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Intertidal Concentrations of Microplastics and Their Influence on Ammonium Cycling as Related to the Shellfish Industry

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Abstract Microplastics are ubiquitous within the marine environment. The last 10 years have seen research directed at understanding the fate and effect of microplastics within the marine environment; however, no studies have yet addressed how concentrations of these particles could affect sedimentary processes such as nutrient cycling. Herein we first determine the concentration and spatial distribution of microplastics within Baynes Sound, a key shellfish-growing area within coastal British Columbia (BC). We also determined sediment grain size and % organic matter (OM) such that we could relate spatial patterns in sediment microplastic concentrations to sedimentary processes that determine zones of accretion and erosion. Using field-determined concentrations of microplastics, we applied laboratory microcosms studies, which manipulated sediment concentrations of microplastics, OM, and bivalves to determine the influence of sediment microplastics on ammonium cycling within intertidal sediments. Concentrations of microplastics determined within the intertidal sediment varied spatially and were similar to those found in other coastal regions of high urban use. Concentrations were independent of grain size and OM suggesting that physical processes other than those that govern natural sediment components determine the fate of microplastics within sediments. Under laboratory conditions, concentrations of ammonium were significantly greater in the overlying water of treatments with microplastics, clams, and OM compared with treatments without microplastics. These preliminary studies suggest that high concentrations of microplastics have the potential to alter key sedimentary processes such as ammonium flux. This could have serious implications, for example, contributing to eutrophication events in regions of the coast that are highly urbanized.

In 2004, the word "microplastics" was first introduced into the literature (Thompson et al. 2004). Ten years since has seen a growing body of literature showing the real threat these particles pose to marine ecosystems and those species including humans that rely on these ecosystems for their services and goods. Microplastics are considered to be <5 mm in diameter; however, the diversity of the types of microplastics, from fibers to spheres, makes it difficult to exactly quantify this contaminant by size alone. Current research has shown that microplastics occur in oceans worldwide and are ingested by marine species thereby providing a possible route for the entry of persistent organic pollutants, which adhere to the microplastics, into marine food webs (Andrady 2011). More recently, Ivar do Sul and Costa (2014) reported that microplastics can transport pollutants over large ocean areas and can contaminate marine biota when ingested. Perhaps of greater concern are the findings of Van Cauwenberghe and Janssen (2014) who determined that *Mytilus edulis* (the blue mussel) and *Crassostrea gigas* (Pacific oyster) contained on average 0.35 and 0.47 microplastics g ww^{-1}, respectively, which translates into an annual dietary exposure for European shellfish consumers of 11,000 microplastics/year. In a similar study, Mathalon and Hill (2014) determined that farmed mussels sampled from the Halifax Harbor, Nova Scotia, contained approximately 75 microplastics/mussel or 10 microplastics g ww^{-1} [assuming a mussel meat wet weight (ww) of 7 g] (Karayucel et al. 2010), which would translate into the

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ingestion of approximately 1700 microplastics/24 mussels (average restaurant serving).

Not yet studied is how microplastics influence key ecosystem processes such as nutrient cycling within surface sediments. Concentrations of microplastics that have been reported recently for coastal sediments include 390, 2000–8000, and 2175–6725 particles kg^{-1} of sediment for Belgium (Claessens et al. 2013), Nova Scotia (Mathalon and Hill 2014), and Lagoon of Venice, Italy (Vianello et al. 2013) coastal sediments, respectively.

Coastal zones are the most valued of earth ecosystems as a result of their role in the storage and cycling of nitrogen and phosphorus (Jickell 1998). Within the intertidal, bivalves, such as the Manila (*Venerupis philippinarum*) and varnish clam (*Nuttallia obscurata*), have been shown to be key agents in influencing the behaviour of nitrogen and phosphorus. Through both filter feeding from the overlying water column as well as deposit feeding, these ecosystem engineers serve to link overlying water and sedimentary processes, i.e., benthic–pelagic coupling providing nutrients to sediments directly through excretion or through biodeposits. Traditionally, sediments have been described by grain size, percent organic matter (OM), oxides, and manganese and iron with these major sedimentary components being key abiotic factors in influencing sediment processes such as nutrient cycling (e.g., Bendell et al. 2010) and metal availability to invertebrates (e.g., Bendell et al. 2002). Given the high concentrations of plastics that now occur within coastal sediments and the potential for these particulates to influence geochemical processes, sediments now must be viewed as being comprised not only of the classic geochemical parameters but also microplastics, which now make up a significant part of sediment. Indeed Holmes et al. (2014) has noted that microplastics should be regarded as a component of the suspended load of estuaries the role of which on contaminant transport requires further study.

Microplastics have been characterised into two groups: primary [plastics originally <5 mm, e.g., polyethylene microbeads used in cosmetic scrubs and pellets used to remove boat rust (Cole et al. 2011)] and secondary [microplastics whose origin is the degradation of larger plastic items, e.g., ropes used for shellfish aquaculture purposes such as antipredator netting, two-strand polypropylene rope (e.g., "oyster blue", and plastic trays and bags)]. There are at least two ways that the presence of microplastics could influence sedimentary geochemical processes: (1) increasing sediment porosity by the addition of "inert" sediment components thereby increasing rates of sediment nutrient flux; and (2) the presence of antibacterial compounds used in cosmetic scrubs could suppress sediment bacterial processes leading to altered cycles for nutrients, such as nitrogen, that are dependent on bacterial processes for conversion into biologically available forms.

Hence, the objectives of our study were twofold: (1) to determine the concentrations and spatial distribution of microplastics within Baynes Sound. Baynes Sound is home to British Columbia's (BC) shellfish industry with >50 % of production originating from this region; and (2) to assess the influence these particles have on the behaviour of ammonium within intertidal sediments through laboratory microcosms studies, in which sediment concentrations of microplastics are manipulated to concentrations determined in objective number 1. To our knowledge this is one of the first studies that has addressed the role of microplastics in altering nutrient fluxes within the intertidal sediments of coastal ecosystems.

Materials and Methods

Study Site

Sediment sampling took place June 12 through 14, 2014, at extreme low tide within Baynes Sound, BC. Shellfish farming within Baynes Sound dates back to the 1940s and is now home to 50 % of the provinces' shellfish industry with Manila clams and Pacific oysters being the main product farmed (Bendell et al. 2014b; Fig. 1a).

Concentrations and Distribution of Microplastics Within Baynes Sound: Field Sampling

Sediment Sampling

We sampled a total of 14 sites: 5 along the east coast of Vancouver Island, 7 along the west coast, and 2 along the east coast of Denman Island (Fig. 1b). Three cores at sites 1–5 and 1 core at sites 6–14 were taken at extreme low tide for microplastic analysis. At each site a corresponding core was also collected for grain size and OM content. A sediment corer (40-cm height and 6-cm internal diameter) was inserted to the maximum depth possible at each site (the deepest being 23 cm at Henry Bay) given the presence of underlying bed rock. Retrieved cores were sectioned into 1- to 2-cm sections in the field, placed into prelabeled zip-close bags, and placed in coolers for transportation back to the laboratory. Sediments were frozen until required for analysis.

Grain Size and Organic Matter

Sediments collected for grain size analysis were sieved through sequential sieves to separate out sediments based on sediment size >2, 2–0.25, 0.25–0.063, and <0.063 mm. OM on a dry-weight basis was determined on approximately 1 g of sediment by loss on ignition (500–550 °C for 10–14 h).

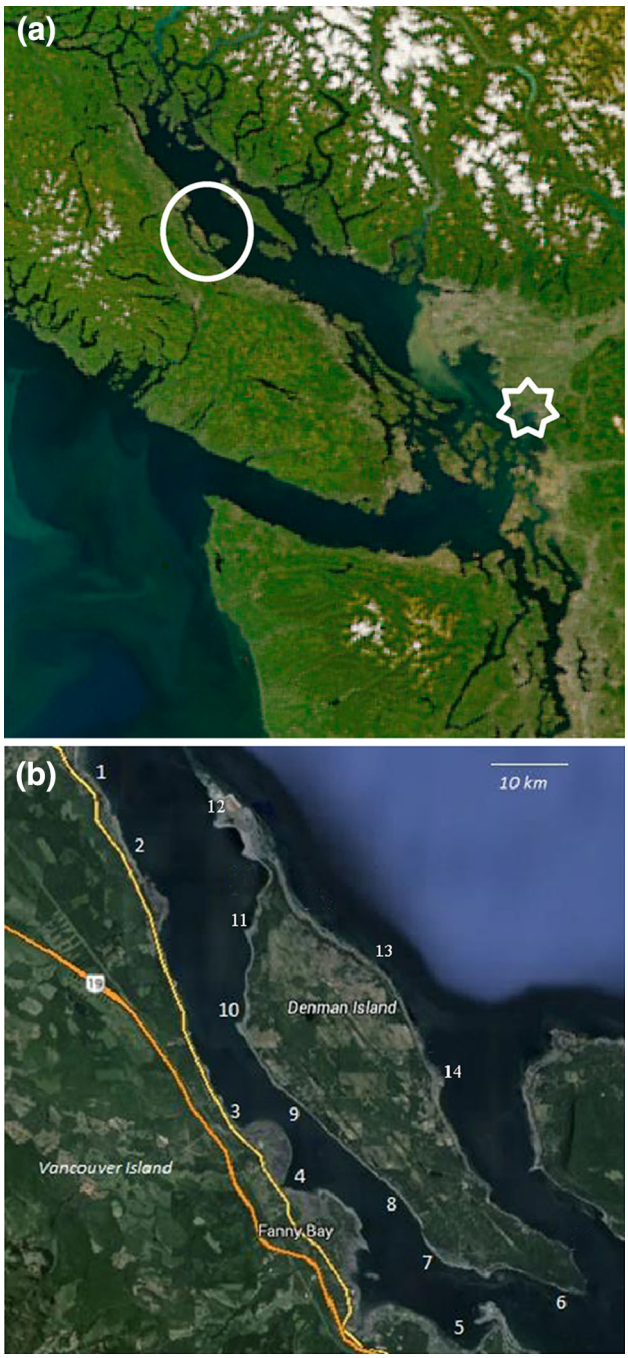

Fig. 1 Location of the study sites. **a** Salish Sea located on the west coast of BC and **b** Baynes Sound with the location of the 14 study sites. *Circled area* is Baynes Sound and Lambert Channel. *Asterisk* Vancouver, BC

Microplastic Recovery

Sectioned sediment was first sieved through a 1 mm aperture sieve to facilitate the recovery of all plastics >1–5 mm in diameter. Sieved sediments were then subjected to the "overflow/flotation" method (Claessens et al. 2013; Vianello et al. 2013; Mathalon and Hill 2014; Nuelle et al. 2014). Between 10 and 100 g of sediment was added to a concentrated sodium chloride solution (300 g L^{-1}) to enable low-density particulates, such as plastics, to separate from the sediment. The sediment–sodium chloride solution was stirred with microplastics decanted at 5 and 30 min. The procedure was repeated three times to ensure the collection of all plastics. To determine the efficiency of the floatation method, five microplastic beads were introduced into 30 g of plastic-free sediments originating from the previously treated sediment. The flotation method in triplicate was then applied as performed for field-collected sediments resulting in a 100 % recovery of the introduced beads. Recovered microplastics were identified at 10× to 12× magnification by microscope.

Influence of Microplastics on Ammonium Cycling Within Intertidal Sediments: Microcosm Studies

We used a microcosm approach based on that of Bendell et al. (2014a, b) to determine the influence of microplastics on ammonium within intertidal sediments. Six aquaria (12-cm long × 6-cm wide × 8-cm depth), three treatments, and three controls were prepared with 775 g of sediment collected from Fillongley Park [site 14 (Fig. 1b), Lambert Channel] and 400 mL of seawater. Microcosms were established for 3 days before manipulation and were constantly aerated throughout the duration of the experiment.

We used microbeads separated from facial scrub (Clearasil Ultra, Rapid Action Scrub) for the manipulations. Based on the product-listed ingredients, microbeads were comprised of polyethylene. The facial scrub was sieved through a 33-μm sieve to retain only the larger particles. Three of the six microcosms had microbeads added to them at the average concentrations found in the field sampling, i.e., 0.045 particles g (ww) sediment^{-1}. Before addition to the sediment, the beads were thoroughly rinsed with distilled deionized water with the intent to remove antibacterial properties.

Three sequential experiments were performed under artificially induced tidal conditions (Fig. 2). In the first set of experiments, only seawater and sediments were present in the microcosms. For the second set, we added OM to the sediment [2 g (dry weight) *Chlorella*]. In the final set of experiments, three Manila clams were added to each microcosm. Clams were purchased from a local seafood outlet and at time of purchase were 24 h postharvest. There were significant accumulations of ammonium in the water of sediment in the treatments with microplastics + OM + clam, which resulted in clam mortality on day 3 of the experiment. The dead clams were removed and fresh clams added for the remainder of the experiment. Tidal conditions were achieved according to the methods of Bendell et al. (2014a). Microcosms were "flooded" with seawater for

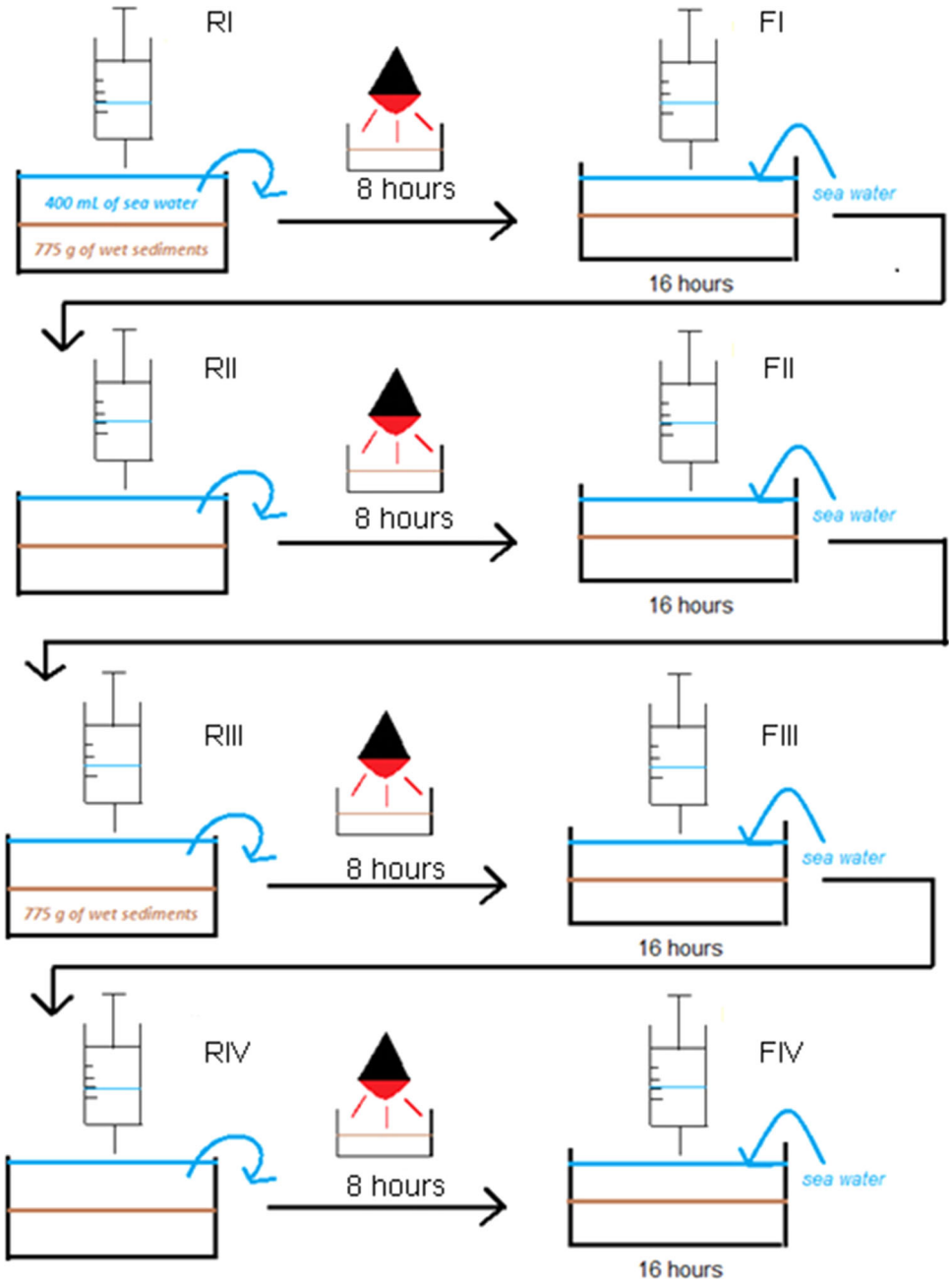

Fig. 2 Experimental protocol for the microcosm experiments

16 h, after which the seawater was removed by syringe and the microcosm then placed under a heat lamp to mimic warming conditions of the surface sediment at ebb tide. Sediment temperature reached 28–30 °C, which are values recorded under field conditions (Bendell et al. 2014a). Three millilitres of pore water and 45 mL of seawater were removed by syringe after 16 h of "inundation" just before removal of the water (R1, RII, RIII, and RIV) and just after the addition of fresh seawater to mimic "flood tide" conditions (FI, FII, FIII, FIV) (Fig. 2). Samples were stored frozen until ammonium analysis by fluorometry by way of an Aquafluor (Holmes et al. 1999).

Data Analysis

Software used to analyze the data included SigmaPlot12 (Systat), and PRIMER v6 (Clarke and Gorley 2006). Statistical significance was accepted at $P < 0.05$.

Organic Matter and Grain Size

The distribution of OM within Baynes Sound was determined by plotting % OM as a function of core depth and site. Data were plotted as sites 1–5 (east side of Vancouver Island), 6–12 (west side of Denman Island, and 13 and 14

(east side of Denman Island). Given the colinear nature of % grain size, we applied principle component analysis (PCA) on arcsine (% square root)—transformed data to determine grain size distribution within Baynes Sound.

Microplastics

We sampled sediment cores for plastics both spatially (site) and by sediment depth. However, the distribution of microplastics within the core was highly variable occurring in some but not all of the 2 cm-sectioned sediments. Therefore, to determine the spatial distribution of microplastics within the sound, we calculated the total weight of sediment within each core summing the dry weight of each section and summing all microplastics recovered within each core section to express the recovery as number of microplastics kg dry weight/sediment for each site. Because data failed the Shapiro–Wilk test for normality ($P < 0.05$), we applied one-way Kruskall–Wallis ANOVA on ranks on the number of microplastics kg dry weight/sediment recovered from sites 1 to 5 (east Vancouver Island), 6 to 8 (south west Denman Island), 9 to 12 (north west Denman Island), and 13 to 14 (east Denman Island). We applied Dunn's method of multiple-comparison procedure to determine the groups that differed from each other.

Microcosm Studies

Ammonium pore water and water column concentrations from the laboratory experiments passed the Shapiro–Wilk test for normality ($P > 0.05$); hence, we applied two way-ANOVA's general linear model (with no interactions) to determine if treatment (with or without plastics) or tide had an effect on water or pore water ammonium concentrations for each of the three experiments, i.e., sediment alone, sediment + OM, and sediment + OM + clams. Tide and treatment had effects for ammonium in the overlying water column; however, no effect was detected for ammonium in pore waters ($P > 0.05$). Hence, for pore water ammonium, all data were pooled across simulated tides, and Student's *t* test between treatments (sediment with plastics vs. sediment without plastics) was applied for the three experiments: sediment alone, for sediment + OM, and for sediment + OM + clams.

Results

OM and Grain Size

Three-dimensional profiling of % OM showed very distinct depositional patterns that were both site and depth dependent (Fig. 3). High concentrations were observed at site 5 at 4-cm depth, sites 8 through 10 at 8-cm depth, and throughout site 13. Depositional patterns determined at sites 8 through 10 suggest a historical source, whereas for site 13 high concentrations occurred throughout the core. Grain size analysis also separated the sites based primarily on particle sizes <2 mm and between 0.25 and 0.063 mm with the first PC accounting for 63.5 % of the variation (Fig. 4a). Sites 2, 9, and 14 were characterised by the

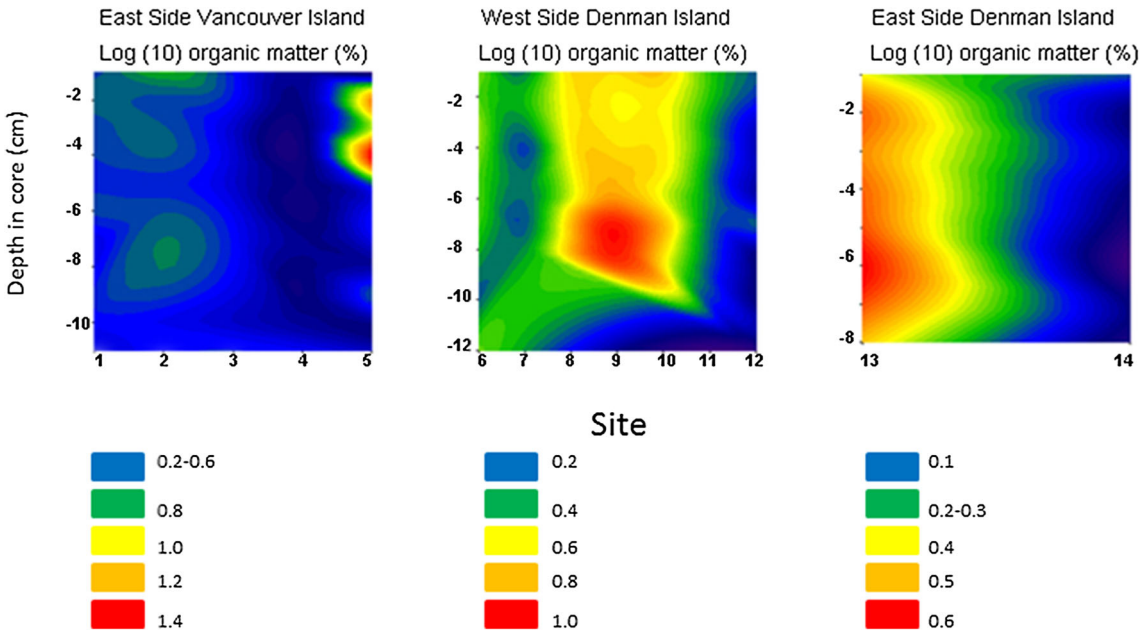

Fig. 3 Distribution of OM within Baynes Sound

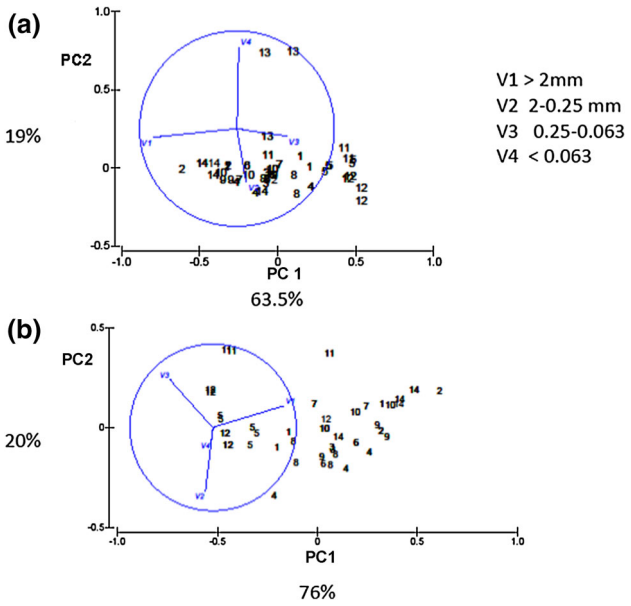

Fig. 4 PCAs of grain size for the 14 sites. **a** PCA with all sites. **b** PCA with site 13 removed

2-mm size fraction, whereas sites 1, 5, 8, 11, and 12 were characterised by a grain size between 0.25 and 0.063 mm. In agreement with the high concentration of OM found at site 13, this site also separated from all sites based on the <0.063-mm size fraction with the second PC accounting for 19 % of the variation in grain size among the 14 sites (Fig. 4a). A second PCA with site 13 removed separated sites 14, 10, 9, 8, 7, and 6 from all other sites based on the >2-mm grain size with the first PC accounting for 76 % of the variation (Fig. 4b). Site 11 separated based on the 0.2- to 0.063-mm grain size with the second PC accounting for 20 % of the overall variation (Fig. 4b).

Distribution of Microplastics

Several kinds of microplastics were recovered from sediments within Baynes Sound. Fibres and pieces of transparent plastics were positively identified, notably the presence of blue rope (Oyster Blue), which is used extensively by the oyster-farming industry (Fig. 5). The distribution of microplastics was spatially dependent (Fig. 6a) with greater numbers being recovered from the northeast end of Denman Island (i.e., sites 9 through 12) compared with all other sites ($P < 0.05$, Kruskall–Wallis one-way ANOVA on ranks; Fig. 6b). Median number of particles kg^{-1} dry weight of sediment for the four regions within Baynes Sound were 24.6, 33.8, 85, and 0 for east Vancouver Island, west Denman Island, northeast Denman Island, and southeast Denman Island, respectively.

Influence of Microplastics on Ammonium Cycling: Microcosm Studies

Ammonium concentrations in the overlying water column were tide dependent for the experiments comprising sediment only and sediment + OM and were treatment dependent for the treatment comprising sediment + OM + clam treatment (Fig. 7a–c; Table 1). No effect was detected for ammonium in pore waters (Fig. 8a–c). Pooled samples (i.e., tide effect removed) indicated that pore water ammonium in the sediment-only experiment significantly differed ($P < 0.05$; Student's t test) with greater concentrations being recovered from sediments with microbeads present compared with sediment without microbeads (Fig. 9).

Discussion

The accumulation of plastic debris within our marine environment is now recognized as being one of the greatest anthropogenic threats facing our ocean ecosystems (Ivar do Sul and Costa 2014). A review of 101 peered-reviewed articles concluded that all marine groups are at eminent risk of interacting with microplastics (Ivar do Sul and Costa 2014). Reviewed studies were grouped into four broad categories: (1) plankton samples, (2) sandy and muddy sediments, (3) vertebrates and invertebrate ingestion, and (4) chemical pollutant interactions. Here, we present the concentrations and distribution of plastics within Baynes Sound, a region that has experienced shellfish aquaculture since the 1940s. We also assessed the possible influence of microplastics on

Fig. 5 Polypropylene rope (*oyster blue*) and an example of microplastics recovered from sediment sampled from Baynes Sound (Color figure online)

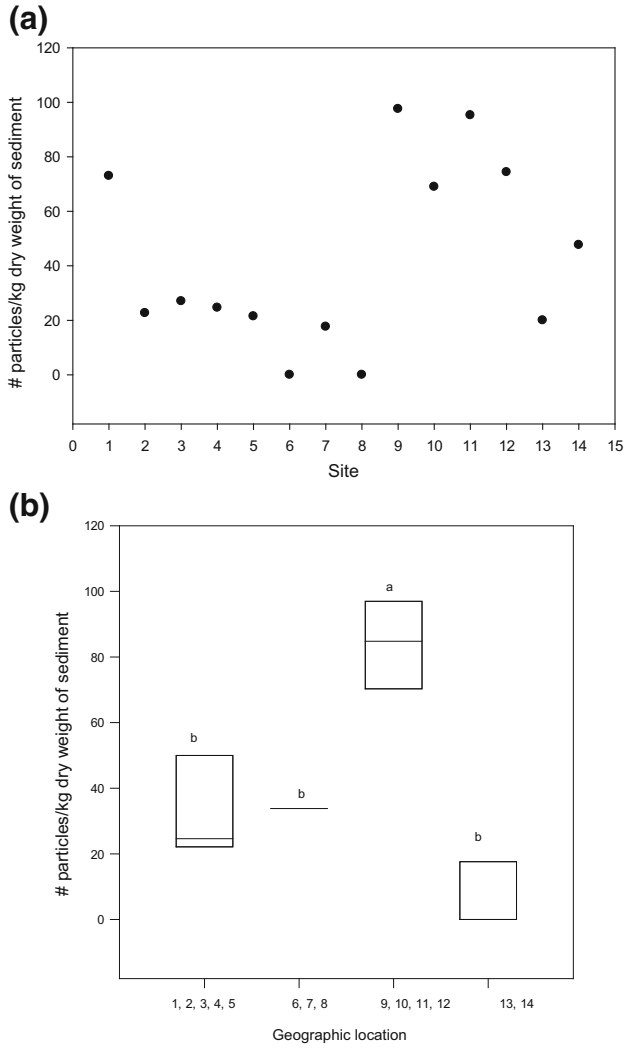

Fig. 6 Distributions of microplastics within the sound **a** by site and **b** by region. Values are ranges and medians. Kruskall–Wallis ANOVA on ranks on number of microplastics kg dry weight/sediment recovered from sites 1 to 5 (east Vancouver Island), 6 to 8 (southwest Denman Island), 9 to 12 (northwest Denman Island), and 13 to 14 (east Denman Island) indicates that sites 9 to 12 are significantly different from all others ($P < 0.05$; Dunn's method of multiple comparison)

the cycling of ammonium within intertidal sediment, and, to our knowledge, this is the first study to do so.

Distribution of Microplastics Within Baynes Sound

The overall average of microplastics determined in the intertidal sediments of Baynes Sound was 0.045 particles g (ww) sediment^{-1} or 76,500 particles m^{-3} [assuming a sediment density of 1.7 g cm^3 (Tenzer and Gladkikh 2014)]. These values are similar to those reported for coastal sediments elsewhere in the world (Ivar do Sul and

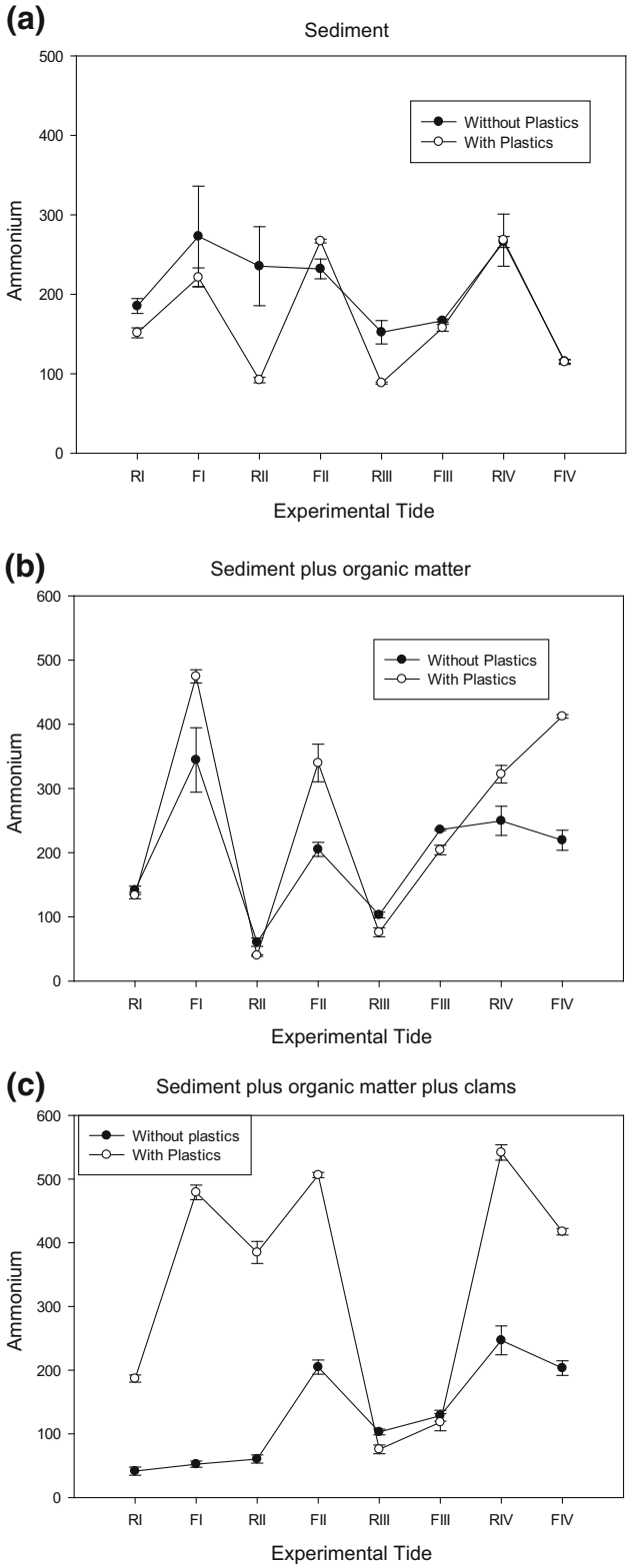

Fig. 7 Ammonium concentrations (μg L^{-1}) in overlying waters of the three microcosm experiments. **a** Sediment only. **b** Sediment + OM. **c** Sediment + OM + clams. Values are averages of $n = 3$ with SE

Table 1 Results of two-way ANOVA Kruskall–Wallace for overlying water ammonium concentrations for the three microcosm experiments

Factor	*F*	*P*
Sediment only		
Treatment	2.93	0.13
Tide	4.78	0.028
Sediment + OM		
Treatment	3.10	0.122
Tide	7.58	0.008
Sediment + OM + clams		
Treatment	13.21	0.008
Tide	2.056	0.181

Costa 2014) but lower than those recovered by Mathalon and Hill (2014). The distribution of the microplastics was region dependent with the greater number recovered from the northeast side of Denman Island. As also noted by Mathalon and Hill (2014), who found no relationship between microplastic concentrations and grain size, the region where the greatest number of particles were found (northeast Denman Island) coincided with those sites characterized by sediments of the largest grain size (2 mm; sites 9 though 11). Depositional zones with the smallest size fractions and greatest amounts of OM (sites 5 and 13) did not have the expected greater number of microplastics.

How microplastics physically interact with the sedimentary abiotic environment has yet to be understood. Given their highly diverse shapes and various physical properties, such as density and surface area, predicting where they will ultimately settle out within the intertidal sediment will be challenging. A general pattern would be expected based on tidal and wave energy where regions of high energy would be less prone to microplastics accumulation compared with those with low energy. Wave/tide energy could in part be the reason of no recovery of microplastics from sites 7 and 8, which are more prone to tidal and wave action compared with samples from other sites Mathalon and Hill (2014) also found that high microplastic concentrations were observed in protected tidal mud flats. These investigators further suggested that microplastics can become associated with microbial films, thus decreasing their ability to be washed from the tidal flat with the tides. Clearly, much study is needed on the physical fate of microplastics within intertidal sediments.

Influence of Microplastics on Cycling of Ammonium Within Intertidal Sediments

Microcosm manipulations indicated that the presence of microplastics (as microbeads) could have a profound effect

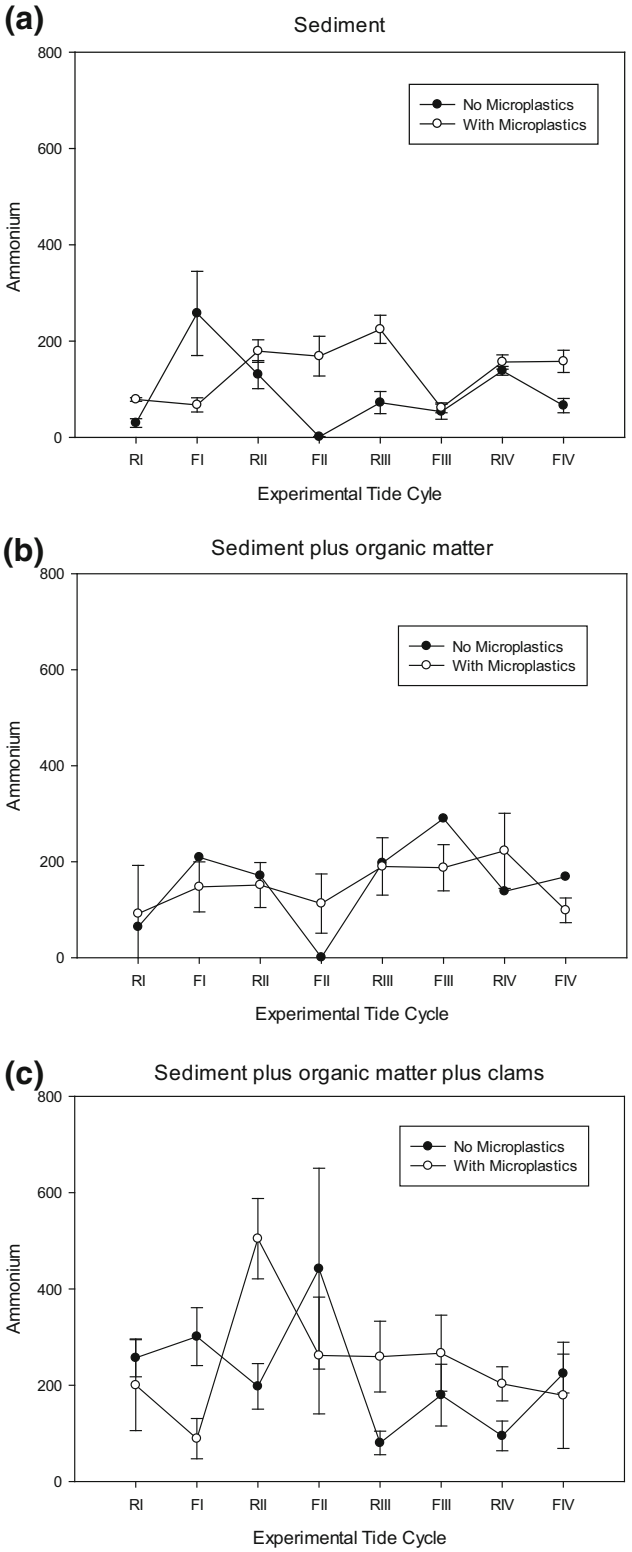

Fig. 8 Ammonium concentrations (μg L^{-1}) in pore waters of the three microcosm experiments. **a** Sediment only. **b** Sediment + OM. **c** Sediment + OM + clams. Values are averages of $n = 3$ with SE

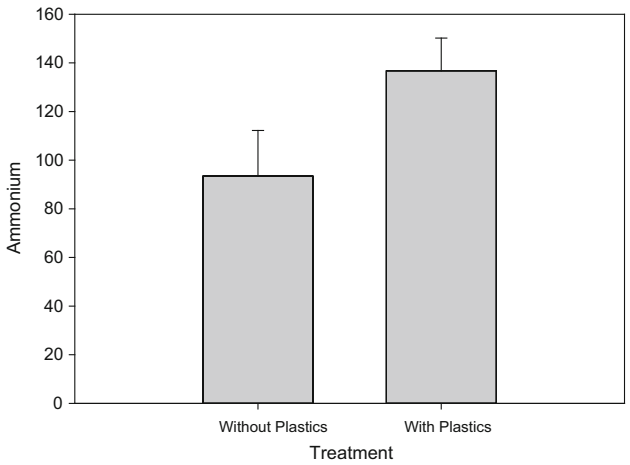

Fig. 9 Overall ammonium concentrations (μg L^{-1}) in sediment-only treatments with and without microplastics

on the cycling of ammonium within the intertidal. Simulated tide events also influenced the amounts of ammonium recovered in the surface water but not in pore water. The greatest amounts of ammonium in the overlying water were recovered after simulated "flood tide", i.e., after the 8-h exposure period. Bendell et al. (2014a) reported similar findings for ammonium sampled from intertidal sediments in which flood tides generally contained greater amounts of ammonium within interstitial waters compared with ebb tides. Bendell et al. (2014a) concluded that ammonium accumulated within the sediment during low tide is discharged to coastal seas as a "pulse" on flood tides. Findings of the current study support this conclusion as well.

Microcosms that were populated by bivalves rapidly accumulated ammonium within the overlying water compared with those without microplastics. Pore water ammonium concentrations also appeared to be influenced with greater recovery of ammonium in sediments with microplastics compared with those without. Ammonium cycling within sediments is bacteria dependent. Bivalves excrete ammonium directly as inorganic ammonium through urine, the composition of which can range from 60 to 100 % ammonium depending on the species of bivalve, and indirectly as organic ammonium (feces and pseudofeces) (Bendell et al. 2014a). The pool of ammonium within the sediment and overlying water will be determined by the rate of ammonification of the organic pool and the rate of nitrification of the inorganic pool. Ammonification will increase the pool, and nitrification will decrease the pool. Hence, accumulations of ammonium in pore water and overlying waters suggest that the bacterial process by which ammonium is removed through denitrification has been interrupted. A similar observation was reported by Bendell et al. (2014a) in their studies in which microcosms containing bivalves and sterile sediments had greater accumulations of ammonium in the overlying water compared with those with bivalves and unsterilized sediments.

The accumulations of ammonium in the microplastic-treated sediments could be a result of the combination of increased sediment porosity facilitating the fluxes of ammonium to pore waters and the overlying water (physical processes) and antibacterial properties of the microbeads suppressing bacterial processes (biological processes) required for the normal functioning of the nitrogen cycle. Carson et al. (2011) reported that the physical presence of small plastic debris changed water movement and heat transfer though beach sediments by increasing sediment porosity. Although the microbeads were thoroughly cleansed before addition to the sediment, accumulations of ammonium within the water suggest that not all of the antibacterial properties were removed.

Summary and Conclusions

Concentrations of microplastics determined in sediments from Baynes Sound, a key shellfish-growing region in BC, were comparable with those found in other urbanized coastal regions. Recent research on the distribution of microplastics in the subsurface seawater of the Pacific Northwest found that concentrations ranged from 8 to 9200 m^{-3}, with concentrations of approximately 4000–5000 m^{-3}, of which 80 % were fibrous occurring within Baynes Sound (Desforges et al. 2014). That fact the shellfish industry is a major source of plastics to the marine environment has been shown by Denman Island community groups, who have been conducting beach clean-ups within Baynes Sound for 10 years. Each year, 3–4 tonnes of debris are collected, 90 % of which are plastics and Styrofoam originating from the shellfish industry (Bendell et al. 2014b). Examples of plastics collected include "Oyster Blue" plastic rope, which likely contributes to the high fibrous composition of the microplastics as determined by Desforges et al. (2014), plastic net shell bags, and plastic oyster pouches and baskets. Based on the findings of previous studies (e.g., Mathalon and Hill 2014), shellfish farmed from this region are likely contaminated by the presence of these microplastics within their tissues. Microcosm studies suggest that microplastics, notably microbeads, have the potential to significantly alter the cycling of key nutrients, such as ammonium, within intertidal sediments. This could have significant consequences in that increased amounts of ammonium within the water column can lead to eutrophication events and could serve to trigger red tides. Highly urbanized regions where untreated effluent is directly discharged into the coastal environment could be particularly at risk.

Our study represents only a preliminary study to address the role of microplastics in altering sediment geochemical fluxes. Controlled laboratory experiments performed at the microcosm scale can only provide direction as to what may be occurring under field conditions. Clearly this is an area well deserving of much more attention.

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