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Abstract

Seagrasses have global distribution and are highly productive and economically valuable habitats. They are sensitive and vulnerable to a range of human-induced pressures, including ongoing exposure to marine litter, such as microplastic particles (<5mm). In this study, a *Zostera marina* bed in Deerness Sound, Orkney was selected to determine whether microplastics accumulate in seagrass beds and adhere to seagrass blades. Sediment, seagrass blade, biota and seawater samples were collected. 280 microplastic particles (0.04 to 3.95mm (mean = 0.95mm ± 0.05 SE)) were observed in 94% of samples collected (n = 111). These were visually categorised into type (fibre, flake, fragment) and colour, and 50 were successfully identified as plastic using ATR-FTIR. Fibres contributed >50% of the total microplastics observed across all samples. This is the first known study on *Z. marina* to describe microplastic loading within a seagrass bed and to identify microplastic adherence to seagrass blades.

Highlights

- *Z. marina* seagrass beds act as a sink for microplastics with higher loadings than bare sandy sediment sites.
- First known observation of microplastics adhering to *Z. marina* blades.
- Microplastic identified in sediment, on seagrass blades, in associated biota and in seawater samples.
- Fibres contributed >50% of the total microplastics observed.
- Of the 54 samples analysed by ATR-FTIR, 92.5% of samples were successfully identified as plastic, with poly(ethylene) recorded as the most common (34%).

Keywords: Microplastics, Seagrass, *Zostera marina*, Orkney

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1. Introduction

Microplastic particulates represent a significant proportion of debris originating from anthropogenic activities on land or at sea and/or discharged into coastal or marine environments (Löhr et al., 2017; UNEP and NOAA, 2011). The term microplastic describes a plastic particle <5mm in size and defines a range of particle shape combinations (fibres, fragments of various dimensions, pellets and spheres) and colours (Arthur et al., 2009; Wright et al., 2013a). Plastic pollution in the ocean is recognised as a major global environmental issue (Sutherland et al., 2011), as over time, plastic degradation typically results in fragmentation into increasingly smaller pieces by physical abrasion from wave action, photodegradation (Barnes et al., 2009) or biological processes (Andrady, 2011; Cole et al., 2011). Microplastics are widely dispersed throughout the marine environment, accumulating in pelagic and sedimentary habitats (Thompson et al., 2004). Sediment in particular, has been suggested as a potential long-term sink for plastic (Browne et al., 2010; Claessens et al., 2011; Nuelle et al., 2014). An understanding of the distribution and accumulation of this form of pollution is crucial for gauging environmental risk (Martin et al., 2017).

Microplastics are considered ubiquitous, with high concentrations recorded in the most remote marine regions, including deep-sea sediments and in Arctic sea ice (Peeken et al., 2018). This is due to the dispersal of buoyant plastics cast ashore by winds or ocean currents, concentrating or trapping floating debris around islands and onto shorelines (Laist, 1987; Thiel et al., 2013). Although the highest number of microplastics are often associated with urbanised or industrial areas, regions of low human activity can be subject to similar levels of contamination (Alomer et al., 2016, Blumenröder et al., 2017). The Orkney Islands, with an estimated population of 22,000 (National Records of Scotland, 2018), are a network of small, remote islands, situated off the Northeast coast of Scotland. Evidence suggests they are subject to microplastic contamination similar to areas of high urbanisation (Blumenröder et al., 2017) due in part to transport of microplastics on oceanic currents leading to deposition in these more remote areas driven by complex local hydrographic conditions.

Marine litter can impact all coastal environments and although obvious litter can be observed on rocky shores and sandy beaches, inshore habitats such as seagrass are also susceptible (d'Avack et al., 2014; Balestri et al., 2017). Colonizing shallow coastal waters, seagrasses are wide-ranging and are documented, with the exception of Antarctica, on coastlines of all continents (DeAmicis, 2012; Short, 2007). They are considered a complex marine ecosystem of great ecological and economic importance on a global and local scale (Nordlund et al., 2017; Ruiz et al., 2009). Seagrasses provide habitat, shelter and function as a nursery, spawning and foraging grounds for a diverse assemblage of fauna, including commercially important species (Bertelli and Unsworth, 2014; Thomson et al., 2014; OSPAR, 2009) and have a role in the 'blue carbon' solution, as a natural carbon sink (DeAmicis, 2012). A study conducted by Scottish Natural Heritage (SNH) identified *Zostera marina* as the most common species

of seagrass in Orkney (Thomson et al., 2014). *Zostera* species have an extensive root-rhizome system, which oxygenates and stabilizes the sediment, minimising resuspension and preventing erosion by promoting the accumulation and binding of sediment (Jackson et al., 2013; Mcleod et al., 2011; OSPAR, 2009). Seagrasses are effective attenuators of wave energy, decreasing the physical stress on the sediment-water interface (Bradley and Houser, 2009; Mcleod et al., 2011). The creation of this hydrodynamic state encourages sediment to settle and prevents local sediment resuspension (Fonseca and Cahalan, 1992), providing a natural form of coastal protection. This sediment can support a rich infauna of polychaete worms, burrowing anemones and bivalve molluscs, which are often associated with seagrass (d'Avack et al., 2018). These blades may be colonised by epiphytic diatoms, algae, hydroids and bryozoans (d'Avack et al., 2018) and grazers, which in turn provide food for higher order predators (DeAmicis, 2012; Heck et al., 2008; Hily et al., 2004). There is limited knowledge on ingestion of microplastics by grazing species, with most documenting planktonic grazers such as copepods and zooplankton (Cole et al., 2013; Setälä et al., 2014). However, recently, microplastic ingestion by gastropods including mud snails, periwinkles and limpets (Karlsson et al., 2017; Naji et al., 2018) has been investigated.

Direct microplastic ingestion is likely due to accidental consumption of particles through indiscriminate feeding strategies (e.g. filter and deposit feeders) (Cole et al., 2013) or through misidentification as food (de Sá et al., 2015). Following ingestion, microplastics can infiltrate food webs (Browne et al., 2008; Wright et al., 2013b) resulting in trophic transfer from contaminated prey to predator (Farrell and Nelson, 2013; Nelms et al., 2018). Commercially important species are of particular concern due to risks of plastic pollution to human consumers (Bonello et al., 2018) as microplastic uptake can also occur via other pathways. Microplastic particles have been found in the gills of crabs (Watts et al., 2014; GESAMP, 2015) and the circulatory system of mussels (*Mytilus edulis*) following migration from the gastrointestinal tract (Browne et al., 2008). Therefore, the use of whole-body analysis can be beneficial, particularly for trophic transfer studies (Karlsson et al., 2017) to capture all microplastics within the biota (Leslie et al., 2013).

Researchers have called for further investigation into the impact of plastic waste on seagrass bed habitats (European Commission, 2011), however, no known published research has studied microplastic loading within the sediment of a seagrass bed or microplastic adherence to the surface of *Z. marina* blades. As a result, there is limited knowledge available to determine if seagrass act as a microplastic trap, in the same way seagrasses accumulate sediment. Furthermore, microplastics may adhere to seagrass blades in biofilms, providing another route of trophic transfer to seagrass grazers. Therefore, this study focusses on the Orkney mainland, with the aim of conducting a study of microplastic accumulation within a *Z. marina* bed. The aims of this study were: (1) determine whether seagrass beds act as sink for microplastics; (2) ascertain whether microplastics adhere to seagrass blades; (3) examine mobile biota associated with the seagrass bed to confirm ingestion of microplastics. The findings of this

study provide important information on the susceptibility of seagrass beds to microplastic pollution and the potential impacts to associated fauna within these habitats.

2. Methods

2.1 Sample Location

At peak low tide on June 13th, 2018, a small team of snorkelers laid a 100m transect parallel to the shore within Deerness Sound (58.952433, -2.778626) to determine variance of seagrass abundance (Table 1). This area of study was selected, as (Scottish Natural Heritage) SNH had previously identified *Z. marina* within the region in 2014. The study site is located near the mouth of the Sound, with a substrate comprising mainly of sand. The sample location contained sparse kelp species, including *Chorda filum* and *Saccharina latissimi*. Prior to conducting the survey, the conditions and depth were checked and appeared to be similar across the 100m transect. All samples were frozen at -20°C for preservation prior to examination.

2.1.1 Seagrass samples

Percentage coverage of seagrass was recorded using a quadrat ($1m^2$) with two replicate samples taken every 10m across the 100m transect line (n = 20) to estimate seagrass abundance and identify if an association exists between seagrass coverage and microplastic loading. Three seagrass blades were carefully removed from each side of the transect line every 10m (n = 60) to identify species and determine if any microplastics were adhered to the blades. Samples were individually stored in zip-lock bags, which had been pre-rinsed with distilled water prior to use in the field to avoid cross contamination (see Section 2.4.3).

2.1.2 Sediment samples

Sediment samples were collected from each side of the transect line (n = 20) to 5cm depth every 10m using a sterile 50ml plastic centrifuge tube, applied as a miniature corer and individually stored in zip-lock bags. Measures to limit cross-contamination were strongly adhered to for all samples collected in the field (as discussed in Section 2.4.3). Control sediment samples were collected in clear sandy areas where no seagrass was present and within sandy sediment at the boundary of the seagrass bed (n = 5) (Table 2).

2.1.3 Biota samples

Following the collection of sediment samples, a $1m^2$ area at each 10m point, on either side of the transect was searched for grazing organisms both on the seagrass and on or within the sediment. Grazing organisms were carefully removed by hand from seagrass blades within the search area, whilst sediment directly beneath the seagrass was physically searched to identify any living organisms both visually and using the 50ml miniature corer. Any organisms discovered were individually stored in clean, 50ml skirted centrifuge tubes (Triple Red®), which had been pre-rinsed with distilled water prior to use in the field (see Section 2.4.3).

2.1.4 Seawater samples

To quantify buoyant microplastics on the sea surface and suspended within the water column, seawater samples were collected using a plankton net tow (200µm), with an adapted method from Cole et al. (2014). Conducted at the end of the survey, to prevent seabed disturbance of sediment and biota, surface water samples were obtained using a plankton net towed along the transect line (in both directions), at a walking pace of approximately $0.5ms^{-1}$ for a period of 2 min with a weighted bottle connected to the cod end of the net. A walking pace was necessary, as the water was too shallow to use a vessel. The mid-water column sample was collected using body weight to push down on the net, whilst moving it through the water. The slow tow speed and the washing of the plankton net between tows provided sufficient assurance that any difference in sample collection efficiency due to variation in tow speed, tow time or the net size was negligible. Seawater samples were stored in sealed 200ml plastic bottles during collection to minimise contamination. The bottles had been pre-rinsed with distilled water prior to use in the field (see Section 2.4.3).

2.2 Tissue Digestion Protocol

Organisms collected (sediment biota (20 individuals) and seagrass associated biota (4 individuals)) were digested to aid visual analysis of microplastics within the small sized organisms. The current absence of a standardised method for extracting microplastics from biological tissues, required protocol testing using enzymatic and chemical approaches. Preliminary tissue digestion experiments were carried out on test samples of similar biota (gastropods and amphipods), as identified in the seagrass bed, using existing procedures identified in peer-reviewed published literature. To improve digestion, gastropod species were initially removed from their shell using forceps (which had been cleaned with distilled water) and the inside of the shell was rinsed with distilled water to remove any loose biological tissue. The soft tissue, including gut contents and the whole body of the amphipods were digested using the method according to Leslie et al. (2013). Tissue protocol trials involved both chemical (10% KOH) and enzyme digestions (Proteinase-K). Proteinase-K (50°C for 18h) was more expensive and the least efficient in fully digesting the biota. The protocol with the greatest efficiency was 10% KOH (50°C for 2h) and was subsequently administered to all tissue digestions in this study. Furthermore, most documented polymers show resistance to this chemical digestant (Dehaut et al., 2016; Foekema et al., 2013; Rochman, 2015).

2.3 Microplastic Extraction from Samples

2.3.1. Seawater samples

Seawater was filtered using methods from Lusher et al. (2015). The ceramic filtration funnel was rinsed with distilled water before and after samples to prevent cross-contamination. Using a vacuum pump microplastics were removed from the solution and transferred on to a clean glass fibre filter paper

(FisherbrandTM, ⌀70mm, pore size: 0.7µm). Samples were stored in labelled Petri dishes (⌀100mm) and dried overnight, ready for microscope examination.

2.3.2 Seagrass samples

Prior to further analysis, all seagrass samples were visually identified to determine species. Using a Zeiss Stemi DRC stereomicroscope (magnification range 10x - 20x) samples were placed onto a clean Petri dish and manipulated using sterile forceps.

Seagrass samples were washed with 35ml of distilled water in a clean 50ml skirted centrifuge tube, by shaking the tube vigorously for 30s. The samples were then removed from the tube and placed in a sterile tray, where each blade of seagrass was scraped with the blunt side of a scalpel blade to remove any microplastics attached to the seagrass or biofilms on the blades. Any biofilms removed were washed back into the centrifuge tube using a glass pipette filled with distilled water. Samples were then filtered using a vacuum pump onto glass fibre filter paper and stored in Petri dishes for microscope analysis. As three blades were removed per replicate site, the number of microplastics identified were combined to represent one replicate. Occasionally, filter papers would clog when exposed to samples containing more debris and as a result, multiple filter papers were required.

2.3.3 Biota samples

Biological organisms collected from the sediment and seagrass were visually identified and measured (length, mm) using a Leica M125 C stereomicroscope (magnification range 8x - 100x) before digestion. Microplastics identified in the marine biota were recorded as number of microplastics per individual, as weight could not be accurately calculated. Each specimen was individually digested following the method outlined in Section 2.2. To neutralize the solution, 20ml of distilled water was added to each flask and swirled manually for 30s and left to cool to room temperature in the fume hood for 5 min. Samples were then filtered using a vacuum pump onto glass fibre filter papers, stored in Petri dishes and left to dry overnight in the fume hood, before analysis.

2.3.4 Sediment samples

Extraction of plastic particles from sediment samples was carried out following Blumenröder et al. (2017) density separation protocol. Ceramic crucibles were thoroughly cleaned with distilled water and weighed, prior to adding the sediment. Samples were covered and dried overnight in an oven at 50°C, until no moisture remained. The crucibles were weighed to determine the dry weight and covered with aluminium foil. A highly saturated solution of 100ml NaCl (384g L^{-1}) was added to the samples and vigorously agitated using a Teflon coated magnetic stirrer for 30s and then left to rest for 2 min, to allow the sediment to settle. The top section of the solution containing the microplastics (approximately 30ml) was carefully removed using a glass pipette connected to a vacuum pump by a silicon hose and transferred into a glass round-bottomed boiling flask. The extracted solution was vacuum filtered

through a glass fibre filter and placed in a covered Petri dish and left to dry overnight. Each sample was washed twice, and the results combined. Microplastics were expressed as, number of microplastics per kilogram of sediment (dry weight).

2.4 Microplastic Analysis of Samples

2.4.1 Microplastic quantification and visual sorting

All quantification and visual sorting of microplastics was conducted by the same operator throughout this study. All filter papers were observed under a high-powered Leica M125 C stereomicroscope (magnification range 8x - 100x) and items assumed to be microplastics were photographed using LASV4.8 software. Microplastics identified from the fieldwork samples were measured (length, mm), counted, photographed and categorised in terms of colour and shape; fibres (thread-like microplastics), flakes (thin microplastic shavings, possibly originating from larger objects) and fragments (small irregular shaped microplastics), and carefully removed, using fine forceps to a clean glass fibre filter. Fragments of algae on some of the filter papers from the seagrass samples were examined before being carefully removed with fine forceps during microscope examinations and discarded to better view the filter paper.

Microscopy was utilised during this study as a simple and cost-effective approach to microplastic identification. Exclusively using microscopy has been documented in instances where Fourier-Transformed Infrared Spectroscopy (FTIR) analysis was unavailable (Collignon et al., 2012; Song et al., 2015). Items of interest were visually identified as microplastics based on shape, size and colour. Fibres were inspected under a microscope to determine if they were of a plastic origin. Using visual techniques recorded by Dochia et al. (2012), fibres with a 'twisted ribbon shape' were considered cotton and removed from analysis.

2.4.2 Polymer verification

Microplastic polymer identification was conducted with samples >1.5mm in size (n = 54), using Attenuated Total Reflectance (ATR-FTIR; Thermo Fisher Nicolet IS). A Perkin-Elmer press was used to flatten microplastics to increase the surface area, before applying ATR-FTIR at a range of 450-4000cm^{-1}. The polymers were identified from the spectra generated and compared to known polymer types (Fig 3).

2.4.3 Contamination mitigation

During field collection and laboratory work, steps were taken to mitigate, where possible, the risk of post-sample contamination following the recommendations of Catarino et al. (2016), Cole et al. (2014) and Blumenröder et al. (2017). Prior to conducting fieldwork, all equipment used for collection or storage, was rinsed thoroughly with distilled water and sealed. The plankton net tow was thoroughly

rinsed between samples, to prevent cross-contamination. To minimise and monitor post-contamination of the samples, items of clothing prone to shedding fibres were avoided and any synthetic clothing was covered by a 100% cotton lab coat. All surfaces used in the laboratory were cleaned daily prior to conducting any work and all stereomicroscopes were cleaned prior to opening Petri dishes. Using a technique described by Woodall et al. (2015) a clean glass fibre filter paper was dampened using a sterile glass Pasteur pipette, placed into a clean Petri dish and left open on the workbench for the same duration as samples were uncovered, to assess atmospheric contamination. Prior to conducting laboratory work, equipment and glassware were rinsed thoroughly with distilled water and covered with aluminium foil. Equipment including a vacuum pump, forceps and scalpel were cleaned thoroughly between each sample with distilled water to prevent cross-contamination. All glassware and Petri dishes were cleaned in the dishwasher and rinsed out with distilled water, before reuse. To assess microplastic contamination from equipment, control samples (n = 2) were taken of the distilled water in its container. The uncontaminated distilled water was then used as a control solution to run blank tests for microplastic analysis to determine any contamination in the 50ml plastic centrifuge tubes (n = 20), the 200ml plastic bottles (n = 2) and the zip-lock field sampling bags (n = 20) prior to use. All tissue digestions were conducted in the fume hood with bench control filters papers. Control samples were taken of 10% KOH and filtered onto glass fibre filter paper and analysed for microplastic contaminants.

2.5 Data and Statistical Analysis

Statistical analysis was conducted using R software package 3.5.1 (R Core Team, 2018) and a probability level of ≤ 0.05 was considered to indicate statistical significance. All data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Bartlett test). Generalised Linear Models (GLMs) of log-transformed count data were utilised to assess the factors influencing microplastic distribution in samples. This included initially comparing total microplastic count data of sediment samples and seagrass blade samples. Microplastic count data were compared between sediment and seagrass samples using type of microplastic (fibre, flake, fragment) as a factor. A post-hoc Tukey (HSD) test was used to determine if the differences were statistically significant. This approach was also used to identify if location and seagrass abundance influenced the type of microplastic (fibre, flake, fragment) and the number of microplastics recorded.

3. Results

3.1 Microplastic Observations

Items of interest visually identified as microplastics, were observed in the majority of samples analysed (94.37%). A total of 280 individual plastic items were counted across all samples, ranging from 0.04 to 3.95mm (mean = 0.95mm ± 0.05 SE) in size. In this study, 20 out of 25 individuals of marine biota analysed contained microplastics. Observations on the number of microplastics recorded in each organism extracted from the seagrass blades and the sediment are documented in Tables 3 and 4 respectively. Microplastic fibres contributed >50% of the total microplastics observed, with an overall mean of 69.12% ± 5.77 SE across all samples, the majority of these were identified as blue in colour (45.52% ± 6.48 SE). With respect to the number of samples collected, sediment from the seagrass bed recorded the greatest number of microplastics (Table 2). The average number of microplastics per kilogram of sediment (dry weight) was recorded as 300 kg^{-1} ± 30 SE within the seagrass bed sediment and 110kg^{-1} ± 20 SE within the sediment controls. The average length of seagrass blades was recorded as 166mm ± 13 SE (3 seagrass blades per replicate sample, total n = 60). The average number of microplastics found on a seagrass blade was 4.25 ± 0.59 SE (n = 60).

3.2 Microplastic in Sediment and Seagrass Samples

3.2.1 Total number and type of microplastics in sediment

The number of microplastics were significantly higher in seagrass sediment samples compared to bare sediment ($F = 2.052$, $df = 1$ and 22, $p = 0.040$). Within-site variability was identified along the transect line at 10m, 20m, 30m, 40m, 50m and 60m ($F = 2.741$, $df = 1$ and 24, $p<0.02$) (Fig 1), highlighting the heterogenous nature of microplastic contamination. Microplastic abundance in the sediment was not significantly related to the density of the seagrass canopy at the sample location.

Whilst category of microplastic (fibre, flake, fragment) did not significantly vary along the transect line in both bare sandy sediment and seagrass bed sediment ($F = 2.208$, $df = 1$ and 24, $p>0.1$), the 60m sample site on the transect line had a significantly higher ratio of microplastic fragments to fibres, than any other 10m sample site, again highlighting the heterogeneous distribution of microplastics in the environment.

3.2.2 Microplastics adhering to the seagrass blades

All seagrass blade samples showed microplastic loading, irrespective of location on the transect line. A significant difference was identified between the number of microplastics recorded adhering to the blades and the location along the transect line at 10m, 30m, 40m, 50m and 60m ($F = 2.547$, $df = 1$ and 22, $p<0.05$) (Fig 2). The number of microplastics adhered to seagrass blades (4.25 ± 0.59 SE) was not significantly related to the length of those blades (166 ± 13mm) ($F = 1.806$, $df = 1$ and 59, $p = 0.060$),

nor to the category of microplastic (fibre, flake, fragment) regardless of placement on the transect line ($F = 2.52$, $df = 1$and 22, $p = 0.992$).

3.3 Analysis of Microplastic Type and Colour

Throughout microscopic examination, photographs of microplastics were collated and compared. Similarities of the type and colour of microplastics were observed between locations on the transect line (e.g. between sediment samples) and between water, sediment and biological samples (Fig 3). The occurrence of similar type and colour of microplastics throughout the study site demonstrates their ubiquity and suggests similar microplastic fragments were recorded in biota, sediment and adhering to the surface of the seagrass blades, irrespective of substrate or organism type.

3.4 Polymer Type

Of the 280 microplastic samples collected from the study site in Orkney, only those >1.5mm (n = 54) could be analysed using ATR-FTIR spectrometry. Of those samples analysed, 50 were positively identified as known polymers: 34% poly(ethylene), 22% poly(propylene), 14% polyamide, 10% polyether urethane, 10% polyester, 8% poly(styrene) and 2% poly(trimellitic) (Fig 4).

3.5 Microplastic Cross-Contamination

No microplastic contaminants were observed on the dampened filter paper controls, during this study. Throughout sample processing, the dampened filters were only exposed to atmospheric contaminants for the same duration as the samples were uncovered. Microscopic observation of controls identified only sand particles and small pieces of algae.

4. Discussion

This is the first known study to describe microplastic loading within a seagrass bed and to identify microplastic adherence to seagrass blades in a *Z. marina* bed. Whilst it can be difficult to compare samples from different substrate types (sediment, seagrass, biota, water), it is increasingly clear that microplastics are ubiquitous in the environment with microplastics observed in the majority of samples (94.37%) with the exception of four sediment-associated biota. Microplastics were quantified and categorised by type (fibre, flake or fragment) and colour. Overall, the similarity between groups of different variables (sediment, seawater, biota and seagrass blade) is shown in not only the type of microplastic but also the colour observed, such as white fragments recovered from sediment and adhering to seagrass blades; and turquoise fragments identified within the seawater and the sediment. The similar microplastic fragments and flakes were later shown to originate from the same polymer type. This corroborates Thompson et al. (2004) who found similar types of polymers in the water column as in sediments. Microplastics fibres (>50%) represented the main source of contamination across samples (microfibers were the only microplastics recorded in the bare sediment control samples), with an overall mean >69% across all samples, the majority of these fibres were identified as blue in colour (>45%). These blue microfibers were most abundant in sediment samples with the greatest number found within the *Z. marina* bed.

4.1 Microplastic Loading in the Sediment

Microplastic loading was observed within sediments of the *Z. marina* bed and were significantly higher than microplastics observed in bare sandy sediment (control sites) adjacent to the seagrass bed. This demonstrates seagrass beds ability to accumulate and retain microplastic particles, acting as a sink. Whilst within-site differences were observed, a greater number of flakes and fragments were found within the *Z. marina* bed, with the majority of microplastics observed in all sediment samples being fibrous (control sites contained only microplastic fibres). The variability between the 10m intervals on the transect is likely to be related to the structural complexity of the substrate directly above it, with the seagrass blades trapping the microplastic particles from the water column, that will eventually sink to the seabed. This study recorded abundant *Z. marina* along the transect line with no observable difference identified between the number of microplastics and the percentage cover of seagrass. The density of the *Z. marina* seagrass canopy did not appear to fluctuate significantly along the transect line, so microplastic loading in the sediment could not be correlated with seagrass cover. Hansen et al. (2017) recorded areas where seagrass density was high, the flow interaction with the seagrass blades reduced turbulence. This ability to attenuate the wave energy is due to the extent of seagrass coverage (Bradley and Houser, 2009). The seagrass blades can slow the rate at which microplastics suspended in the water-column travel through the seagrass canopy, thus increasing the capacity of seagrass blades to accelerate the sedimentation process (Van Montfrans et al., 1984) and, subsequently, microplastic accumulation.

Moreover, the sediment dynamics within a seagrass bed can obstruct the water flow, limiting the opportunity for microplastics captured within the seagrass bed to re-suspend. The relatively high concentrations of microplastics in sediment compared to the seawater samples, lend support to the suggestion that sediment acts as a sink. The commonly used seawater sampling method applied in this study (Lusher et al., 2015; Reisser et al., 2013) limits the catch rate to larger microplastics than those targeted in sediment sampling. Therefore, the method for extraction from sediment, targets a wider size range of microplastics than many seawater extraction methods. Future study could conduct simulations under different hydrodynamic regimes, under laboratory conditions, to determine suspension and settling rates of different microplastics (high and low density) fibres and particles.

4.2 Microplastics Adherence to the Seagrass Blades

Microplastic particles were discovered on every sample of seagrass collected. While a similar study by Goss et al. (2018) on the seagrass blades from a *Thalassia testudinum* bed in Belize, recorded an average of 3.69 ± 0.99 SE microplastics per blade, these were only recorded on 75% of the seagrass examined. The *Z. marina* blades analysed in this study showed a higher average of 4.25 ± 0.59 per blade (mean ± SE, n = 60), with no significant difference observed between the length of the seagrass blades analysed (166 ± 13mm SE (3 seagrass blades per replicate sample (n = 60)) and the number of microplastics recorded adhering to them. Significant differences in the numbers of microplastics on seagrass blades along the transect line highlight the heterogeneity of not only number of microplastics but also type (i.e. fibre, flake, fragment). This could be a result of the seagrasses ability to slow the flow rate, as microplastics suspended in the water column can meet the seagrass blades. Whilst the physical structure of seagrass blades allow for microplastic adherence, filamentous and calcareous epiphytes already present on the seagrass, as well as biofilm growth already present on plastic particles can further facilitate the adherence of microplastics to seagrass (Van Montfrans et al., 1984). Furthermore, *Z. marina* are naturally dynamic, with a growth season occurring in spring and early summer and leaf loss during the winter (d'Avack et al., 2018). As a result, any microplastics attached to the seagrass can be lost to the seabed or be consumed by sediment-associated organisms. Additionally, currents or wave action can transport seagrass detritus (Heck et al., 2008) potentially spreading the attached microplastics to another location within the seagrass, into the open ocean or washed up onto beaches. Orkney's hydrographical conditions could facilitate the effective distribution of microplastic contaminants around the island (Blumenröder et al., 2017).

4.3 Microplastics in Seawater

A greater number of plastic particles can be observed at high tide compared to low tide (Van Cauwenberghe et al., 2015; Kazmiruk et al., 2018). The number of microplastics identified within the seawater samples collected at low tide might be lower in comparison to other studies conducted at high tide or from a vessel. Although conducting a walking pace tow at a similar speed as Eriksen et al. (2014),

it was not efficient in allowing water to flow into the collecting vessel. This may have been due to a lack of strong current or waves, as although Deerness Sound is sheltered from both wave and tidal action (Thomson et al., 2014), the study site location is closer to the mouth of the Sound and may be influenced by a greater tidal influx.

Surface and mid-water column samples showed a high abundance of fibres in comparison to other sediment and seagrass blades. The prevalence of microplastic fibres on the surface and the mid water column in this study, could be due to an increase in buoyancy attributable to the higher surface to volume ratio (Karlsson, 2015). However, fibres were abundant in many of the samples, with microfibres the only microplastic observed in the sediment control sites. The high number of fibres settling on the sediment and seagrass could be a result of a decreased buoyancy from fouling organisms or entrapped in settling detritus (Barnes et al., 2009; Eriksen et al., 2014). Consequently, this can lead to marine organisms inadvertently ingesting microplastics, which have settled to the seabed, or on the seagrass blades.

4.4 Occurrence of Microplastics in the Seagrass and Sediment Biota

In this study 83.33% of marine biota analysed (n = 24) contained microplastics. This correlates well with Karlsson (2015) who identified that 85% of marine biota they studied (including amphipods and gastropods) contained microplastics. These results also align well to earlier studies where whole-body analysis of biota have been performed (Leslie et al., 2013). However, in the present study, a limited number of grazers were collected from the seagrass blades, as most were identified on the kelp species within the area such as *Saccharina latissimi. Gibbula* species have been reported to graze upon the epiphytes present on seagrass blades (Van Montfrans et al., 1984). It is possible the microplastics ingested by the collected *G. cineraria,* are adhering to the seagrass blades by filamentous and calcareous epiphytes or biofilms growing over the plastic particles or they ingested the microplastics from the kelp prior to grazing on the seagrass. Future study could compare the number of microplastics recorded on the seagrass blades to any microplastics identified on kelp, where they co-occur. Goss et al. (2018) found the microplastic adhering to the surface of the seagrass blades were either partially or fully overgrown by a diverse epibiont community. Furthermore, the average number of microplastic observed in the seagrass biota suggests grazing species may be more susceptible to inadvertently ingesting microplastics. The consumption of microplastics by small organisms can facilitate their transport to higher trophic levels, including commercially important species (Heck et al., 2008). Although living seagrass is commonly consumed (Heck et al., 2008), residual seagrass blades can directly enter the food web as detritus. Van Montfrans et al. (1984) observed unconsumed material from *Z. marina* leaves dropped to the sediment below. Therefore, any microplastics attached to the seagrass blades can become available as a food source for macrodetritivores (e.g. amphipods) and decomposers. Remy et al. (2015) reported the ingestion of artificial fibres (not synthetic) by amphipods living in the macrophytodetritus

of the seagrass species *Posidonia oceanica*. However, their study identified no connection between the artificial fibres and the seagrass. Observations on the number of microplastics recorded in each of the sediment-associated biota, showed no link between the number consumed and the size of the organisms (Table 3 and 4). The average size of microplastics within the biota were relatively small in comparison to the microplastics found within the sediment, seawater and on the seagrass. The biota are more likely to consume these micro-sized plastic particles due to the organism's small size and these microplastics are within the same size range as their food (Leslie et al., 2013).

4.5 Polymer Identification

In this study, only ATR-FTIR spectroscopy was available and as a result, a limited number of items of interest were successfully confirmed as a plastic particle. However, 50 of the 54 samples analysed were identified as plastic. Analysis of flake and fragments was carried out using the Hummel polymer library, which allowed comparisons between the spectra of the items of interest against the library. Analysis found all white microplastic fragments to be poly(ethylene), all turquoise fragments were polyether urethane and all red fragments were identified as poly(propylene). The similarities for each of the coloured fragments identified, suggests that all white, turquoise and red may have originated or fragmented from the same source respectively. Brightly coloured fibres are generally confidently defined as "plastic", however further analysis by Blumenröder et al. (2017) using FTIR suggests the colour of the microfibre is not a reliable indicator of polymer type. Overtime weathering or exposure to UV radiation can result in photodegradation of plastic (Thompson et al., 2004; Barnes et al., 2009).

5. Conclusion

Monitoring microplastic loading in ecologically important seagrass beds is vital to address the biological impact of microplastics within this ecosystem and determine the potential trophic transfer in marine food webs. This study in Orkney provides the first known observation of microplastic loading in a *Z. marina* seagrass bed and microplastic adherence to seagrass blades. The prevalence of microplastics throughout the biological samples highlights their ubiquity within the marine environment and increased probability of ingestion, in particular by marine organisms grazing on seagrass. The limited number of studies to confidently compare data demonstrates the requirement for standardised methods for microplastic extraction and quantification in marine biota and the removal of microplastic particles from seagrass blades. Furthermore, microplastic loading must be addressed on a larger scale, to determine the accumulation of microplastics at additional sites within the Orkney Islands and on mainland Scotland.

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Table 1. Seawater depth, co-ordinate locations of transect tape and sediment control sites.

Sample Type	Location	Latitude	Longitude	Depth (m)
Transect Line	Start Point (0m)	58.9521172	-2.778039	0.95
	End Point (100m)	58.953056	-2.777789	1.15
Sediment Controls	Sand 1[1]	58.951956	-2.779975	0.97
	Sand 2[1]	58.951717	-2.779706	0.82
	Sand 3[1]	58.951472	-2.779053	0.50
	Adjacent 1[2]	58.951975	-2.778422	0.79
	Adjacent 2[2]	58.95185	-2.778172	0.65

[1] Sand controls collected in clear sandy sediment.
[2] Adjacent controls sampled within sandy sediment at the boundary of the seagrass bed.

Table 2. The number of microplastics per sample and the average length of microplastics observed (mean ± SE).

Sample Type	Sample (n)	Average No. of Microplastics	Average Microplastic Length (mm)
Sediment Controls	5	3.40 ± 0.50	1.12 ± 0.19
Seagrass Sediment	20	5.65 ± 0.55	1.15 ± 0.07
Seagrass Blades	60	4.25 ± 0.59	0.91 ± 0.08
Seawater*	2	7.50 ± 1.50	1.10 ± 0.24
Sediment-associated Biota	20	1.60 ± 0.32	0.35 ± 0.05
Seagrass-associated Biota	4	4.50 ± 0.96	0.60 ± 0.13

*Surface and mid-water column samples were combined due to lack of replicates.

Table 3. Number of microplastics observed within each *Gibbula cineraria,* collected from the seagrass blades.

Location	Species	Size (mm)	No. of Microplastics per Individual	Fibre	Flake	Fragment
30m R2*	*Gibbula cineraria*	6	5	1	0	4
	Gibbula cineraria	7.9	3	2	0	1
60m R2*	*Gibbula cineraria*	6.3	7	5	0	2
	Gibbula cineraria	4	3	2	1	0

* R2 represent replicate point 2 at each location on the transect line.

Table 4. List of the marine biota collected within the sediment of the seagrass bed.

Location	Species	Size (mm)	No. of Microplastics per Individual	Fibre	Flake	Fragment
20m R1*	*Gibbula cineraria*	4	5	4	0	1
	Bittium reticulatum	8.5	2	2	0	0
30m R2*	*Gammarus* species	2.7	1	1	0	0
	Gammarus species	3	1	1	0	0
	Rissostomia membranacea	3.9	4	2	1	1
40m R1*	Unidentified *Amphipoda*	2.5	1	1	0	0
40m R2*	*Nephtys* species	46	3	2	0	1
	Gibbula cineraria	2.7	0	0	0	0
60m R1*	*Gammarus* species	3	2	2	0	0
	Chaetogammarus species	3.2	1	0	0	1
70m R1*	*Lysianassa* species	1.3	0	0	0	0
	Unidentified *Amphipoda*	0.97	2	0	2	0
	Unidentified *Amphipoda*	1.1	2	1	1	0
80m R1*	*Gammarus* species	2	1	1	0	0
	Unidentified *Amphipoda*	1	1	1	0	0
80m R2*	*Lysianassa* species	1	0	0	0	0
90m R1*	*Chaetogammarus* species	2	1	1	0	0
	Unidentified *Amphipoda*	2	1	0	1	0
	Unidentified *Amphipoda*	1.8	0	0	0	0
100m R2*	*Eulalia viridis*	52	4	3	1	0

* R1 and R2 represent replicate points 1 and 2 at each location on the transect line, respectively.

Figure captions

Figure 1. Mean number of microplastics (± SE, n = 25) recorded in sediment at each point on the 100m transect line and type of microplastic identified (fibre, flake, fragment). The line graph shows the average percentage cover (%) of seagrass at each sample point within the seagrass bed. Significance are represented by * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

Figure 2. Mean number of microplastics (± SE, n = 20) found adhering to seagrass blades, categorised by type of microplastic (fibre, flake, fragment) and location on the seabed. Significance are represented by * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

Figure 3. Examples of similar microplastics found across sample locations and the corresponding ATR-FTIR. White poly(ethylene) fragments found in sediment (**A**) 30m and (**B**) 70m, and on seagrass blade surface (**C**) 30m and (**D**) and 70m.

Figure 4. Proportion of polymer types of positively identified as a microplastic using ATR-FTIR spectroscopy

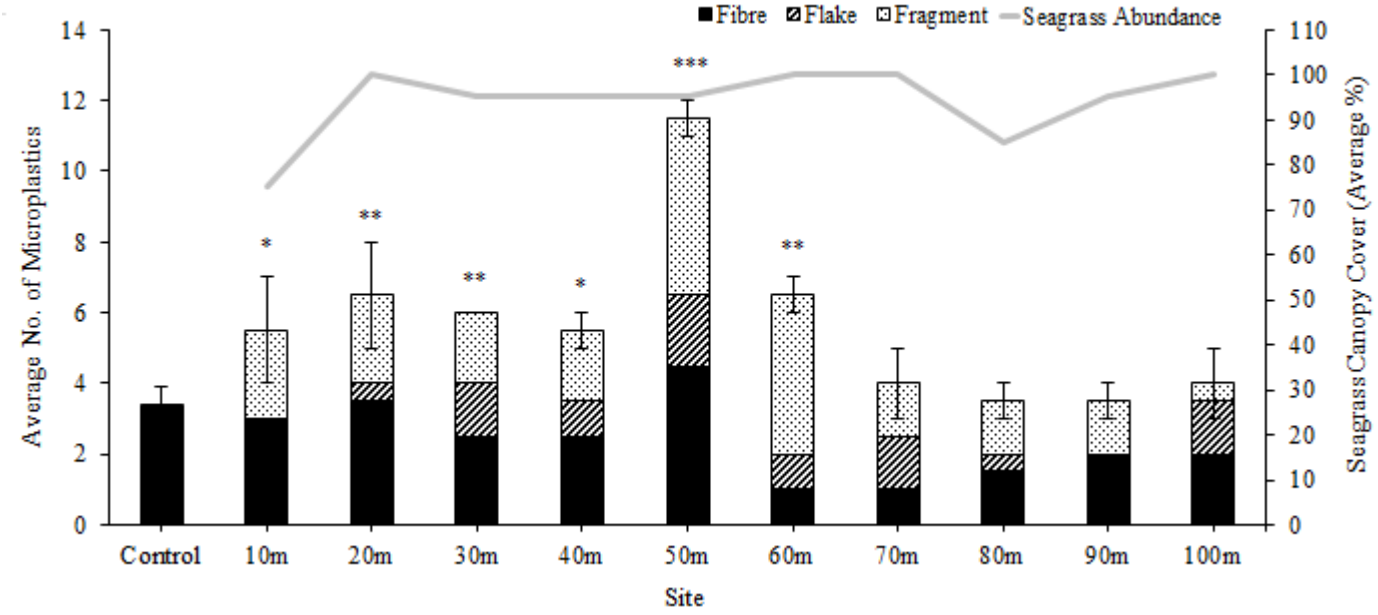
Fibre
Flake
Fragment
Seagrass Abundance
Average No. of Microplastics
Seagrass Canopy Cover (Average %)
Site
Control
10m
20m
30m
40m
50m
60m
70m
80m
90m
100m
0
2
4
6
8
10
12
14
0
10
20
30
40
50
60
70
80
90
100
110
*
**
**
*
***
**

Fig 1.

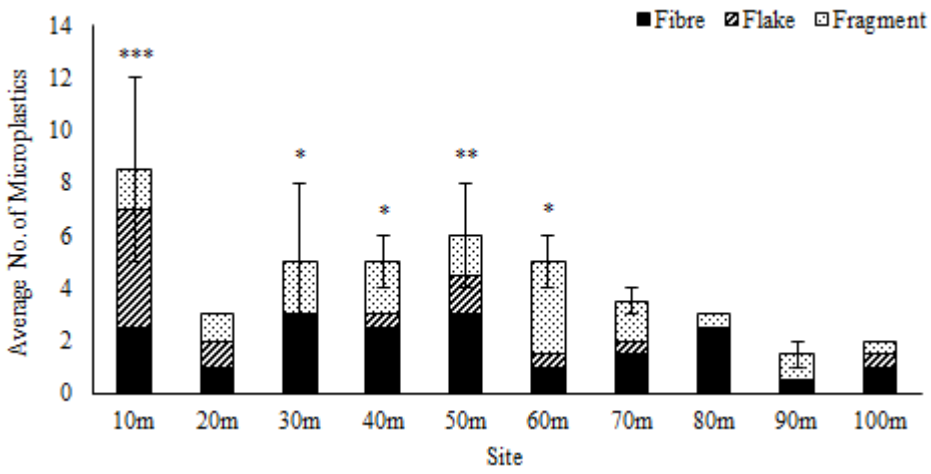
Fibre
Flake
Fragment
Average No. of Microplastics
14
12
10
8
6
4
2
0
***
*
**
*
*
10m
20m
30m
40m
50m
60m
70m
80m
90m
100m
Site

Fig 2.

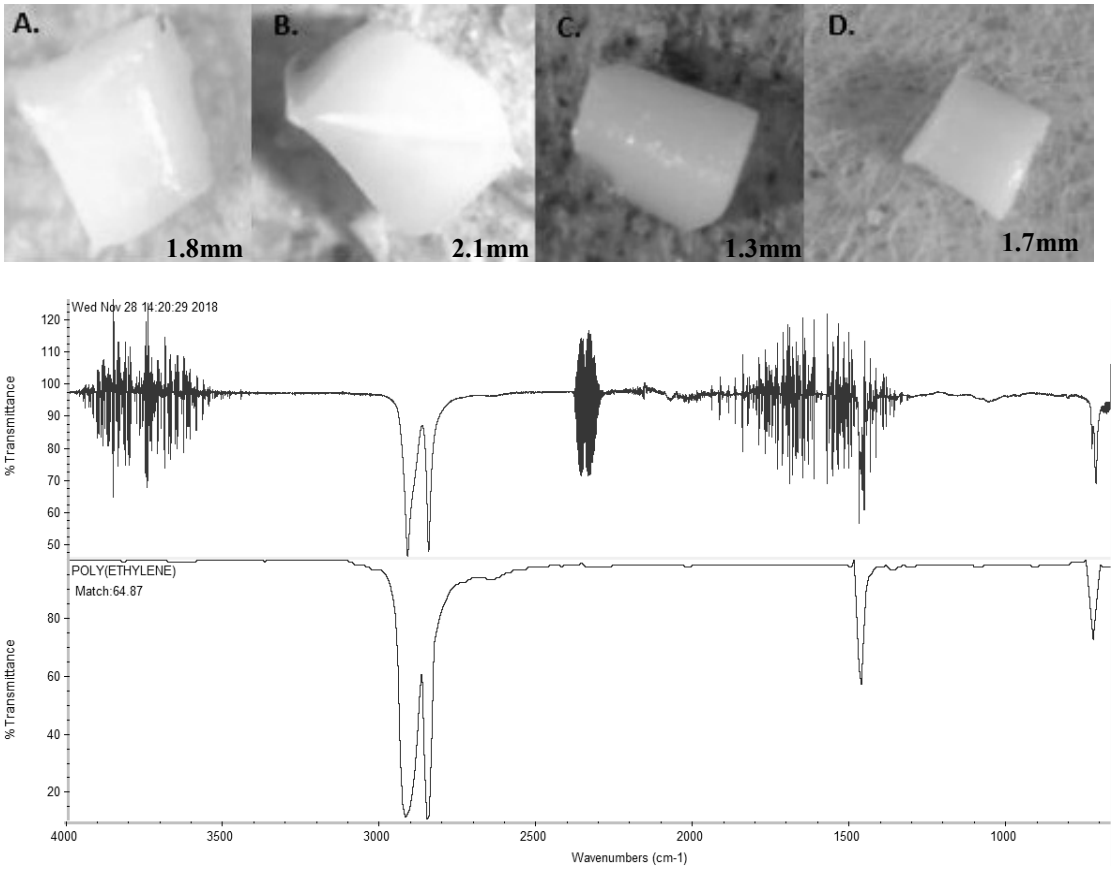
A.
1.8mm
B.
2.1mm
C.
1.3mm
D.
1.7mm
Wed Nov 28 14:20:29 2018
% Transmittance
POLY(ETHYLENE)
Match:64.87
% Transmittance
Wavenumbers (cm-1)

Fig 3.

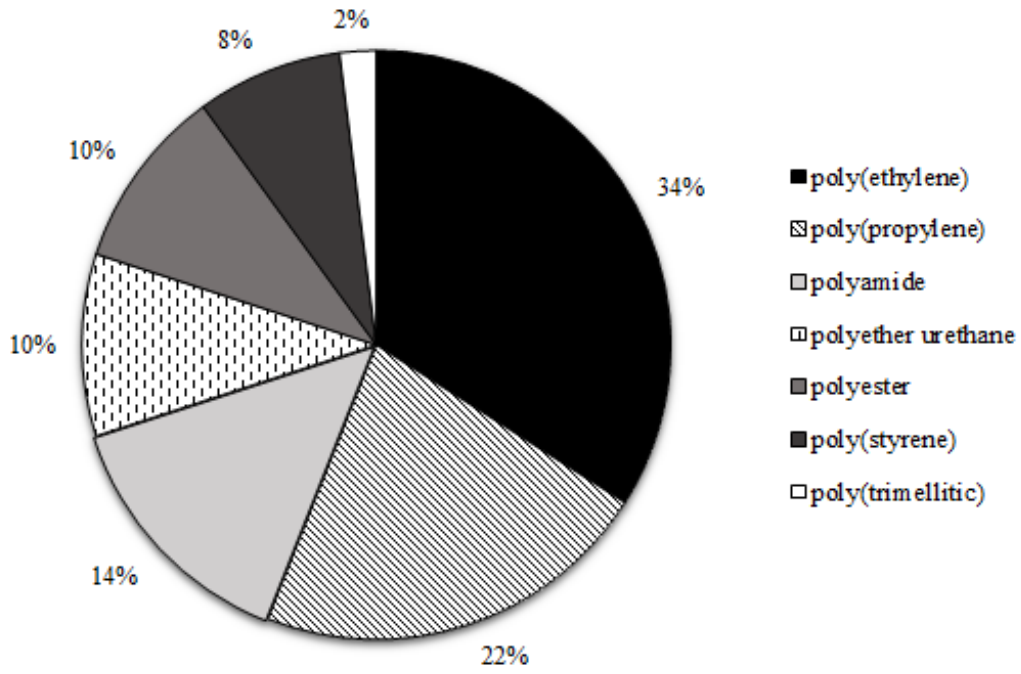
2%
8%
10%
34%
10%
14%
22%
poly(ethylene)
poly(propylene)
polyamide
polyether urethane
polyester
poly(styrene)
poly(trimellitic)

Fig 4.