LOW LEVELS OF MICROPLASTICS RECORDED FROM THE EDIBLE PERIWINKLE, *L. LITTOREA* ON THE WEST COAST OF IRELAND

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ABSTRACT

Microplastics (MPs) are an environmental pollutant of increasing concern, particularly within intertidal habitats. However, little research has been carried out to assess MP levels in intertidal fauna. Despite being at particular risk of encountering MPs through grazing, rocky shore gastropods are one such groups that have been relatively understudied in relation to MP pollution. The authors explored MP abundance in the gastropod *Littorina littorea* from four sites within Galway Bay in West Ireland. To do this, 50 *L. littorea* individuals were collected from four rocky shores of varying wave exposure, with two sites located in the north of the bay and two in the south. An additional 50 individuals were taken from a commercial exporter, to determine MP levels in *L. littorea* intended for human consumption. MPs were recovered from 60.4% of the samples and the average MP level was 2.14 MPs/gram. MP levels were found to differ significantly between sites, with the two northern sites having the greatest MP abundances. Results are discussed in relation to differences in environmental conditions and the possible implications of MP levels for selection of harvesting sites.

INTRODUCTION

Microplastics (MPs), as recently defined by Frias & Nash (2019), are recognised as a pollutant of increasing environmental concern (Thompson *et al*, 2004; Cole *et al*, 2011). Since their first noted occurrence in the 1970s, MPs have been recorded in most, if not all, of the Earth's marine ecosystems, with plastic fibres reported from the equator to the polar regions (e.g. Barnes *et al*, 2009; Lusher *et al*, 2015), and from the sea surface to deep ocean sediment (e.g. Van Cauwenberghe *et al*, 2013; Song *et al*, 2014). As global plastic production is expected to increase in the future (Thompson, 2004; Cózar *et al*, 2014), the problem of MP pollution is expected to persist and likely to magnify, especially given the mostly non-biodegradable nature of the plastic polymers responsible.

Though MPs are now common in most marine ecosystems, they are likely to be disproportionately prevalent and problematic in intertidal regions, as they are transported down rivers and accumulate first on beaches, rocky shores, and boulder fields (Williams & Simmons, 1997; Rech, 2014). Rocky shores may be at particular risk, as shores dominated by benthic macroalgae may trap and hold MPs by virtue of their complex topography (Scoffin, 1970; Mudd *et al*, 2010). Also, rocky shores have been suggested to aid in the breakdown of primary plastics into secondary MPs through mechanical action and abrasion against the shore (Hidalgo-Ruz *et al*, 2012, Cole *et al.*, 2011). The presence of these pollutants on rocky shores has the potential to induce negative effects on any organisms that encounter them, especially considering their small size range (1-5000µm; Frias & Nash, 2019), which is conducive to being ingested by an array of marine fauna such as polychaeta, bivalvia, and crustacea (Wright *et al*, 2013; Green, 2016; Tosetto, 2016). However, intertidal gastropods may be at particular risk as many of these organisms are likely to encounter and ingest MPs through their grazing activity (Norton *et al*, 1990). One such intertidal gastropod is the common edible periwinkle *Littorina littorea* (Linnaeus, 1758).

L. littorea is an herbivorous Caenogastropod native to rocky shores throughout the North-East Atlantic. The species is often the dominant algal grazer on its host shores, where it feeds on various brown and green algae (Watson & Norton, 1985). Through this process, it reduces algal cover and allows space for other species to colonise the exposed rock (Lubchenco, 1983), thus playing a vital role in the succession of rocky shore organisms. In addition to its ecological role, *L. littorea* is a commercial species of significant economic importance to many coastal communities throughout North-West Europe (Cummins *et al*, 2002).

To date, little research has been carried out to assess current MP levels in wild *L. littorea* populations, despite evidence that *L. littorea* consume MPs from the surface of contaminated macroalgae i.e. *Fucus vesiculosis* and through pedal mucus associated with trail-following (Gutow *et al*, 2015; Gutow *et al*, 2019). MP ingestion and retention may have several negative effects for *L. littorea* based on what has been observed in other species. These potential effects include a reduction in energy reserves (Watts *et al*, 2015), clogging of digestive and respiratory organs (Wright *et al* 2013), and a potential increase in mortality rate (Green, 2016). Persistent organic pollutants (POPs) present in weathered MPs may also lead to increased mortality, as *L. littorea* rely on chemical cues to avoid predators such as the green shore crab *Carcinus maenas* (Hadlock, 1980). A disruption to this predator avoidance mechanism caused by MP leachate has recently been demonstrated by Seuront (2018).

To the authors knowledge, baseline levels of MP in *L. littorea* have not been assessed and so the potential threat that this emergent pollutant poses to the species is currently unknown. The aim of the current study is to determine baseline MP levels in *L. littorea* within Galway Bay based on shore type (Lewis, 1964).

METHODOLOGY

Sample sites and collection

Four sampling sites were selected within Galway Bay; two sites were selected in the north of the bay and two in the south of the bay (Figure 1). The north sites were Mutton Island (53° 15’ 42.65” N, 9° 3’ 13.703” W) and Ballynahown (53° 14’ 0.4416” N, 9° 32’ 36.114” W), while the south sites were The Flaggy Shore (53° 09' 26.8" N, 9° 06' 52.1" W), and Blackhead (53°09'05.4" N, 9°16'07.8"W), all located on the West coast of Ireland and within the Galway Bay SAC. Mutton Island and Ballynahown were sampled in November 2017 while The Flaggy Shore and Blackhead were sampled in April and May 2019 respectively. Mutton Island is a sheltered shore composed of mixed sediment, (sand, rock, and gravel), with several rocky outcrops that are inhabited by *L. littorea*. The site is in the North-East of the bay, backed by Galway City and adjacent to a cause way to a wastewater treatment facility. Ballynahown is a moderately-exposed bedrock and boulder shore located in the North-West of Galway Bay in a sparsely populated coastline. The upper shore is composed of exposed granite bedrock while the lower shore is composed of large granite boulders. The Flaggy Shore is a moderately-sheltered rocky shore in the South East of the bay, opposite Galway City, and is the most representative of a typical Atlantic rocky shore, exhibiting a full vertical zonation of fucoid algae. Blackhead is a very exposed shore located in the South West of the bay and is composed of exposed limestone bedrock that slopes towards the waterline in steep terraces.

Figure 1: Map of the sampling site locations within Galway Bay (●) and the location of the bay on the west coast of Ireland (inset).

Fifty *L. littorea* individuals were collected haphazardly by hand from each site. They were collected in the mid to lower shore where the gastropod is known to inhabit rocks and *Fucus vesiculosis* when present. A random sample of 50 *L. littorea*, collected by periwinkle pickers from an unknown site on the north-west coast of Ireland, was also obtained from Breizon Ltd., an exporter based in west Galway in February 2019. Once returned to the laboratory, the samples were graded using digital callipers to ensure the shell height was ≥12mm. This removed maturity as a possible confounding factor (Williams, 1964; Yamada, 1987). The samples were frozen overnight at -20°C.

Digestion and microscopy

Shell height and overall weight were recorded for each individual. Samples were then removed from their shells by cracking the shell with a pestle and mortar, detaching the columellar muscle with forceps, and carefully uncoiling the whole body. Removing the soft body in this way ensured no intestinal breakages and subsequent loss of ingested MPs. The soft bodies were rinsed with ultrapure water to remove any external MP contamination or shell fragments. The soft bodies were weighed, transferred to glass Schott bottles, and digested at room temperature for 24 hours using 10% potassium hydroxide (KOH). Once the biological material was fully digested, the samples were filtered through individual glass fibre filter pads (Whatman GF/C). In order to speed up the filtration process and to prevent clogging of the filter paper, the digested supernatant was first removed using a pipette and filtered, followed by the digested sediment, reducing the processing time. The pipette was rinsed through with ultrapure water between samples. Throughout this process, airborne contamination was monitored using blank filter pads. All glassware was rinsed in nitric acid and triple rinsed in ultrapure water prior to initial use, and triple rinsed in ultrapure water between digestions. Once the filtrations were complete, the filter pads were inspected under a stereo microscope to identify any recovered MPs. The MPs recorded were classified according to type e.g. nurdle, microbead, fragment, fibre, etc. based on Frias *et al.*, (2018) and Bessa *et al.*, (2019). In addition, colour and measurements such as width and length in the case of fibres and area in the case of fragments were also recorded.

Statistical analysis

The MP abundance data were tested for normality using the Kolmogorov-Smirnov test with Lilliefors correction and were found to be non-normal. Based on this, a non-parametric Kruskal-Wallis test was used to determine if there were significant differences in

MPs/individual between sites, followed by pairwise Mann-Whitney U-tests to determine which sites differed significantly upon comparison of mean ranks. Alpha for each comparison was adjusted using Bonferroni correction.

To explore the relationship between *L. littorea* weight and shore exposure, a 2-tailed Spearman rank-order correlation was carried out on the variables soft body weight and shore exposure. Soft body weight was found to be highly correlated with shore exposure. Because of this, the number of MPs/gram of soft body weight was calculated to determine if exposed shore *L. littorea* consumed relatively more MPs than sheltered shore individuals. Because soft body weight and shore exposure were highly correlated, ordinal regression was used to predict the shore exposure of the commercial sample based on weight.

A Kruskal-Wallis test was used to determine if there were significant differences in the abundance of MPs/gram between sites with pairwise Mann-Whitney U-tests being carried out to assess where any significant differences lay. To determine if there was a significant difference in MP abundance based on sex for each site, a Mann-Whitney U-test was performed, without the Ballynahown data as the gastropod's sex was not recorded for this site.

A 2-tailed Spearman rank-order correlation was carried out to test if there was a relationship between MP abundance and *L. littorea* weight in order to reveal if larger *L. littorea* consumed more MPs. Alpha for each test was set at 0.05. Statistical analyses were carried out using SPSS Version 25 (IBM Corp., 2017).

RESULTS

MPs, in the form of fibres and fragments, were recovered from all the samples in this study, with fibres compromising 97% of all MPs recorded. The highest percentage of gastropods with MPs recorded was from Blackhead, where 66% of the sample had between 1 and 3 MPs recovered. This was followed in *ex aequo* by The Flaggy Shore (60%) and the Commercial sample (60%), and by Ballynahown (58%) and Mutton Island (58%) in *ex aequo* as well. The vast majority of the MPs recovered were in the form of fibres (~97%) with the length the fibres ranging between 60m and 15585µm. The average fibre length recorded across all sites was 1865.03 ± 1834.19µm. Of the MPs recovered, most were black (35%), followed by blue (34%) and red (11%), with the remaining MPs being yellow, white, orange, brown, or colourless.

The highest mean levels of MPs/individual in Galway Bay were recorded from the northern sites at Ballynahown (2.40 ± 2.11) and Mutton Island (2.02 ± 2.07), in comparison to the southern sites at Black Head (0.98 ± 0.88) and The Flaggy Shore (0.59 ± 0.90) and the commercial sample (1.30 ± 1.76) from outside of the bay (Table 1).

Table 1. The levels of MPs recorded from sites in Galway Bay and a commercial sample from the north-west coast of Ireland.

Site	Total microplastics	Mean microplastics/ individual	Mean microplastics/gram	Mean microplastic length (µm)	Mean soft body weight (g)
Ballynahown	120	2.40 ± 2.11	2.80 ± 2.85	1987.29 ± 1849.41	0.98 ± 0.26
Mutton Island	101	2.02 ± 2.07	2.11 ± 2.94	2182.95 ± 2400.29	1.28 ± 0.34
Blackhead	49	0.98 ± 0.88	2.96 ± 2.92	988.87 ± 1173.86	0.39 ± 0.18
Flaggy Shore	29	0.59 ± 0.90	0.59 ± 0.91	1653.95 ± 784.71	1.05 ± 0.21
Commercial	65	1.30 ± 1.76	2.24 ± 3.15	1732.53 ± 857.70	0.63 ± 0.22

As the data did not meet the assumption of normality, a non-parametric Kruskal-Wallis test was carried out to compare MPs/individual between sites, which revealed that MPs/individual differed significantly based on site ($p = 0.001$). Post hoc pairwise Mann-Whitney U-tests with Bonferroni correction revealed that Ballynahown differed significantly from both the southern sites (Flaggy Shore ($p = <0.001$), and Blackhead ($p = 0.005$)) and the Commercial sample ($p = 0.009$) while the second northern site, Mutton Island, and the Flaggy Shore in the south were also significantly different ($p = <0.001$), see Figure 2.

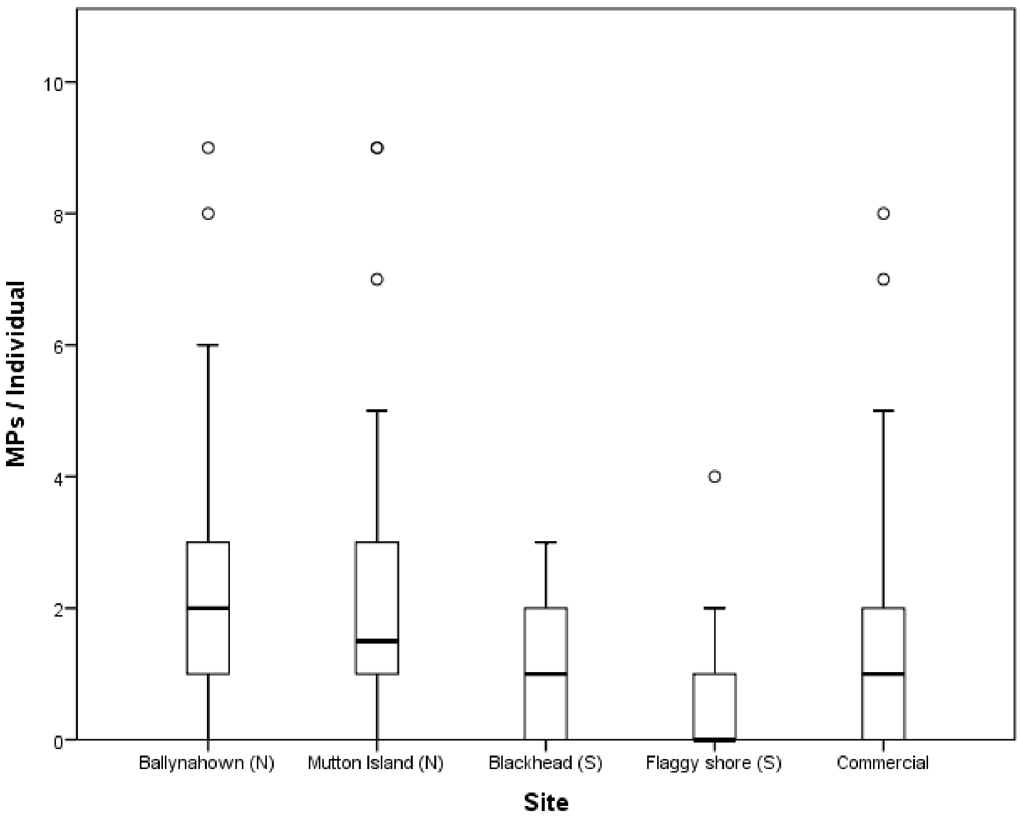

Figure 2. Boxplot of mean MPs/individual based on site. The commercial data are included for comparison. Site location within the bay is denoted by N (north) and S (south).

2-tailed Spearman's correlation showed a strong positive relationship between shore exposure and soft body weight ($r_s = 0.737$, $p = <0.001$; figure 3). Therefore, ordinal regression was used to estimate the shore exposure of the commercial sample based on weight and plotted with the other sites (Figure 3). Based on this prediction, and visual assessment of Figure 3, the commercial data were estimated to originate from a site with a Lewis exposure of 2-3.

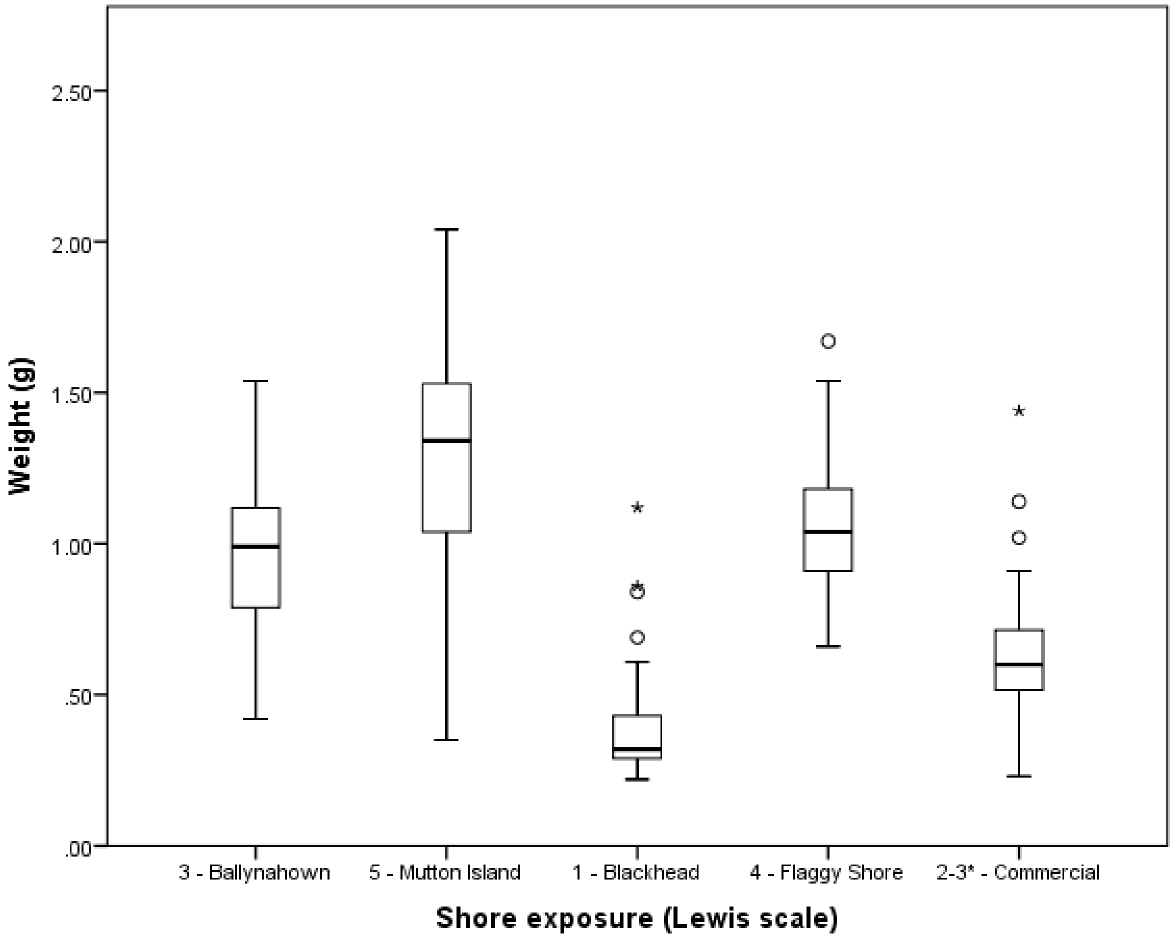

Figure 3 A boxplot of soft body weight and shore exposure for each site, illustrating the shore exposure of the commercial data from the north-west coast as being between 2-3 on the Lewis exposure scale.

As shore exposure was shown to be correlated with body weight, the number of MPs/gram of soft body weight was calculated for each sample. When adjusted to account for weight, the two most westerly and exposed sites in the bay, Blackhead and Ballynahown, had the greatest MPs/gram (2.96 ± 2.92 and 2.80 ± 2.85 respectively), followed by the commercial sample (2.24 ± 3.15 MPs/gram). Mutton Island in the inner bay had a similarly high level (2.11 ± 2.94 MPs/gram), with The Flaggy Shore again showing the lowest levels of MPs (0.59 ± 0.91

MPs/gram). The data did not meet the assumption of normality, and a non-parametric Kruskal-Wallis test was carried out to compare MPs/gram to test for significance between sites. This test showed that MPs/gram varied based on site ($p = <0.001$). Pairwise Mann-Whitney U-tests with Bonferroni correction revealed that the Flaggy Shore had significantly lower MP levels than all other sites ($p < 0.01$) with no significant differences in MPs/gram between the other sites ($p > 0.05$; Figure 4).

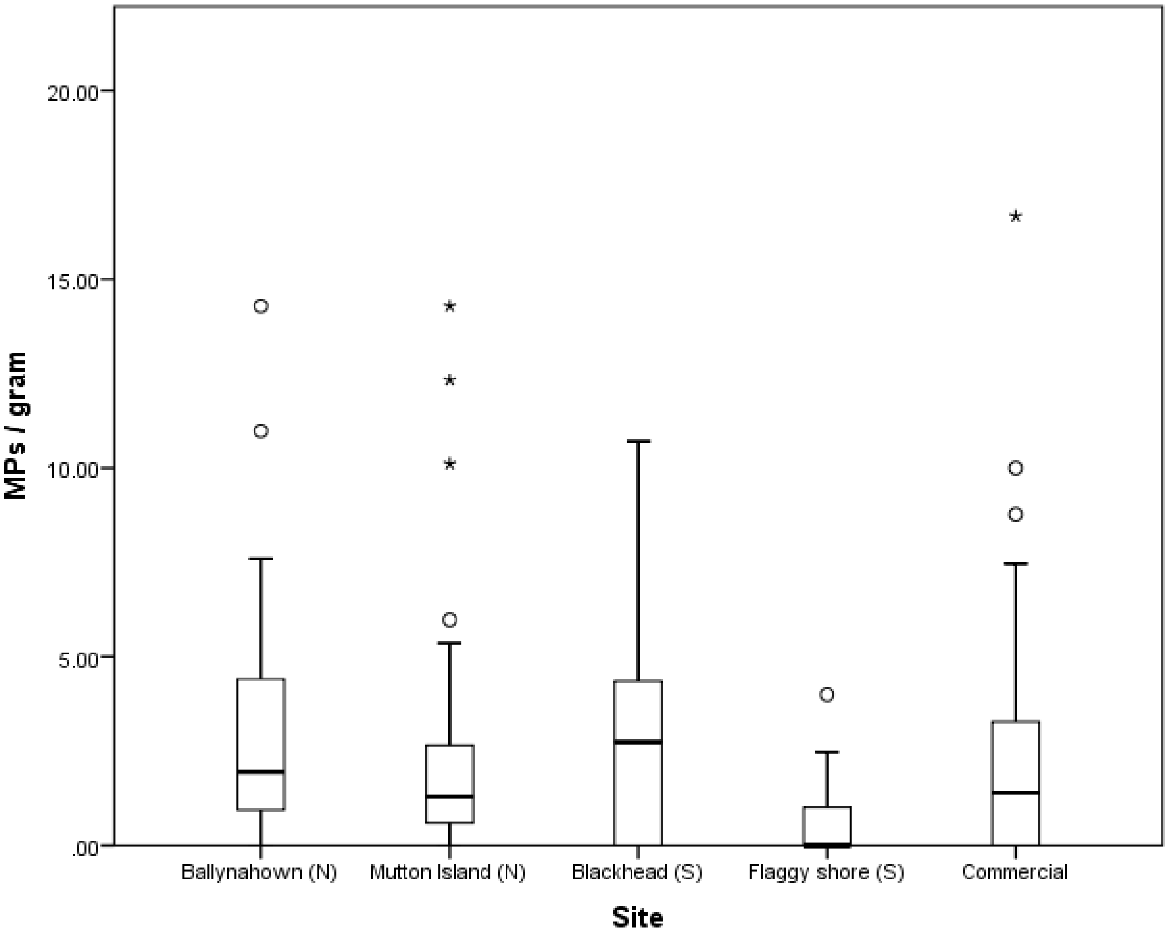

Figure 4 Boxplot of MPs/gram based on site. Site location within the bay is denoted by N (north) and S (south). Commercial data are again included for comparison.

A Mann-Whitney U-test revealed no significant difference in MP abundance between male and female samples of *L. littorea* (p = 0.696; figure 5).

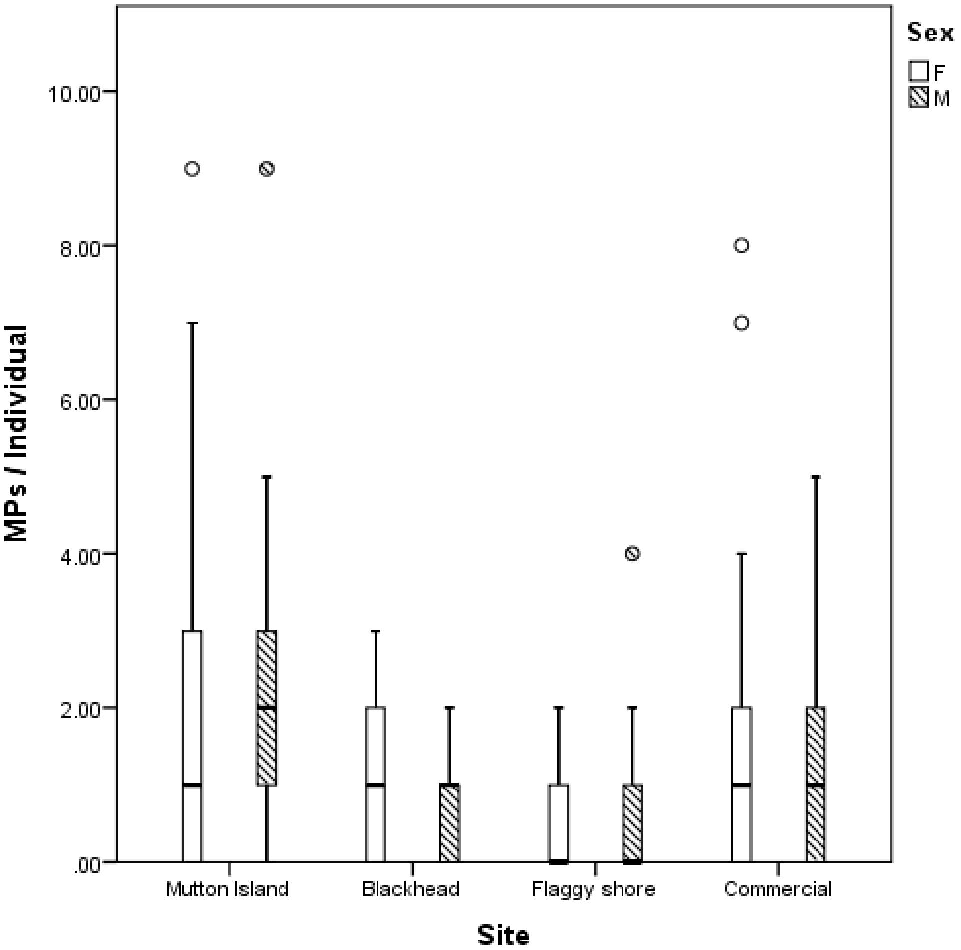

Figure 5 MPs/gram based on the variables ‘sex’ and ‘site’.

A 2-tailed Spearman’s correlation found no relationship between soft body weight and MP abundance ($r_s = -0.013$, p = 0.837).

DISCUSSION

This study has confirmed the presence of MPs in the edible periwinkle, *L. littorea,* in Galway Bay, where MPs were detected at all sites. The vast majority (~97%) of this MP pollution were fibres, which suggests that most of the MP pollution encountered by grazing gastropods on rocky shores was secondary MPs, resulting from the breakdown of for example larger plastic items, shredding from clothes, and wear and tear of ropes (Andrady, 2011; Napper & Thomson, 2016). This is further supported by the large percentage of blue fibres recovered, which potentially originate from ropes used in fishing and aquaculture and are commonly recovered in MP studies (Welden & Cowie, 2017). The relatively high percentage of fibres recorded here is consistent with Martin *et al* (2017), who found that fibres comprised 85% of MPs recovered from sediment cores.

There is relatively little comparative literature with which to compare the levels of MPs in gastropods. However, Leslie *et al* (2013) found that *L. littorea* from the Dutch coast contained 20 MPs/gram dry weight. This value vastly exceeds the average MP level observed in the current study where the average across all sites is 2.14 MPs/gram wet weight), especially considering that Leslie *et al* (2013) presents the results as MPs/gram of dry weight. Heavily industrial and agricultural water bodies such as the Scheldt Estuary (Baeyens *et al*, 1998) may contribute to higher MP levels in the coastal waters of the Netherlands in comparison to the relatively low levels of surrounding agriculture and industry in Galway Bay. In relation to other intertidal species however, the MP levels recorded here are relatively high. Van Cauwenberghe *et al* (2015), in a study of six Northern-European sites, found that *Mytilus edulis* and *Arenicola marina* contained on average 0.2 ± 0.3 and 1.2 ± 2.8 MPs/gram respectively. However, this may be associated more with differences between feeding groups, as opposed to differences in ambient MP levels (Setälä *et al*, 2016).

The moderately sheltered site the Flaggy Shore had the lowest MP abundance of all sites in the study while the moderately exposed site Ballynahown had the highest overall levels. That Ballynahown had the highest MP levels was somewhat unexpected as the site has recently been shown to have low levels of nutrient pollution, especially when compared to the Mutton Island site (Atalah & Crowe, 2012). This suggests that nutrient pollution may not be a suitable proxy for MP pollution. The relatively high MP levels recorded at Ballynahown are consistent with Martin *et al*, (2017), who recorded mean MP levels of 7.67 ± 2.09 MPs per sediment core within the North Sound of the Bay, close to Ballynahown, with the authors suggesting that the north sound of the bay may be a deposition area for MPs. Circulation in the bay, where the main body of water leaves via the North Sound, and or ocean-based sources of MPs may have resulted in the high levels at this site. Mutton Island, by virtue of its proximity to a wastewater treatment plant (WWTP), the River Corrib estuary and Galway City, was expected to have the highest levels of MP pollution available to gastropods, as previous studies have found that MP concentrations tend to increase with proximity to urban areas (Frias *et al*, 2014; Frère *et al*, 2017). However, though proximity to urban areas has been suggested to be the main predictor of MP abundance, water circulation patterns within the bay and shore topography may also be determining factors (Rocha-Santos & Duarte, 2014) in the distribution of MPs. Mutton Island is a very sheltered shore with a mixed substrate, composed mostly of small gravel, sand, and mud, and as a result of this, MPs may be bound in this sediment matrix as opposed to the rocky outcrops that *L. littorea* tend to inhabit. Ballynahown, in contrast, is composed of bedrock with large rocks and boulders present. This substrate is dominated by fucoid algae that may trap and hold MPs (Gutow *et al*, 2015), making them bioavailable for grazers such as *L. littorea*. In addition, Ballynahown is composed of coarse-grained granite boulders that have been shown to accommodate a greater algal mat than other rock types (Guidetti *et al*, 2004), potentially allowing more microplastics to become trapped in the complex topography. This may explain, in part, the observed differences between Ballynahown and the other sites.

The Flaggy shore and Blackhead were found to have comparatively lower levels of MPs than the other two sites. Both of these sites are located in the south shore of Galway Bay and hence, the relatively low MP abundances recorded at these sites may be related to the movement of water and plastic debris within the bay. For both the northern sites (Ballynahown and Mutton Island) and the southern sites (Flaggy Shore and Blackhead), the western sites located in the outer bay recorded higher MP abundances than their eastern inner-shore counterparts. This may be due to the proximity of these outer sites to the incoming Atlantic Ocean or it may be that water circulation patterns concentrate MP debris at these outer bay sites, depositing them and making them bioavailable for intertidal grazers (O' Donncha *et al*, 2015; Martin *et al*, 2017).

When adjusted to compensate for weight differences between sites, Blackhead had the highest average MP abundance (2.96±2.92 MPs/gram) while Flaggy Shore had the lowest levels (0.59±0.91 MPs/gram). The differences in MP levels observed here are due to differences in *L. littorea* weight between sites caused by variation in wave exposure. Both exposed shores (Blackhead and Ballynahown) had the highest MP levels relative to soft body weight while the sheltered sites of Flaggy Shore and Mutton Island had the lowest levels. This suggests that *L. littorea* on exposed shores may be more susceptible to the potential harmful effects of MP ingestion as they have been shown to ingest a higher number of MPs relative to their body weight. This may lead to more profound impacts for exposed shore *L. littorea* such as blockages (Wright *et al*, 2013) increased energy demand caused by the residence of non-food items in the gut, higher energy allocation to regulation of non-food items, increased immune function, and potentially higher sorption of harmful chemical compounds from exposure to weathered MPs (Hartmann *et al*, 2017), even if MPs are not being retained (Teuten *et al*, 2007). Green (2016) also demonstrated a legacy effect of MPs, with *L. littorea* displaying a reduction in recruitment following exposure to high density polyethylene (HDPE) and polylactic acid (PLA), indicating that MP ingestion has the potential to alter rocky shore community structure. This alteration may be more profound on wave-exposed rocky shores based on the evidence presented here.

MP levels were not found to be related to *L. littorea* weight. This was somewhat unexpected as larger individuals would have a greater capacity to consume and concentrate MPs. However, it may be the case that larger *L. littorea* also have greater capacity for regulation of non-food items and a greater ability to excrete MPs than their smaller counterparts. In feeding experiments, Gutow *et al* (2015) noted that no MPs were recovered from the hepatopancreas of MP contaminated *L. littorea*. As the hepatopancreas is the main food assimilation organ, this suggests that MPs are not retained, but rather excreted. MPs recovered from faecal pellets in the same study further supports this (Gutow *et al*, 2015). However, their study only considered microbeads and fragments. As the factors that influence MP retention are likely to be complex and heavily dependent on MP morphology, it is unknown if MP fibres are more likely to be retained. In addition, the size of the ingested MPs is likely to be important in determining if they are retained or not.

The average fibre length of the recovered MPs was found to vary between sites. Blackhead had the smallest average fibre length and the lowest average soft body weight of all the sites. Conversely, Mutton Island had the longest average fibre length and the greatest average soft body weight. This suggests that while MP abundance is not related to weight, the length of the ingested fibres potentially is. This is intuitive, as smaller snails are likely to consume smaller fibres when compared to larger snails. The broad size range of fibres recovered in this study also has important implications, as size is the main factor by which *L. littorea* separate food and non-food items following ingestion (Fretter & Graham, 1962) with particles in the micrometre size range likely being rejected (Walker, 1972; Gutow *et al*, 2015). However, larger

MPs may be passed into the midgut gland where retention and accumulation may be possible. The fibre lengths recorded here are similar to those recorded by Leslie *et al* (2013).

MPs were found in 60% of the commercial sample of *L. littorea*. As the *L. littorea* harvesting industry is unregulated in Ireland, there is no way of knowing exactly what sites are being harvested (Cummins *et al*, 2002). This is concerning, given that differences in MP abundance between sites has been demonstrated in the current study. However, more sampling of different shore types with replicates is required in order to determine the exact environmental variables that dictate MP abundance on rocky shores and thus to inform harvesting practices. The comparatively lower levels of MPs in the commercial samples may be due to the samples originating from relatively unpolluted sites. However, *L. littorea* harvesters may collect individuals and store them in mesh bags for up to 2 weeks prior to transporting them to the exporter. During this time, the *L. littorea* may have partially purged any ingested MPs (Gutow, 2015), leading to the appearance of lower levels of MP pollution. However, retention experiments would be required to confirm if this is the case.

Conclusion

This study has confirmed the presence of MPs in *L. littorea* from a variety of shore types. However, more research is required to elucidate the effects of such ingestion on physiology, histology and behaviour. Overall, the levels of MPs found in Galway Bay *L. littorea* are comparatively lower than those found in other areas e.g. Netherlands (Leslie *et al*, 2013). Given that global plastic production is increasing, and current oceanic plastic will continue to persist and further fragment into MPs, the level of MP pollution in Galway Bay *L. littorea* may rise in the future unless there are mitigation measures taken. Because of this, there is a need for continued monitoring of MP pollution in species such as *L. littorea* where there is baseline data.

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