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A preliminary analysis of ingestion and egestion of microplastic fibres in the acorn barnacle *Balanus glandula*

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ABSTRACT

Microplastic contamination is a growing threat facing marine ecosystems, of which a prominent source is the fibres from synthetic clothing. Ingestion and egestion (excretion) of microfibres, and whether these have short-term effects on behaviours such as feeding rate, have yet to be studied in many organisms, especially non-bivalve filter feeders. To determine if a common, filter feeding, intertidal invertebrate can ingest microfibres, we studied the acorn barnacle (*Balanus glandula* Darwin 1854). We collected *B. glandula* from four locations near Bamfield, British Columbia, Canada, exposed half of them to a high concentration (~70,000 microfibres/L) of brightly coloured polyester microfibres for 24 h in unfiltered seawater (while the other half received a non-exposure treatment), and measured the feeding rates of the barnacles before and after the exposure. An average of 1.2 ± 1.9 fibres per barnacle were present in the gastrointestinal tracts of the plastic treatment group before depuration, and 0.3 ± 0.6 fibres per barnacle were found in the corresponding control group. Prior to depuration, 50% of the 20 barnacles in the plastic treatment ingested at least one microfibre, while a 15% ingestion rate was observed in the control group. There was no detectable short-term effect of microfibre ingestion on feeding rate. A 48-h post plastic exposure depuration period was used to evaluate whether microplastics were egested. No difference in egestion was found between those assessed directly after exposure and those that underwent depuration. Furthermore, a low depuration rate of 0.05 microfibres per 48 h suggests that barnacles may require longer than 48 h to egest microfibres. If representative, these results indicate that acorn barnacles ingested few microfibres even when exposed at very high concentrations, which supports the idea that they are at low risk for microplastic contamination and would not be a suitable indicator species.

1. Introduction

Plastic pollution is a global concern, as the input of plastics into the environment and the associated impacts are growing steadily. An estimated 359 million metric tons of plastic were produced globally in 2018 (PlasticsEurope, 2019), and 4.8–12.7 million metric tons of plastic waste created by coastal countries is believed to have entered the marine environment in 2010 (Jambeck et al., 2015), with an order of magnitude increase predicted by 2025 if waste management infrastructure does not improve. Once plastics enter the marine environment, they fragment into smaller pieces that may persist for years to decades if floating at the surface (Julienne et al., 2019; Zhu et al., 2020), or for centuries if they are transported to depth and/or deposited into the sediment (Ward et al., 2019a). When these fragments are less than 5 mm in length along their longest dimension, they are defined as a microplastic (MP) and can be categorized as primary or secondary depending on whether they were produced at that size or have degraded from larger objects, respectively. Fragments, films, pellets (spheres) and fibres are prevalent MP shapes (GESAMP, 2019).

Plastic fibres, or microfibres (MFs), are pervasive in the environment and are often the most commonly occurring type of MPs found in seawater and marine biota (Barrows et al., 2018; Walkinshaw et al., 2020). They can be composed of a variety of synthetic polymers, including acrylic, polyethylene, polypropylene, polyamide (Nylon) and polyester (Browne et al., 2011). MFs are primarily generated by the shedding of fibres from textiles, especially during laundering (Henry

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et al., 2019; Mishra et al., 2019). A single garment can create more than 1900 fibres per wash, from materials including polyester fleece (Browne et al., 2011; Pirc et al., 2016; Sillanpää and Sainio, 2017).

Many marine animals are already known to consume MPs, including MFs, whether via direct ingestion, inhalation, or indirect digestion through trophic transfer (Zhang et al., 2019). The consumption of these particles may not currently be of concern for some animals at existing rates of exposure (Foley et al., 2018), but the effects of MPs and MFs less than 100 μm in length are still poorly understood. Effects on individuals, populations, and ecosystems are likely to become more evident as annual plastic production is continuing to increase exponentially. Furthermore, MPs represent a complex mixture of polymers, additives, and adsorbed chemicals, with additional properties including shape, size, and colour, that will potentially have species-specific effects on ingestion and egestion rates, as well as toxicity (Haegerbaeumer et al., 2019; Rochman et al., 2019). However, some of the most commonly occurring types of MPs, including polyester fibres, have been underrepresented in laboratory exposure studies (Carlos de Sá et al., 2018).

Small animals feeding at lower trophic levels are at the greatest risk of MP exposure (Walkinshaw et al., 2020), and are thus the most affected by the potential toxicological effects of MPs and associated chemicals (Foley et al., 2018). Potential fitness effects include reduction in feeding and reproductive success, oxidative stress, genotoxicity, neurotoxicity, and delayed growth (Carlos de Sá et al., 2018; Galloway et al., 2017). If organisms (e.g. fish or terrestrial isopods) can quickly egest the ingested MPs, however, they may not experience substantial fitness effects at environmentally relevant doses (Jovanović et al., 2018; Kokalj et al., 2018). Nonetheless, MFs (as compared to other shapes of MPs) may have an increased residence time in the gut of filter feeding organisms (Ward et al., 2019b), as well as have a greater potential for toxicity due to their shape and high surface-to-volume ratio, resulting in a higher rate of chemical leaching (Gray and Weinstein, 2017). If MFs or their associated contaminants can accumulate in the tissues and cells of these organisms, a potential pathway exists to higher trophic level organisms; since MP ingestion has been recorded in many organisms at a wide range of trophic levels, from sea urchins to whales, transfer through marine food chains is a pertinent ecological concern (Zhang et al., 2019).

To investigate the risk that MFs may pose to small, benthic marine invertebrates, we utilize a common, filter feeding invertebrate, *Balanus glandula* Darwin 1854, to examine whether they ingest MFs, whether ingestion of MFs affects feeding rate (cirral beats per minute), and whether the MFs ingested are retained or egested. *B. glandula,* a highly abundant, filter feeding, sessile invertebrate inhabiting the rocky intertidal shore of the northeast Pacific coast, is a common food source for other invertebrates and fish (e.g. predatory marine snails, striped seaperch *Embiotoca lateralis*, and pile perch *Rhacochilus vacca*), and functions to increase habitat complexity (Connell, 1970; Cruz Sueiro et al., 2011; Hueckel and Stayton, 1982; Navarrete et al., 2000). Their role in coastal food webs, abundance, and easily measurable feeding behaviour (the sweeping motion of the cirral fan), make *B. glandula* an ideal organism to study the effects of MFs on filter feeding behaviour.

We hypothesized that *B. glandula* would ingest MFs as has been reported in filter feeding bivalves (Covernton et al., 2019; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014), as well as other species of barnacles (Goldstein and Goodwin, 2013; Xu et al., 2020). We also hypothesized that feeding rate (cirral beats/min) would decrease with MF ingestion, as barnacles might suffer negative short-term fitness effects from the plastic fibres. Additionally, we hypothesized that the majority of MFs would be egested after a 48-h depuration period, as they are likely too large to be incorporated into barnacle tissues. This study has broader implications as to the effects of MFs on marine organisms, the ingestion of MPs by other filter feeders, the bioaccumulation potential of MPs and MFs to higher trophic level organisms, and the potential for barnacles to be an indicator species for MP contamination.

2. Materials and methods

2.1. Study sites and collection of animals

We collected *B. glandula* from four locations near Bamfield Marine Sciences Centre, Bamfield, British Columbia, Canada during the fall of 2017: Eagle Bay (48° 50.010 N, 125° 08.764 W), Aguilar Point (48° 50.186 N, 125° 08.586), Strawberry Point (48° 49.946 N, 125° 07.775 W), and the Bamfield Marine Sciences Centre (BMSC) foreshore (48° 50.120 N, 125° 08.185 W) (Fig. 1). The four sites were selected based on varying degrees of exposure to wave action (which may have potential to affect feeding rate), with Eagle Bay and Strawberry Point being the most protected, the BMSC foreshore being fairly protected, and Aguilar Point being the most exposed. We laid out a 10 m transect parallel to the waterline and randomly sampled two locations along the transect. If no appropriate barnacle rocks were at the location (i.e. a rock with at least 10 spaced-out barnacles on a single side), then the nearest suitable barnacle rock was selected. Transect heights ranged between 1.3 and 2.4 m above mean lower low water. A total of eight rocks with at least 10 live barnacles on a single side (to simplify counting during video analysis) were collected from the four study sites ($n = 8$). We selected ten live *B. glandula* individuals on each rock, with aperture lengths between 3 and 5 mm. The barnacles were selected to maximize the distance between individuals, with a minimum distance of 4 mm between apertures. We removed all other adjacent barnacles from the rock to reduce feeding competition and facilitate video analysis.

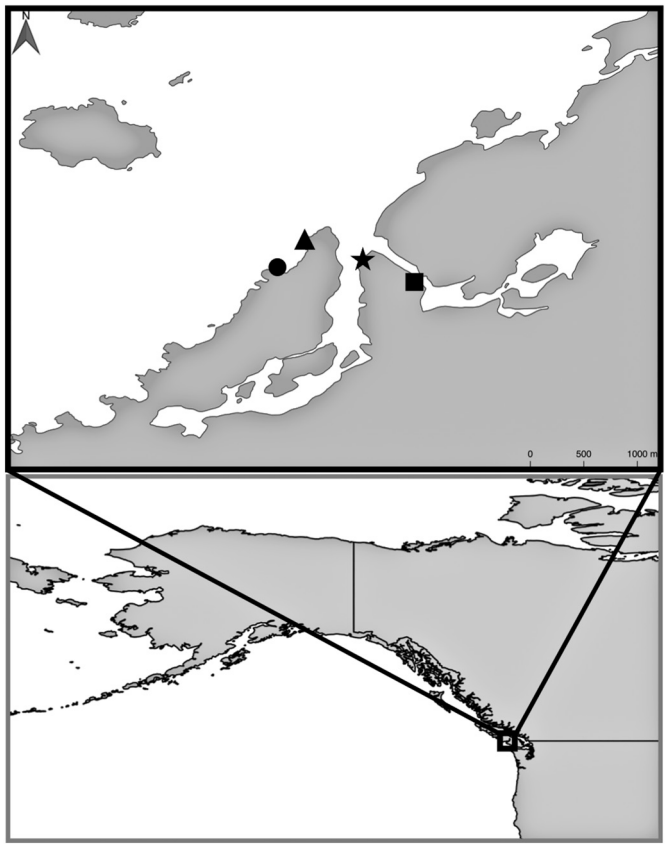

Fig. 1. Collection sites of rocks with *Balanus glandula* from four locations near Bamfield Marine Sciences Centre, British Columbia, Canada. Circle: Eagle Bay (48° 50.010 N, 125° 08.764 W), triangle: Aguilar Point (48° 50.186 N, 125° 08.586), star: the Bamfield Marine Sciences Centre foreshore (48° 50.120 N, 125° 08.185 W), and square: Strawberry Point (48° 49.946 N, 125° 07.775 W).

2.2. Experimental design

We placed eight glass aquaria (51 × 31 × 26 cm) side by side in two sea tables and filled each tank with 13 L of unfiltered seawater containing no additional planktonic food, maintained at 10.9–12.8 °C (Fig. 2). We secured a pump in the top left corner of each tank to circulate the seawater throughout the closed system and attached a clear plastic funnel (10 cm diameter) to the pump's outflow to better distribute the flow throughout the tank. The average flow rate in the tanks was 0.27 m/s. We randomly assigned study site and treatment (plastic or control) to each tank, totalling four plastic treatment tanks and four control tanks, and allowing for four experimental replicates. For the plastic treatment, we added brightly coloured, pink polyester plastic MFs (of unstandardized fibre lengths) obtained from a fleece blanket using a razor blade, at a concentration of 37 mg/L, to the tanks. Due to the ability of fibres to intertwine, a fibre count was difficult to attain, but was estimated to be approximately 70,000 fibres/L, based on the calculations of Pirc et al. (2016), who used a comparable polyester fabric in their study. Brightly coloured MFs are effective for MP studies as they remain easily distinguishable from other natural and plastic fibres (Browne et al., 2011). To reduce the risk of contamination, pink clothing was not worn at any point. We vacuum filtered (Whatman 11 μm cellulose filters) one litre of source seawater for use as a procedural blank and recorded the count of pink fibres present to ensure there was not already an abundance of pink MFs. No pink MFs were observed within the source water.

2.3. Feeding rate

To standardize hunger levels and increase the likelihood of feeding, we withheld food from the barnacles for 12 h prior to feeding trials by removing them from the seawater, then placed each barnacle rock in a flume (apparatus to recirculate seawater at a specified flow rate through a 90 × 15 × 14 cm tank). The barnacle rocks were centered in the flume in groups of one or two (depending on barnacle location and visibility on the rock), with unfiltered seawater pumped from an inlet adjacent to the BMSC, flowing at 0.27 m/s for 1 h. The flow was created from an external pump, and the average flow rate was measured by timing the distance travelled of a submerged object three times immediately prior to the beginning of the feeding trials. We secured a GoPro Hero 3+ above the flume and recorded the barnacles feeding for 1 min at the end of each 1-h trial. We assessed feeding rate by counting the number of cirral beats (sweeping motion of the cirri) of each barnacle in the 1-min videos. We then placed the rocks in their respective treatment aquaria for 24 h under an 12:12 h light-dark cycle. Following the treatments, we assessed feeding rate again in the flume using the same methods as above. Change in feeding rate was calculated for all 80 individuals by subtracting feeding rate before plastic exposure or control treatment from feeding rate after exposure, as cirral beats per minute.

2.4. Plastic ingestion/egestion

After the final feeding trial in the flume, we randomly selected five of the ten barnacles from each rock, which were removed and frozen in glass vials at −20 °C for later analysis of plastic consumption. The remaining five barnacles on each rock were placed in clean glass aquaria (51 × 31 × 26 cm) with closed-circulating seawater and an air stone for an additional 48-h depuration period, to examine whether the MFs remained inside the barnacles or were egested. The water was not changed during the depuration period. We then removed the remaining 40 barnacles from their rocks and euthanized them by freezing at −20 °C. All frozen barnacles were thawed and dissected to examine the number of pink MFs they ingested, by removing the gastrointestinal tract (stomach and intestines) of each frozen barnacle from the calcified plates. We counted all pink MFs (and excluded other coloured MFs) under a compound microscope (Olympus CX31) at 400× total magnification. The same two individuals processed each barnacle sample slowly and systematically, providing high certainty that most or all pink fibres were enumerated. Prior to this we observed the source fleece blanket MFs under the microscope to ensure accurate identification of the pink fibres. The depuration rate was calculated for both the plastic exposed and control groups by averaging each group's MF counts before depuration and subtracting the group's average MF count after depuration.

2.5. Statistical analysis

We used a linear mixed-effects model (LMM) to estimate the relationship between plastic ingestion and feeding rate. We examined the change in feeding rate per individual barnacle before and after 24 h of exposure to plastic. Each aquarium was treated as a random effect to avoid pseudo-replication. We used a generalized linear mixed-effect model (GLMM), assuming a Poisson error distribution, to examine fibre presence in barnacles before and after egestion. We again included each aquarium as a random effect. Data analysis was conducted using the R statistical program (R Core Team, 2016) and the lme4 and ggplot2 packages (Bates et al., 2015; Wickham, 2016).

3. Results

3.1. Plastic ingestion

Pink MFs were found in the gastrointestinal tracts of *B. glandula* (Fig. 3). The count of fibres per individual barnacle ranged from 0 to 9 for the plastic treatment and 0 to 2 for the non-exposure treatment (1.2 ± 1.9 and 1.2 ± 2.6 fibres before and after depuration, and 0.3 ± 0.6 and

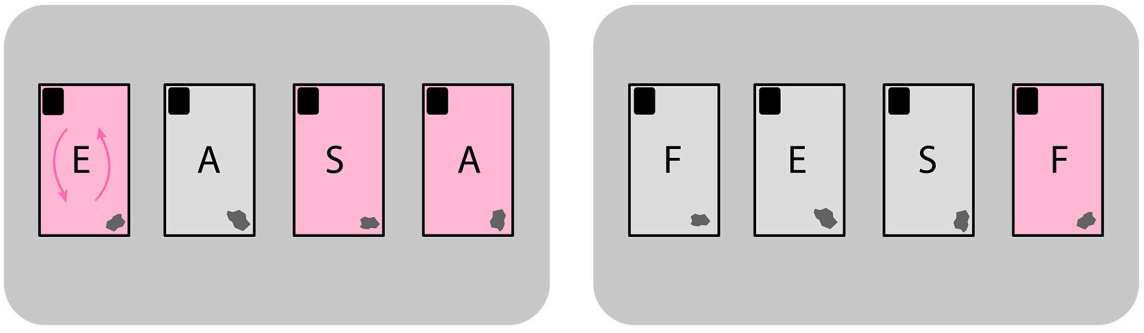

Fig. 2. Experimental tank setup showing the randomly assigned locations: Eagle Bay (E), Aguilar Point (A), Strawberry Point (S) and the Bamfield Marine Sciences Centre foreshore (F), and treatments: pink aquaria indicating tanks with the plastic treatment and grey aquaria indicating the control tanks. Pumps (black rectangles) in the upper left corners of each tank were fitted with funnels to disperse water flow (pink arrows). Each tank had a rock (grey object) with ten barnacles on it in the bottom right corner.

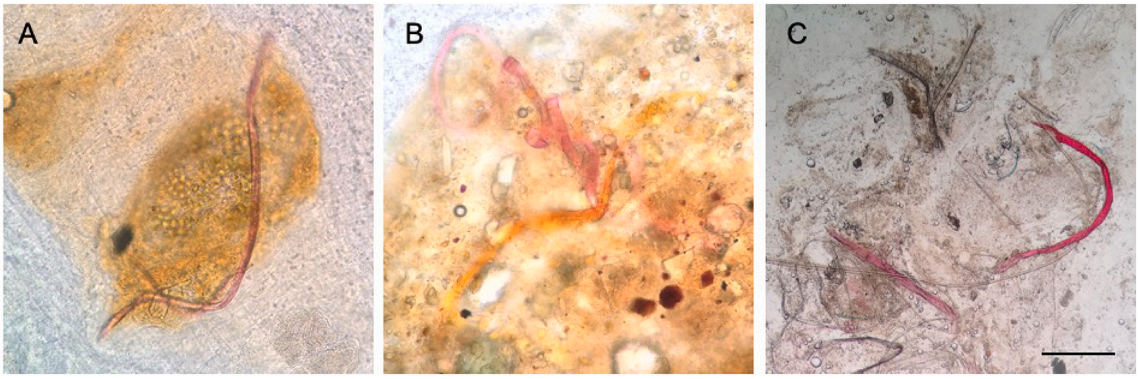

Fig. 3. Pink microfibres in the gastrointestinal tract of *Balanus glandula*, viewed at 400× total magnification on a compound microscope. (A) A single pink plastic microfibre, (B) a pink plastic microfibre and a yellow fibre, and (C) two pink plastic microfibres among smaller blue fibres. The scale bar represents 50 μm, and only experimentally-introduced pink microfibres were enumerated.

0.05 ± 0.2 fibres, average ± standard deviation, respectively). Of the 20 barnacles in the plastic treatment (before depuration), 50% ingested at least one pink fibre. Of the 20 barnacles in the corresponding control group (not exposed to MFs), 15% ingested pink fibres. For barnacles in the plastic treatment (before depuration), *B. glandula* individuals sampled from Eagle Bay had the highest total fibre count of 15 fibres (3 ± 3) for all the barnacles from that site; Aguilar Point, 4 fibres (0.8 ± 1.3); the BMSC foreshore, 4 fibres (0.8 ± 0.8); and Strawberry Point, 1 fibre (0.2 ± 0.4).

3.2. Feeding rate

There was no significant difference in the feeding rate of *B. glandula* after 24 h of plastic exposure (LMM, $t = 0.26$, $p = 0.81$; Fig. 4).

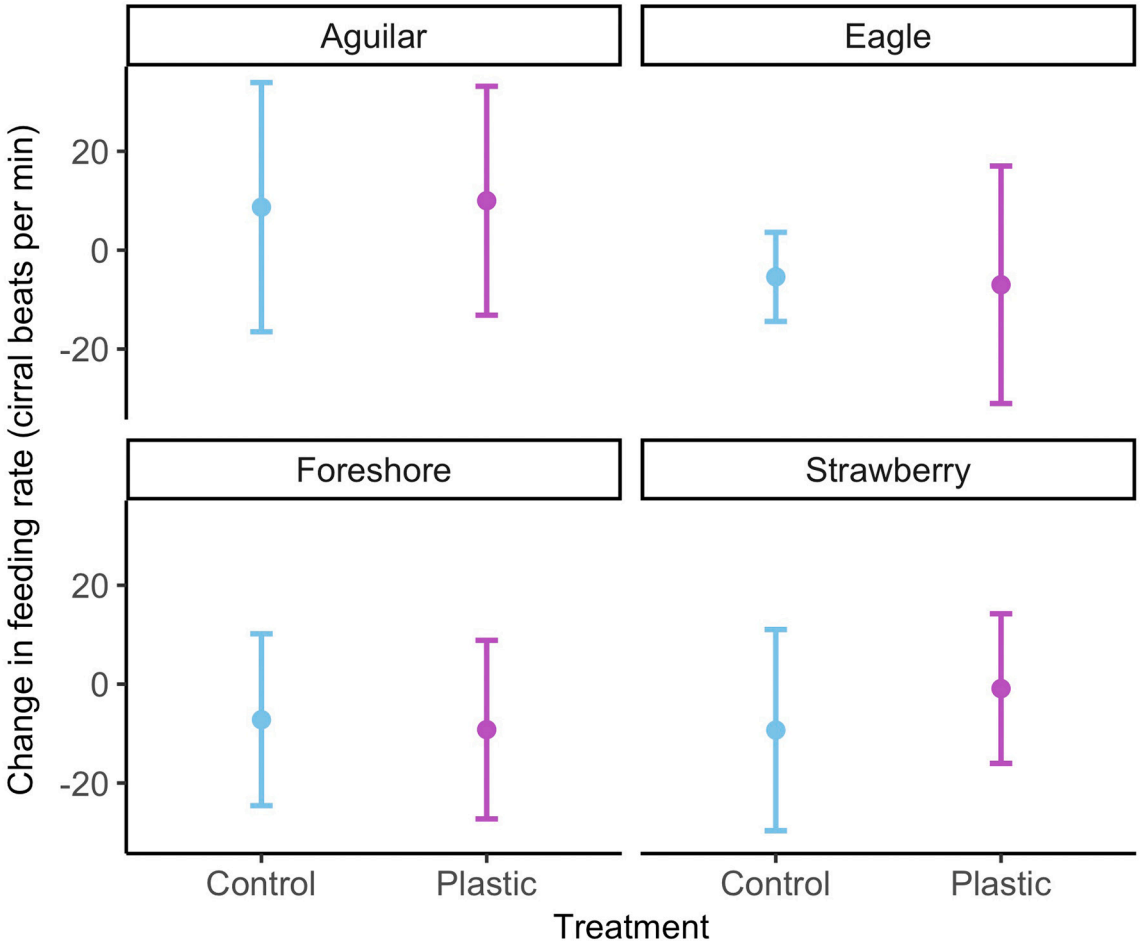

Fig. 4. The average change in feeding rate of *Balanus glandula* (calculated by subtracting feeding rate before plastic exposure or control treatment from feeding rate after, as cirral beats per minute) for the plastic treated (at ~70,000 microfibres per litre) and control groups at each collection location. The locations are intertidal sites near Bamfield, British Columbia, Canada: Aguilar Point, Eagle Bay, Bamfield Marine Sciences Centre foreshore, and Strawberry Point. Barnacles were exposed to microplastic fibres for 24 h and feeding rate was determined before and after this treatment at constant flow. The error bars represent standard deviation.

3.3. Plastic egestion

There were significantly more individuals with ingested pink MFs in the plastic treatment than in the control group, both before and after depuration trials (GLMM, $z = 2.37$, $p = 0.02$; Fig. 5); however, no significant difference was detected in the number of MFs in the group given 48 h to egest the plastics compared to the group that was assessed directly after exposure (GLMM, $z = 1.47$, $p = 0.14$; Fig. 5). Within the plastic treatment group, ten barnacles (50%) contained MFs immediately after plastic exposure, whereas only five barnacles (25%) contained MFs after the depuration trials. In the control group, three barnacles (15%) had MFs following plastic exposure, while one barnacle (5%) contained MFs after depuration. The average depuration rate for the plastic treatment was 0.05 MFs per 48 h, and for the control was 0.2 MFs per 48 h.

4. Discussion

The present study examined whether *B. glandula* ingest plastic MFs, if presence of MFs in the seawater affects their feeding rate, and whether the MFs are retained or egested over a 48-h period. The barnacles were exposed to varied length MFs at a concentration of 37 mg/L, which corresponds to approximately 70,000 fibres/L (Pirc et al., 2016). While this fibre count is an estimate, it can safely be considered a very high MP exposure compared to environmental concentrations in seawater, with recent average concentrations in the Northeast Pacific estimated to be 2.1 ± 2.2 particles/L (Desforges et al., 2014), ranging from 0 to 4 MPs/L (Covernton et al., 2019). Of the 20 barnacles in the plastic treatment (before depuration), 50% ingested MFs, and there was no detectable effect of MP ingestion on feeding rate. After the 48-h depuration trial following plastic exposure, the plastic treatment group did not statistically differ from the barnacles before depuration, suggesting that they may retain MFs.

We found that the average count of fibres ingested per plastic treated individual barnacle (before depuration) was 1.2 ± 1.9 fibres, and that 50% of the barnacles in the plastic treatment ($n = 20$) ingested at least one MF (to a maximum of nine MFs per barnacle). While barnacle species have largely been absent from the growing record of MP-ingesting organisms, a comparable study was conducted on gooseneck barnacles (*Lepas* spp.). Goldstein and Goodwin (2013) found that 33.5% of the gooseneck barnacles in the North Pacific Subtropical Gyre (an area of high marine plastic debris accumulation) ingested MPs (to a maximum of 30 MPs per individual), and concluded that MP ingestion by barnacles is likely a common occurrence. The discrepancy of maximum MP gut concentration between *B. glandula* (maximum of nine MPs) and *Lepas* spp. may be a function of the gut area (as *Lepas* spp. are larger in body size) and the much longer plastic exposure time (that occurred in situ rather than in a laboratory). There is a more extensive record of in situ MP ingestion in other filter feeding invertebrate organisms, namely bivalve molluscs (Covernton et al., 2019; Van Cauwenberghe and Janssen, 2014); for example, of Pacific oysters (*Magallana gigas*) collected from US fish markets, 33% were found to contain anthropogenic debris (plastic and textile fibres) in their guts (Rochman et al., 2015). However, while there is potential for balanoid barnacles to reject unsuitable food particles (Anderson, 1981; Geierman and Emlet, 2009), their feeding mechanisms differ from the highly selective ingestion behaviours of bivalves (Ward et al., 2019b). For this reason, bivalves are not effective bioindicators of MP pollution, which is not the case for all species of barnacles (Xu et al., 2020), and more research should be done on feeding selectivity in barnacles before relevant comparisons can be made with bivalves and other filter feeders. Due to the low number of average ingested fibres per barnacle, at such a high plastic exposure concentration, we do not recommend *B. glandula* as an indicator species for MP contamination. While determining MP occurrence in various species is an important starting point, it is critical to investigate how ingested MPs may impact the behaviour and fitness of animals.

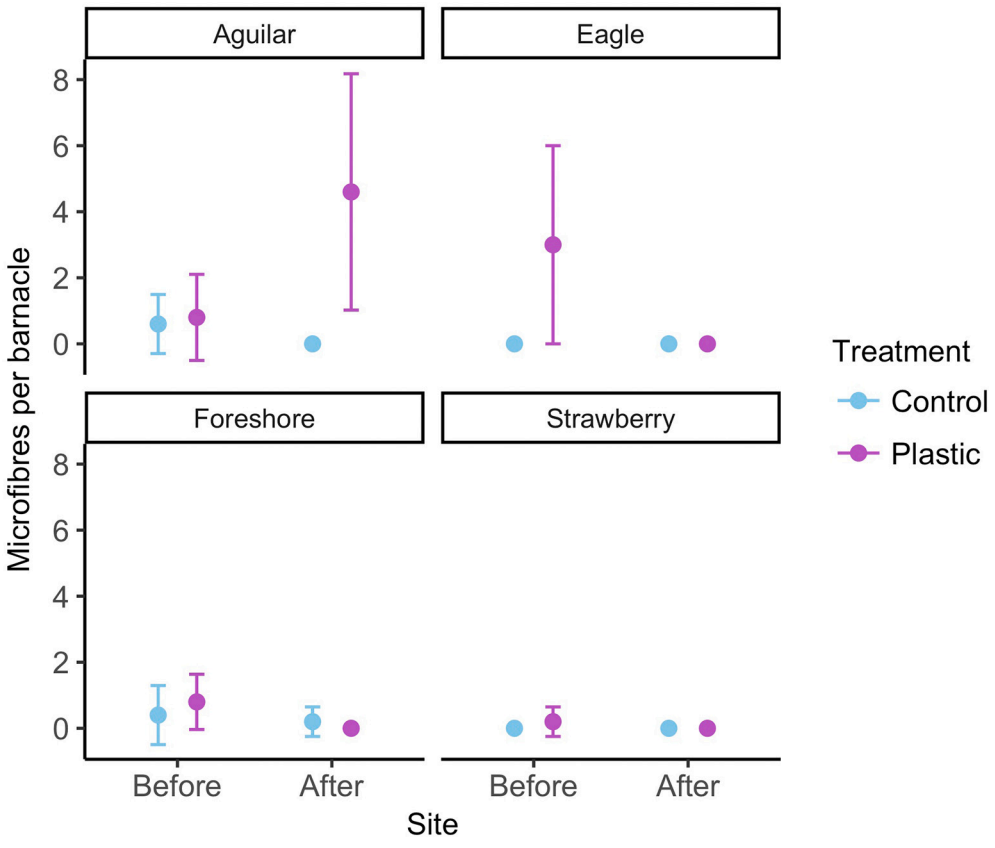

Fig. 5. The mean number of pink microfibres per barnacle gastrointestinal tract in plastic and control treatments before and after the 48-h egestion period. Barnacles were exposed to plastic at a concentration of ~70,000 microfibres per litre. Barnacle intertidal collection sites near Bamfield, British Columbia are denoted accordingly (Aguilar Point, Eagle Bay, the Bamfield Marine Sciences Centre foreshore, and Strawberry Point). Barnacles in the plastic treatment were previously exposed to pink microfibers in circulating tanks for 24 h. The error bars represent standard deviation.

We hypothesized that the barnacles would slow or cease feeding due to the potential for negative short-term fitness effects, or pseudo-satiation from the added particles without nutritional value. However, exposure to MPs for 24 h had no significant effect on the feeding rate of *B. glandula* (Fig. 4). Similar experiments with freshwater amphipods (*Gammarus* spp.) also found no detectable difference in feeding rate following exposure to high concentrations (up to 4000 particles/mL and 13,000 fibres/cm^2) of MP beads (Weber et al., 2018) and MFs (Blarer and Burkhardt-Holm, 2016), respectively. These comparable results were unexpected as it has been suggested that fitness implications and toxicological effects from MP exposure are most prevalent in small animals feeding at lower trophic levels, e.g. amphipods and barnacles (Foley et al., 2018; Walkinshaw et al., 2020); however, longer-term studies are likely required to investigate these effects. While these results on deposit-feeding amphipods might provide a relevant trophic comparison to the present study, filter feeding organisms (e.g. mussels and clams) typically consume more MPs than non-filter feeders (Setälä et al., 2016). When examining the pelagic, filter feeding copepod *Calanus helgolandicus*, Cole et al. (2015) found that short-term (24 h) exposure to 20 μm MP beads (75 MPs/mL) led to a decrease in both the number of algal cells and total carbon biomass consumed, and multi-day exposure led to smaller egg sizes and reduced hatching success. These negative impacts on feeding behaviour and reproduction could be similarly investigated with a long-term MP exposure and monitoring study on *B. glandula*. Since the cirral beating behaviour of barnacles allows them to both feed and respire (through circulation of seawater), satiation will not necessarily cause cessation of beats (Anderson and Southward, 1987). The multifaceted nature of cirral beating, coupled with the high variability observed between individual barnacles (Fig. 4), indicates that feeding rate may not be a reliable measure of the effects of a stressor such as MP exposure.

In our study, barnacles previously exposed to high concentrations of MFs were observed to retain most fibres rather than egest them over a period of 48 h (Fig. 5), which does not lend support to our hypothesis or the findings of many other studies of lower trophic level marine and freshwater crustaceans (e.g. isopods and amphipods; Blarer and Burkhardt-Holm, 2016; Hämer et al., 2014; Weber et al., 2018). Particles in the nano- and micrometer range may be the hardest to egest (Hale et al., 2020; Lee et al., 2013), likely due to their ability to pass through the lining of the intestines; for example, small MPs (0–80 μm) have been found incorporated into the digestive gland tissues of the mussel *Mytilus edulis* (Von Moos et al., 2012). Incorporation of MPs into the tissues of organisms increases the potential for bioaccumulation of MPs and their associated toxins in higher trophic level organisms (Zhang et al., 2019). In addition, the type of MP affects its ability to be egested, and MFs may have an increased residence time in the guts of filter feeding animals compared to those with other feeding strategies (Ward et al., 2019b). Au et al. (2015) investigated timing of MP egestion in a freshwater amphipod (*Hyalella azteca*) and found MFs to have a longer gut residence time (and greater toxicity) than fragmented polyethylene MP particles. Here, we found a 25% decrease in MF presence following the 48-h depuration trial, but no difference in the number of MFs in each individual, and an average depuration rate for the plastic treatment of only 0.05 MFs per 48 h; however, direct comparisons with reported values are difficult due to the high variability in both the study organism and MP type. In addition, the depuration trial was not completely resistant to contamination, as evidenced by the Aguilar Point barnacles that had a higher number of ingested fibres following the depuration period (Fig. 5). Since the depurated barnacles are new individuals being compared to other individuals from the same site, there is potential for such variation to occur with a small sample size, as well as the possibility of egestion followed by re-ingestion by other barnacles; therefore, more stringent depuration protocols should likely be applied in future MP egestion studies. It is also possible that the 48h were not a sufficient length to investigate barnacle egestion, as the barnacles might egest more MFs if given a longer-term depuration period.

While no significant effects of MF ingestion on the behaviour of *B. glandula* were detected, it is important to note that the potential for long-term impacts have not yet been investigated. Here, sublethal acute effects of MFs were measured by investigating changes in feeding rate following a short MF exposure period, and consequently any effects on the reproductive success or population dynamics of *B. glandula* remain unknown. The main conclusions suggested by this work are that acorn barnacles ingest few microfibres even when exposed at very high concentrations, which supports the idea that they are at low risk for microplastic contamination and would not be a suitable indicator species for marine plastic pollution. Similar studies in the future should take precautionary measures to avoid airborne MF contamination (during MF addition to the plastic exposure tanks), as this is the most likely source of ingestion (10%) in the actively feeding non-exposure barnacles. For the present study, MFs not found within the gastrointestinal tract were assumed to have been completely egested and not fragmented into smaller particles and incorporated into the tissues; however, directly examining the feces may allow more definitive conclusions to be made regarding MF egestion. As extremely small MPs can be incorporated into the tissues of the digestive glands of other organisms, leading to inflammatory cellular responses and associated health declines (Von Moos et al., 2012), further studies should be conducted at a nanoplastic scale. For example, planktonic larvae of the barnacle *Amphibalanus amphitrite* had higher mortality rates when exposed to a high concentration of plastic leachates (Li et al., 2016), and ingested nanoparticles with potential to persist to adult life-stages (Bhargava et al., 2018). A study by Yu and Chan (2020) investigated life history traits and feeding of the larval stages of *A. amphitrite* and found environmentally relevant MP concentrations to have little effect. Future research should address the effects of MP ingestion on the larval stages of *B. glandula* to accurately assess the impacts on fitness. Although MP ingestion is well-studied in many filter feeding bivalves (Covernton et al., 2019; Van Cauwenberghe and Janssen, 2014; Ward et al., 2019b), it is important to continue studies of other low trophic level organisms, as they have been identified to be at the greatest risk of ingestion and associated fitness effects (Walkinshaw et al., 2020).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Anderson, D.T., 1981. Cirral activity and feeding in the barnacle *Balanus perforatus* Bruguiere (Balanidae), with no comment on the evolution of feeding mechanisms in thoracican cirripedes. Philos. Trans. R. Soc. B. Soc. B. 291, 411–449. https://doi.org/10.1098/rstb.1981.0004.

Anderson, D.T., Southward, A.J., 1987. Cirral activity of barnacles. In: Southward, A.J. (Ed.), Barnacle Biology. A.A. Balkema, Rotterdam, pp. 135–147.

Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of *Hyalella azteca* to acute and chronic microplastic exposures. Environ. Toxicol. Chem. 34, 2564–2572. https://doi.org/10.1002/etc.3093.

Barrows, A.P.W., Cathey, S.E., Petersen, C.W., 2018. Marine environment microfiber contamination: global patterns and the diversity of microparticle origins. Environ. Pollut. 237, 275–284. https://doi.org/10.1016/j.envpol.2018.02.062.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48 doi:10.18637/jss.v067.i01.

Bhargava, S., Siew, S., Lee, C., Shu, L., Ying, M., Neo, M.L., Lay, S., Teo, M., Valiyaveettil, S., 2018. Fate of nanoplastics in marine larvae: a case study using barnacles, *Amphibalanus amphitrite*. ACS Sustain. Chem. Eng. 6, 6932–6940. https://doi.org/10.1021/acssuschemeng.8b00766.

Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*. Environ. Sci. Pollut. Res. 23, 23522–23532. https://doi.org/10.1007/s11356-016-7584-2.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179. https://doi.org/10.1021/es201811s.

Carlos de Sá, L., Oliveira, M., Ribeiro, F., Lopes Rocha, T., Futter, M.N., Barcelo, D., 2018. Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? Sci. Total Environ. 645, 1029–1039. https://doi.org/10.1016/j.scitotenv.2018.07.207.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49, 1130–1137. https://doi.org/10.1021/es504525u.

Connell, J.H., 1970. A predator-prey system in the marine intertidal region. I. *Balanus glandula* and several predatory species of Thais. Ecol. Monogr. 40, 49–78.

Covernton, G.A., Collicutt, B., Gurney-Smith, H.J., Pearce, C.M., Dower, J.F., Ross, P.S., Dudas, S.E., 2019. Microplastics in bivalves and their habitat in relation to shellfish aquaculture proximity in coastal British Columbia, Canada. Aquac. Environ. Interact. 11, 357–374. https://doi.org/10.3354/aei00316.

Cruz Sueiro, M., Bortolus, A., Schwindt, E., 2011. Habitat complexity and community composition: relationships between different ecosystem engineers and the associated macroinvertebrate assemblages. Helgol. Mar. Res. 65, 467–477. https://doi.org/10.1007/s10152-010-0236-x.

Desforges, J.P.W., Galbraith, M., Dangerfield, N., Ross, P.S., 2014. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. Mar. Pollut. Bull. 79, 94–99. https://doi.org/10.1016/j.marpolbul.2013.12.035.

Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631, 550–559. https://doi.org/10.1016/j.scitotenv.2018.03.046.

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastics throughout the marine ecosystem. Nat. Ecol. Evol. 1, 0116. https://doi.org/10.1038/s41559-017-0116.

Geierman, C., Emlet, R., 2009. Feeding behavior, cirral fan anatomy, Reynolds numbers, and leakiness of *Balanus glandula*, from post-metamorphic juvenile to the adult. J. Exp. Mar. Biol. Ecol. 379, 68–76. https://doi.org/10.1016/j.jembe.2009.08.003.

GESAMP, 2019. Guidelines for the Monitoring and Assessment of Plastic Litter and Microplastics in the Ocean. http://www.gesamp.org/publications/report-of-the-45th-session-of-gesamp-2019 (accessed 30 April 2020).

Goldstein, M.C., Goodwin, D.S., 2013. Gooseneck barnacles (*Lepas* spp.) ingest microplastic debris in the North Pacific Subtropical Gyre. PeerJ 1, e184. https://doi.org/10.7717/peerj.184.

Gray, A.D., Weinstein, J.E., 2017. Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (*Palaemonetes pugio*). Environ. Toxicol. Chem. 36, 3074–3080. https://doi.org/10.1002/etc.3881.

Haegerbaeumer, A., Mueller, M.T., Fueser, H., Traunspurger, W., 2019. Impacts of micro- and nano-sized plastic particles on benthic invertebrates: a literature review and gap analysis. Front. Environ. Sci. 7, 1–33. https://doi.org/10.3389/fenvs.2019.00017.

Hale, R.C., Seeley, M.E., La Guardia, M.J., Mai, L., Zeng, E.Y., 2020. A global perspective on microplastics. J. Geophys. Res. Ocean. 125, 1–40. https://doi.org/10.1029/2018JC014719.

Hämer, J., Gutow, L., Köhler, A., Saborowski, R., 2014. Fate of microplastics in the marine isopod *Idotea emarginata*. Environ. Sci. Technol. 48, 13451–13458. https://doi.org/10.1021/es501385y.

Henry, B., Laitala, K., Klepp, I.G., 2019. Microfibres from apparel and home textiles: prospects for including microplastics in environmental sustainability assessment. Sci. Total Environ. 652, 483–494. https://doi.org/10.1016/j.scitotenv.2018.10.166.

Hueckel, G.J., Stayton, R.L., 1982. Fish foraging on an artificial reef in Puget Sound, Washington. In: Hobart, W. (Ed.), Marine Fisheries Review. National Oceanic and Atmospheric Administration, Washington, pp. 38–44.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771. https://doi.org/10.1126/science.1260352.

Jovanović, B., Gökdağ, K., Güven, O., Emre, Y., Whitley, E.M., Kideys, A.E., 2018. Virgin microplastics are not causing imminent harm to fish after dietary exposure. Mar. Pollut. Bull. 130, 123–131. https://doi.org/10.1016/j.marpolbul.2018.03.016.

Julienne, F., Delorme, N., Lagarde, F., 2019. From macroplastics to microplastics: role of water in the fragmentation of polyethylene. Chemosphere 236, 1–8. https://doi.org/10.1016/j.chemosphere.2019.124409.

Kokalj, A.J., Horvat, P., Skalar, T., Kržan, A., 2018. Plastic bag and facial cleanser derived microplastic do not affect feeding behaviour and energy reserves of terrestrial isopods. Sci. Total Environ. 615, 761–766. https://doi.org/10.1016/j.scitotenv.2017.10.020.

Lee, K.W., Shim, W.J., Kwon, O.Y., Kang, J.H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. Environ. Sci. Technol. 47, 11278–11283. https://doi.org/10.1021/es401932b.

Li, H.X., Getzinger, G.J., Ferguson, P.L., Orihuela, B., Zhu, M., Rittschof, D., 2016. Effects of toxic leachate from commercial plastics on larval survival and settlement of the barnacle *Amphibalanus amphitrite*. Environ. Sci. Technol. 50, 924–931. https://doi.org/10.1021/acs.est.5b02781.

Mishra, S., Charan Rath, C., Prasad Das, A., 2019. Marine microfiber pollution: a review on present status and future challenges. Mar. Pollut. Bull. 140, 188–197. https://doi.org/10.1016/j.marpolbul.2019.01.039.

Navarrete, S.A., Menge, B.A., Daley, B.A., 2000. Species interactions in intertidal food webs: prey or predation regulation of intermediate predators? Ecology 81, 2264–2277. https://doi.org/10.1890/0012-9658(2000)081[2264:SIIIFW]2.0.CO;2.

Pirc, U., Vidmar, M., Mozer, A., Kržan, A., 2016. Emissions of microplastic fibers from microfiber fleece during domestic washing. Environ. Sci. Pollut. Res. 23, 22206–22211. https://doi.org/10.1007/s11356-016-7703-0.

PlasticsEurope, 2019. Plastics – The Facts 2019: An Analysis of European Plastic Production, Demand and Waste Data. https://www.plasticseurope.org/application/files/9715/7129/9584/FINAL_web_version_Plastics_the_facts2019_14102019.pdf (accessed 30 April 2020).

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 1–10. https://doi.org/10.1038/srep14340.

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., Hung, C., 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. 38, 703–711. https://doi.org/10.1002/etc.4371.

Setälä, O., Norkko, J., Lehtiniemi, M., 2016. Feeding type affects microplastic ingestion in a coastal invertebrate community. Mar. Pollut. Bull. 102, 95–101. https://doi.org/10.1016/j.marpolbul.2015.11.053.

Sillanpää, M., Sainio, P., 2017. Release of polyester and cotton fibers from textiles in machine washings. Environ. Sci. Pollut. Res. 24, 19313–19321. https://doi.org/10.1007/s11356-017-9621-1.

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010.

Von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46, 11327–11335. https://doi.org/10.1021/es302332w.

Walkinshaw, C., Lindeque, P.K., Thompson, R., Tolhurst, T., Cole, M., 2020. Microplastics and seafood: lower trophic organisms at highest risk of contamination. Ecotoxicol. Environ. Saf. 190, 1–14. https://doi.org/10.1016/j.ecoenv.2019.110066.

Ward, C.P., Armstrong, C.J., Walsh, A.N., Jackson, J.H., Reddy, C.M., 2019a. Sunlight converts polystyrene to carbon dioxide and dissolved organic carbon. Environ. Sci. Technol. Lett. 6, 669–674. https://doi.org/10.1021/acs.estlett.9b00532.

Ward, J.E., Zhao, S., Holohan, B.A., Mladinich, K.M., Griffin, T.W., Wozniak, J., Shumway, S.E., 2019b. Selective ingestion and egestion of plastic particles by the blue mussel (*Mytilus edulis*) and eastern oyster (*Crassostrea virginica*): implications for using bivalves as bioindicators of microplastic pollution. Environ. Sci. Technol. 53, 8776–8784. https://doi.org/10.1021/acs.est.9b02073.

Weber, A., Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2018. PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*. Environ. Pollut. 234, 181–189. https://doi.org/10.1016/j.envpol.2017.11.014.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Xu, X.Y., Wong, C.Y., Tam, N.F.Y., Liu, H.M., Cheung, S.G., 2020. Barnacles as potential bioindicator of microplastic pollution in Hong Kong. Mar. Pollut. Bull. 154, 1–8. https://doi.org/10.1016/j.marpolbul.2020.111081.

Yu, S.P., Chan, B.K.K., 2020. Effects of polystyrene microplastics on larval development, settlement, and metamorphosis of the intertidal barnacle *Amphibalanus amphitrite*. Ecotoxicol. Environ. Saf. 194, 1–10. https://doi.org/10.1016/j.ecoenv.2020.110362.

Zhang, S., Wang, J., Liu, X., Qu, F., Wang, Xueshan, Wang, Xinrui, Li, Y., Sun, Y., 2019. Microplastics in the environment: a review of analytical methods, distribution, and biological effects. Trends Anal. Chem. 111, 62–72. https://doi.org/10.1016/j.trac.2018.12.002.

Zhu, L., Zhao, S., Bittar, T.B., Stubbins, A., Li, D., 2020. Photochemical dissolution of buoyant microplastics to dissolved organic carbon: rates and microbial impacts. J. Hazard. Mater. 383, 1–10. https://doi.org/10.1016/j.jhazmat.2019.121065.