Beach, McCormack's Beach, and farmed mussels respectively). A one-way analysis of variance was performed to determine if location influenced microplastic concentrations in the 5-mussel subsamples. Overall, there was a significant difference among microplastic concentrations from the three different locations ($p = 0.045$). Pairwise *t*-tests indicated that microplastic concentrations in farmed mussels were significantly higher than concentrations enumerated from Rainbow Haven Beach ($p = 0.030$) and McCormack's Beach ($p = 0.029$), which were not significantly different from one another ($p = 0.965$).

In the second mussel analysis, all mussels were contaminated with microplastics, with the average number per wild mussel observed to be ~126 from McCormack's Beach, ~106 from Rainbow Haven Beach, and ~178 per farmed mussel (Fig. 6). Fig. 7 shows the number of microplastics extracted from each of the 15-mussel samples from McCormack's Beach, Rainbow Haven Beach, and the aquaculture site respectively.

Kolmogorov–Smirnov tests indicated that the null hypothesis that data are normally distributed could not be rejected for any of the sites ($p = 0.17$, 0.99, 0.95 for Rainbow Haven Beach, McCormack's Beach, and the aquaculture location respectively). A one-way analysis of variance was performed to determine if location influenced microplastic concentrations in the 15-mussel samples. Overall, there was a significant difference among microplastic concentrations from the three different locations ($p = 0.001$). Pairwise *t*-test indicated that microplastic concentrations in farmed mussels were significantly higher than in mussels from Rainbow Haven Beach ($p = 0.000$) and McCormack's Beach ($p = 0.003$), which were not significantly different from one another ($p = 0.368$). These results indicate significantly higher microplastic concentrations in farmed mussels compared to wild mussels.

3.4. Grain size distribution

Grain size varied among sites. Mass median diameters were several hundred micrometers at McCormack's Beach and the exposed side of Rainbow Haven Beach, but they were only of order 10 μm in the muddy back lagoon of Rainbow Haven Beach. Despite large differences in grain size, microfiber abundances were similar (Table 2 and Fig. 8).

A linear regression analysis was performed to determine if the grain size distribution was correlated to microplastic concentration. Based on this analysis, there was no evidence that a relationship exists between microplastic concentrations and grain size distributions from each tide line at each beach (*p*-value = 0.421).

3.5. Microplastic extraction efficiency

When the 5-time microplastic extraction was applied to a high tide sediment sample from the exposed side of Rainbow Haven Beach, the majority of microplastics were extracted from the 1st

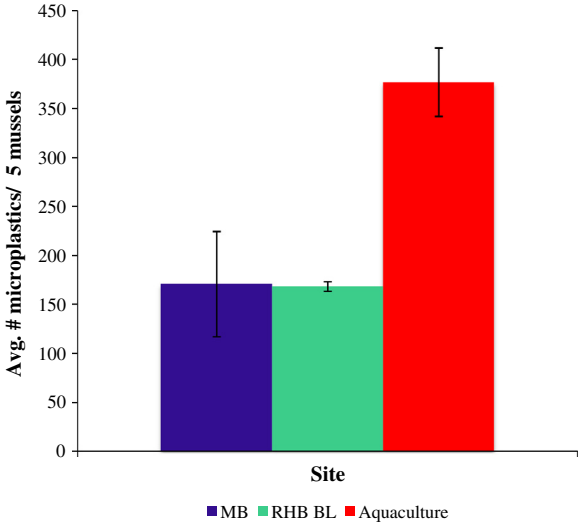

Fig. 5. Average number of microplastic fibers enumerated from two 5-mussel subsamples from McCormack's Beach, Rainbow Haven Beach back lagoon, and an aquaculture site respectively. Error bars on each averaged microplastic concentration represent the standard deviation of each sample.

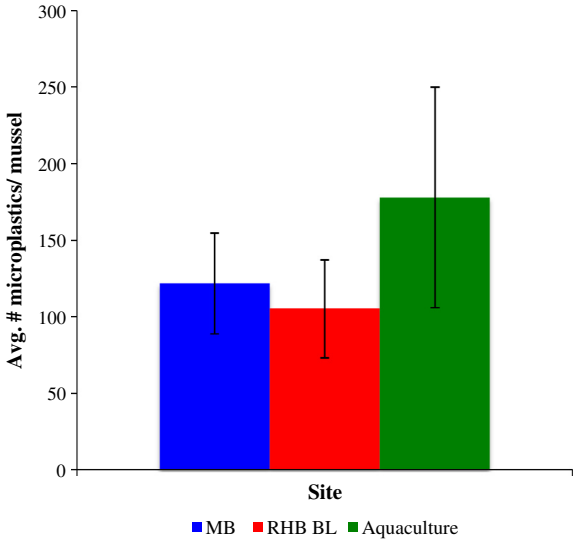

Fig. 6. Average number of microplastic fibers enumerated from 15-mussel samples from McCormack's Beach, Rainbow Haven Beach, and an aquaculture site respectively. Error bars on each averaged microplastic concentration represent the standard deviation of each sample.

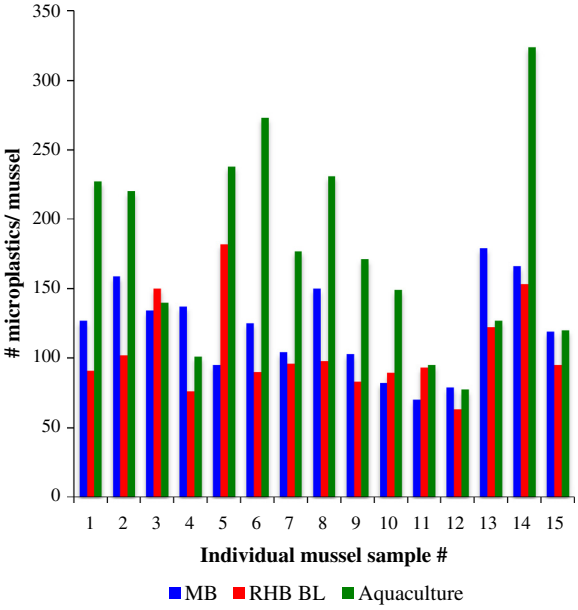

Fig. 7. Total number of microplastic fibers enumerated from 15 individual mussels from McCormack's Beach, Rainbow Haven Beach back lagoon, and an aquaculture site respectively.

Table 2
Average number of microplastics enumerated from six 10 g subsamples, corresponding to the median grain size (μm) calculated from the high, mid, and low tide lines from McCormack's Beach, Rainbow Haven Beach's exposed side, and Rainbow Haven Beach back lagoon.

Beach location	Tidal height	Median grain size diameter (μm)	Avg. # microplastics/10 g sediment
McCormack's Beach	High	218	40
	Mid	207	28
	Low	206	51
Rainbow Haven Beach's exposed side	High	219	74
	Mid	220	39
	Low	174	38
Rainbow Haven Beach back lagoon	High	9	22
	Mid	11	25
	Low	11	60

extraction (~70 microplastics), however, a lesser amount of microplastics were consistently recovered from the 2nd, 3rd, 4th, and 5th extractions (~25 microplastics/extraction) (Fig. 9).

From the 3 control microplastic extraction analyses, it was found that an average of ~100 microplastics were present on filters originating from air circulating throughout the lab (Fig. 10).

4. Discussion

4.1. Beach and tide sediments

The concentrations of microplastics enumerated from the intertidal sediments in this study were higher than Claessens et al. (2011) observed in Belgian Coast sediments, and lower than Liebezeit and Dubaish (2012) observed in sediments from the Frisian Islands in Northwestern European waters. A positive correlation between coastal microplastic concentration and human population density has been demonstrated in multiple locations throughout the world (Browne et al., 2010, 2011). As well, high concentrations of microplastic fibers in the North Atlantic Ocean coincide with increases in global plastic production (Thompson et al., 2004). Since the three locations analyzed in this study were in close proximity to one another, as well as in close proximity to Halifax Harbor, the results that microplastic concentrations were high and not statistically different at the three beaches are reasonable. Analyzing microplastic concentrations from a larger sample size of beach locations that are a greater distance apart and with varying proximity to Halifax Harbor could expose statistical differences in microplastic concentrations at different beach locations.

Microplastic concentrations between the three tidal elevations were not significantly different. However, there was a significant interaction between microplastic concentrations at the 3 beaches and tidal heights. The significant interaction arose from higher microplastic concentrations enumerated at the low tide position at Rainbow Haven Beach back lagoon compared to the high and mid tidal positions. Microplastic concentrations were not significantly different at the different tidal elevations at McCormack's Beach or Rainbow Haven Beach's exposed side.

Liebezeit and Dubaish (2012) found high concentrations of microplastics at the high tide line of exposed beaches. This finding corresponds to microplastic concentrations enumerated at Rainbow Haven Beach's exposed side, which had highest microplastic concentrations at the high tide line. In contrast, the two protected beaches (McCormack's Beach & Rainbow Haven Beach back lagoon) had highest microplastic concentrations at the low tide line (Fig. 3). This corresponds to the findings of Liebezeit and Dubaish (2012), where high microplastic concentrations were observed in protected tidal mud flats. The low tide accumulation of microplastics at protected beaches/tidal flats is thought to be due to the low energy environments that induce higher deposition rates of easily transported, lower density plastics. An additional explanation is that microplastics can become associated with microbial films, thereby reducing their capacity to get washed out of the tidal flat with the tides (Liebezeit and Dubaish, 2012). Perhaps with a larger sample size of exposed and protected beach locations, a stronger relationship could be obtained between the distributions of microplastic concentrations at different tidal heights.

4.2. Low tide sediments and polychaete worm fecal casts

Microplastic concentrations were statistically similar between the two beach sites, as well as between the polychaete worm fecal casts and low tide sediments at McCormack's Beach and Rainbow Haven Beach back lagoon respectively (Fig. 4). This finding suggests that consumption and excretion of plastics by the polychaetes are at steady state, so that ingestion equals egestion. This result does not clarify the residence time of microplastics in the deposit feeders. Further, it suggests that the polychaetes consume microplastics indiscriminately at concentrations present in their environments.

Microplastics are known accumulators of organic hydrophobic contaminants in the water column (Teuten et al., 2007, 2009). Therefore, a primary danger of microplastics is their ability to absorb these organic contaminants, and increase the probability of transporting them into marine biota via ingestion. Different plastic polymers have different capacities to absorb different hydrophobic contaminants; therefore each plastic polymer and organic contaminant must be analyzed individually.

It has been shown that an abundant organic contaminant, phenanthrene, has a high sorption rate to microplastic polymers in the marine environment. If biofouling occurs to phenanthrene-contaminated microplastics, this will cause them to sink and put deposit-feeding organisms at risk of contamination. It has been suggested through feeding experiments that the lugworm, *Arenicola marina*, can ingest microplastics containing phenanthrene, and its metabolic surfactants increase its desorption rate, resulting in tissue contamination (Teuten et al., 2007). This indicates that even if deposit feeders excrete microplastics, it does not necessarily mean that they are not taking up organic contaminants that are absorbed in the microplastics.

Gouin et al. (2011) conducted a study using thermodynamic models to test the partitioning capacities of various Persistent Bioaccumulative Toxic (PBT) substances from polyethylene microplastics to organisms in the food web. Their results showed that as microplastic concentrations increase in the environment, they sorb an increasing amount of PBT substances. Once ingested by an organism, the PBT substances were demonstrated to remain sorbed in the plastic, as their affinity to polyethylene was stronger than their affinity to the organic carbon in the organism. This study suggests a limited importance of microplastics acting as vectors in

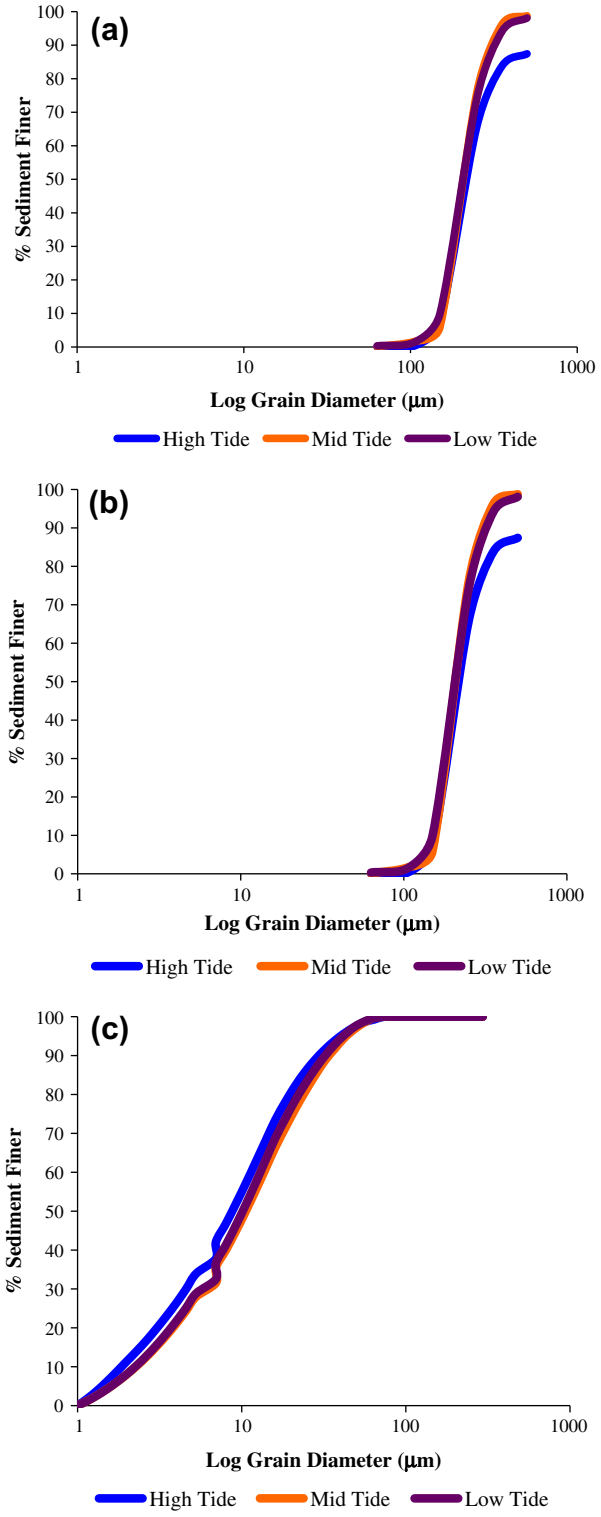

Fig. 8. Grain size distribution of sediment collected at: (a) McCormack's beach, (b) Rainbow Haven Beach's exposed side, and (c) Rainbow Haven Beach back lagoon from the high, mid, and low tide lines, represented as the percent of sediment finer than standard log grain diameters (µm).

transporting contaminants to organisms. That said, gaps in the data include analyzing the kinetics of microplastic digestion through the organism gut, as residence time and extraction efficiency of microplastics from the gut may influence possible tissue contamination. It is also relevant to assess how the size and shape of microplastics influence toxicological responses within organisms.

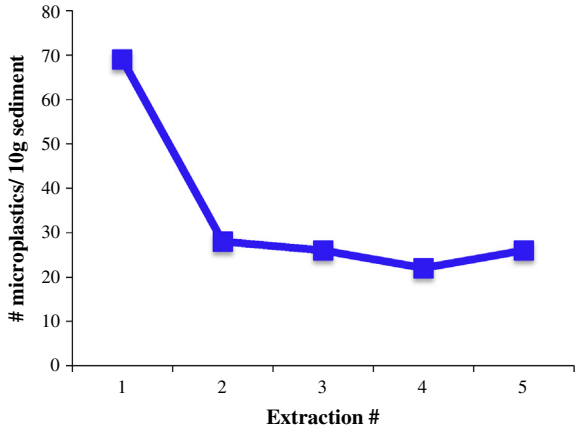

Fig. 9. Number of microplastic fibers recovered from a high tide sediment sample from Rainbow Haven Beach's exposed side, from a total of 5 NaCl solution extractions.

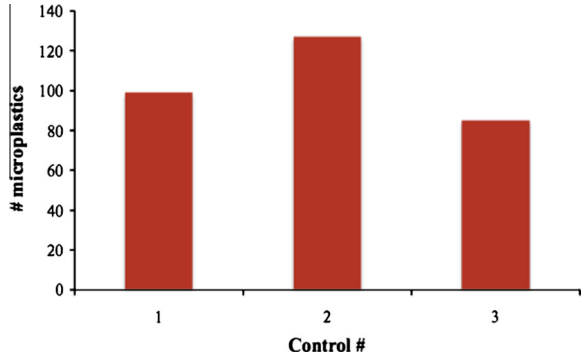

Fig. 10. Total number of microplastics enumerated from 3 control extractions using distilled water.

4.3. Wild mussels and farmed mussels

Microplastic concentrations observed in wild and farmed mussels were significantly different (analysis 1: $p = 0.045$, analysis 2: $p = 0.001$). Average microplastic concentrations in the farmed mussels were higher than in both wild mussel samples (Figs. 5 and 6). Possible reasons for this difference could be due to the fact that farmed mussels were grown on plastic polypropylene lines (Mussel Farmer, 2013, personal communication), the mussels were grown in a coastal location 800 km away from the wild mussels, or perhaps farmed mussels encounter microplastics from the time they are cultured to the time they arrive at the store. Since the farmed mussels originated from a bay with the surrounding human population being approximately 2000 people, the microplastic input in that region would be much less than the input in waters surrounding Halifax Harbor, where the population of Halifax alone is approximately 373,000 people.

In addition to this study, Browne et al. (2008) previously discovered that microplastics can undergo accumulation within the organs of mussels. Through feeding experiments, it was found that microplastics were translocated from the digestive tract to the circulatory system in as little as 3 days of ingestion. As well, accumulation of microplastics in the digestive tract can cause blockages and induce satiation in the organism, leading to a decrease in fitness (Wright et al., 2013). Smaller microplastics (i.e. ~3.0 µm) have higher accumulation rates in mussel tissues compared to larger fragments, indicating that as microplastics continue to degrade, their accumulation rates increase. Microplastics accumulated in

the circulatory system of mussels, but clearance rates of up to 70% were observed in pure seawater (Browne et al., 2008). Although mussels can rid themselves of some microplastics, they are constantly taking them up in the natural environment, therefore their tissues will always be contaminated if microplastics are present in their environment. It may be important to depurate both farmed and wild mussels in clean, plastic-free seawater before human consumption. The persistence of microplastics in the internal organs of mussels can have implications for possible bioaccumulation at higher-trophic-level consumers, including humans. However, bioaccumulation of microplastics has not yet been analyzed due to complex methodologies in testing this phenomenon. That said, microplastics have been detected in higher trophic level organisms including 10 species of fish from the English Channel (Lusher et al., 2012), one third of fish caught from the North Pacific Central Gyre, and in the scat of fur seals and Hooker's sea lions (Wright et al., 2013).

Medical studies have found that the lymph and circulatory systems in rodents and humans can take up certain plastic polymers, such as PVC and polystyrene. The smaller the plastic fragment, the more likely it is to get taken up by cells in vertebrates (Browne et al., 2008). Aside from the dangers of microplastics alone, organic contaminants leached onto microplastics from the surrounding seawater have varying capacities to desorb from plastics within organisms and contaminate organism cells, disrupting cellular functions (Teuten et al., 2009).

4.4. Grain size distribution

In this study, no correlation was detected between grain size distribution and microplastic concentration. This result is similar to that of Browne et al. (2010) and Browne et al. (2011), who also failed to find a relationship between grain size and microplastic concentrations. This could indicate that microplastic distributions in the intertidal zone may not be a function of grain size, but rather a function of microplastic input, density, biological interactions with microbial films, and physical processes such as wave action and tidal patterns.

4.5. Microplastic extraction efficiency

Extraction of microplastic fibers through density separation (floatation using a concentrated NaCl solution), vacuum filtration, and visual sorting under a dissecting microscope is a common and relatively efficient extraction technique (Hidalgo-Ruz et al., 2012). As well, conducting 2–3 extractions allowed for the majority of microplastic fibers in the subsamples to be extracted (Browne et al., 2011). Claessens et al. (2011) did a two time extraction using concentrated NaCl solution with known microplastic concentrations in both sandy and muddy samples, and had 69–98% recovery rates, supporting the efficacy of a 2-time extraction.

Although the NaCl solution (250 g/L) extraction method used in this study was capable of extracting many microplastic fibers, this density was still lower than that of many plastic polymers in the environment. The plastics that float in NaCl solution of the density used in this study are polystyrene (in foam form), and high and low-density polyethylene and polypropylene. Some of the most prominent plastic polymers that are denser than the NaCl solution used in this study are PVCs, polyethylene terephthalates (PETs), and nylon (Hidalgo-Ruz et al., 2012). Therefore, although the first 2–3 extractions are capable of recovering many microplastics, the recovered microplastics are presumed to be the less dense polymers. Denser microplastic polymers should remain at the bottom of the beakers, but these polymers may get extracted if they are in the midst of settling as the supernatant is being pipetted. If only a NaCl solution extraction is applied, then it can be presumed that the denser microplastics will not all be extracted, and with increasing extractions more microplastics will be recovered. Evidence of this effect was apparent when 5 extractions were applied to a high tide sediment sample from the exposed side of Rainbow Haven Beach (Fig. 9). The majority of microfibers were removed from the first extraction, but a lesser number of microplastics was consistently recovered from the 2nd, 3rd, 4th, and 5th extractions. Since sediment, fecal casts, and wild mussel samples were taken from the benthos, it is likely the samples were more contaminated with denser microplastics, which did not all get extracted. A sodium polytungstate solution can be used to efficiently extract denser microplastic polymers (Hidalgo-Ruz et al., 2012). As well, Imhof et al. (2012) suggested using a newly designed device called the Munich Plastic Sediment Separator (MPSS) to reliably separate plastics from sediment, in a liquid medium of zinc chloride (1.6–1.7 kg/L) as the extraction fluid.

Fourier Transformed Infrared spectrometry is useful for determining the chemical composition of microplastics, and therefore gives a better idea of where the microplastics are originating (Claessens et al., 2011; Thompson et al., 2004; Browne et al., 2010). Since this study only focused on the presence of microplastic fibers, overall microplastic abundances observed were lower than the actual total abundances, assuming other types of microplastics were present. Lack of compositional information of microplastics in all samples prevented determination of the primary sources, as well as inferences about potential deleterious effects they could have based on their composition.

After performing three control analyses, it was apparent that microplastic fibers circulating throughout the lab were contaminating the samples used in this study. Microplastic fibers observed in control samples and on the exteriors of petri dishes were shorter and more colorful compared to microplastic strands observed on sample filters, which were longer, and predominantly clear or black in color. Microplastic fibers originating from procedural contamination resembled microfibers found by Davidson and Asch (2011) in empty petri dishes.

Despite this contamination, statistical trends still emerged. These trends include a significant interaction between beach location and tidal elevation, where microplastic concentrations at the low tide line of Rainbow Haven Beach back lagoon were significantly higher than concentrations enumerated at the high and mid tidal elevations. These results correspond to trends observed by Liebezeit and Dubaish (2012) on protected tidal mud flats. As well, microplastic concentrations in polychaete worm fecal casts were not significantly different from the number of microplastics enumerated in low tide sediments from McCormack's Beach and Rainbow Haven Beach back lagoon, respectively. The two mussel analyses yielded the same statistical result; that being significantly more microplastics enumerated in farmed mussels compared to wild mussels. The second mussel analysis yielded stronger statistical differences than the first analysis. This is most likely due to larger sample sizes used in the second mussel analysis, which provided stronger tests. Lastly, a statistically significant relationship did not ensue between grain size and microplastic concentrations, a result similar to previous studies (Browne et al., 2010, 2011). Therefore, although the sediment samples, polychaete fecal cast samples, and mussel samples suffered some microplastic contamination from within the lab, trends still appear in the data.

Laboratory microplastic contamination implies that the results obtained from this study regarding specific microplastic concentrations should be regarded qualitatively, and absolute concentrations should be used with caution. It is important to mention that the filters obtained from the control analyses were extremely clear, and all microplastics present on the filters were visible. Filters obtained from sediment samples, polychaete worm fecal cast samples, and mussel samples all contained debris. Therefore, it is likely that some of the more heavily camouflaged plastics among the various

filter backgrounds were not numerated. This would result in undercounting of microplastics on sample filters. It is essential for scientists to be aware of and avoid airborne microplastics when conducting studies in the future.

5. Conclusions

This study documented the presence of microplastic fibers in intertidal sediments, polychaete fecal casts, and wild mussels adjacent to Halifax Harbor, in addition to coastal, farmed mussels approximately 800 km away from the wild mussel sites. Microplastic concentrations were similar between the 3 beaches analyzed, as they were in close proximity to one another, in addition to all being located on the outskirts of an urbanized harbor. The exposed beach had highest microplastic concentrations at the high tide line. In this high-energy environment, small, relatively low-density plastics remain in suspension until they are stranded at the upper limit of wave action. At the 2 protected beaches analyzed, microplastic concentrations were highest at the low tide line, with statistical significance at Rainbow Haven Beach back lagoon. This low tide accumulation is most likely due to enhanced deposition from reduced wave action, and may also be influenced by interactions with microbial films.

Polychaete worm fecal casts analyzed from the 2 protected beaches had microplastic fiber concentrations resembling those found in low tide sediments from their respective beaches. This is an indication that polychaete deposit feeders are indiscriminately feeding on microplastics, and appear to be excreting most if not all the microplastics they consume. However, polychaetes may still be affected by contaminants that are absorbed in microplastics upon ingestion.

The mussels analyzed in this study contained microplastic fibers, with significantly more microplastics enumerated in farmed mussels compared to wild mussels. Possible reasons for this is may be due to the fact that farmed mussels were grown on polypropylene plastic lines that shed microplastics into the surrounding environment, or this trend could be due to differences in microplastic concentrations in the different locations from which the farmed mussels and wild mussels originated.

The 5-time extraction conducted on a sediment sample revealed that most microplastics were recovered from the 1st extraction, and lower concentrations of microplastics were recovered from subsequent extractions. From the 3-time control extraction using distilled water, it was discovered that the samples used in this study suffered some microplastic contamination from within the lab. Despite this contamination, trends in the data were still detectable. It is important to be aware of and mitigate laboratory microplastic contamination from within the lab by implementing a control sample with every set of samples subjected to H_2O_2 treatment, to better quantify this contamination.

Relevant future work regarding microplastics includes studying how different organic contaminants interact with different microplastic polymers, and more importantly, how they interact within vertebrates and invertebrates. Efforts must be established in the aquaculture industry to regulate the presence of microplastics in farmed organisms, as they are currently unregulated. Suggestions for microplastic regulations could include farming mussels on biodegradable lines to see if changing the net material influences microplastic concentrations within farmed mussels, as well as purifying mussels in seawater before they are sold to the public.

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References

Betts, K., 2008. Why small plastic particles may pose a big problem in the oceans. Environ. Sci. Technol. 42 (24) (pp. 8995–8995).
Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R.C., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45 (21), 9175–9179.
Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, M.D., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ. Sci. Technol. 42 (13), 5026–5031.
Browne, M.A., Galloway, T., Thompson, R.C., 2009. Microplastic—an emerging contaminant of potential concern? Integr. Environ. Assess. Manage. 3 (4), 559–561.
Browne, M.A., Galloway, T.S., Thompson, R.C., 2010. Spatial patterns of plastic debris along estuarine shorelines. Environ. Sci. Technol. 44 (9), 3404–3409.
Cauwenberghe, L.V., Claessens, M., Vandegehuchte, M.B., Mees, J., Janssen, C.R., 2013. Assessment of marine debris on the Belgian Continental Shelf. Mar. Pollut. Bull. 73, 161–169.
Claessens, M., Meester, S.D., Landuyt, L.V., Clerck, K.D., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62 (10), 2199–2204.
Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62 (12), 2588–2597.
Davidson, P., Asch, R.G., 2011. Plastic ingestion by mesopelagic fishes in the North Pacific Tropical Gyre. Mar. Ecol. Prog. Ser. 432, 173–180.
Dawson, M.N., Raskoff, K.A., Jacobs, D.K., 1998. Field preservation of marine invertebrate tissue for DNA analyses. Mol. Mar. Biol. Technol. 7 (2), 145–152.
Gouin, T., Roche, N., Lohmann, R., Hodges, G., 2011. A thermodynamic approach for assessing the environmental exposure of chemicals absorbed to microplastic. Environ. Sci. Technol. 45, 1466–1472.
Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46 (6), 3060–3075.
Hopewell, J., Dvorak, R., Kosior, E., 2009. Plastics recycling: challenges and opportunities. Philos. Trans. R. Soc. B 364 (1526), 2115–2126.
Fossi, M.C., Panti, C., Guerranti, C., Coppola, D., Giannetti, M., Marsili, L., Minutoli, R., 2012. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (*Balaenoptera physalus*). Mar. Pollut. Bull. 64 (11), 2374–2379.
Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P., Laforsch, C., 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnol. Oceanogr. Meth. 10, 524–537.
Koelmans, A.A., Besseling, E., Wegner, A., Foekema, E.M., 2013. Plastic as a carrier of POPs to aquatic organisms: a model analysis. Environ. Sci. Technol. 47, 7812–7820.
Law, B.A., Milligan, T.G., Hill, P.S., Newgard, J., Wheatcroft, R.A., Wiberg, P.L., 2013. Flocculation on a muddy intertidal flat in Willapa Bay, Washington, Part I: A regional survey of the grain size of surficial sediments. Cont. Shelf Res..
Liebezeit, G., Dubaish, F., 2012. Microplastics in beaches of the East Frisian Islands Spiekeroog and Katchelotplate. Bull. Environ. Contam. Toxicol. 89 (1), 213–217.
Lithner, D., Larsson, A., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409 (18), 3309–3324.
Lusher, A.L., McHugh, M., Thompson, R.C., 2012. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67 (1–2), 94–99.
Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminum, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ. Sci. Technol. 35 (2), 318–324.
Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41 (22), 7759–7764.
Teuten, E.L., Saquing, J.M., Knappe, D.R., Barlaz, M.A., Jonsson, S., Bj€orn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., et al., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. 364, 2027–2045.
Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 838, 304.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.