Movement of plastic debris through the benthic marine ecosystem and its interactions with benthic organisms

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I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

Signature:

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Abstract

Plastics, including those of buoyant polymers are increasingly found in high concentrations in benthic sediments where they have the potential to be ingested and interact with a diverse range of benthic marine species. This thesis combines field work and laboratory mesocosm experiments to look at the presence and characteristics of synthetic particles found *in situ* within estuarine benthic habitats, and then investigates experimentally how interactions with benthic species might influence the fragmentation and movement of plastics within a test benthic ecosystem.

Firstly, a field study was undertaken to determine the abundance and particle characteristics of synthetic particles in benthic sediment and within natural populations of the benthic-dwelling polychaete, *Hediste diversicolor*. Sediment (10 samples per site) and worm (30 individuals per site) were collected from three locations of differing anthropogenic influence across South Devon, UK. Samples were analysed for synthetic particle content using density separation for sediments or tissue digestion, followed by particle identification using Fourier-transform infrared spectroscopy (FTIR). All sediment samples analysed contained synthetic (i.e., plastic and/or modified cellulose) particles, but with significantly higher abundance in sediment from the Plym estuary (mean 116 synthetic particles kg^{-1} +/- 18.09 SE) compared to Kingsbridge (mean 62 synthetic particles kg^{-1} +/- 13.55 SE; $P < 0.001$). Of the *H. diversicolor* sampled, 48% of the worms contained synthetic particles with a mean of 0.73 synthetic particles per worm +/- 0.15 SE and no difference in particles per worm by site ($P = 0.30$). The dominant polymer type found within the sediment was polypropylene, accounting for 30 % of the total synthetic particles across the three sites. Semi-synthetic cellulose fibres and polystyrene particles were the most prevalent particle types found within *H. diversicolor*.

The potential role of benthic invertebrates in the fragmentation and movement of plastic litter within a test benthic ecosystem was investigated via a mesocosm study. Biofouled polyethylene (PE) crates (half a crate per tank) were used as the test macroplastic. Mesocosms comprising three benthic species with different functional roles; the purple sea urchin *Paracentrotus lividus,* the blue mussel *Mytilus edulis* and the sediment-dwelling polychaete, *Alitta virens* were

maintained for 13 days under four treatment scenarios (3 tanks per treatment); 1) a no plastic control comprising all species and macroalgae but no added crate, 2) plastic crate, all organisms but macroalgae absent, 3) plastic crate, all organisms and macroalgae present and 4) plastic crate, urchins and ragworms (no mussels) with macroalgae present. In every tank containing a plastic crate and urchins, small plastic fragments were recovered from the water and from the sediment. PE fragments were present within 100 % of urchins (6.3 ± 1.6 (SE) particles per individual), 62 % of mussels (3.5 ± 0.6 (SE) particles per individual) and 65 % of ragworms (2.2 ± 0.5 (SE) particles per individual), confirming urchins are effective at generating plastic fragments (size range 10.2 μm to 5816.6 μm) that are subsequently bioavailable for uptake by benthic-dwelling organisms. In this experimental system, a combination of urchin food availability and mussel presence acted to increase the bioavailability and uptake of plastic fragments into a sediment-dwelling polychaete with a ~ four-fold increase in PE fragments found within ragworms when both macroalgae and mussels were present (3.6 ± 0.5 (SE) particles per individual) compared to mussel absence (0.9 ± 0.2 (SE) particles per individual), and a ~ 1.6-fold increase compared to macroalgae absence (2.2 ± 0.7 (SE) particles per individual).

Overall, this work advances the understanding of how organisms alter the distribution, accumulation and fate of microplastic in the benthic ecosystem while also highlighting the prevalence of synthetic particle contamination in benthic sediments.

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Definitions and Abbreviations

Macroplastic: > 10mm
Mesoplastic: 1 to <10 mm
Microplastic: 1 to <1000 µm
Nanoplastic: 1 to <1000 nm
Synthetic particle: Anthropogenic highly modified cellulose polymers in addition to petrochemical based plastics

ANOVA: Analysis of variance
ASW: Artificial seawater
ATR: Attenuated total reflection
CE: Cellulose
FTIR: Fourier-Transform Infrared
KOH: Potassium hydroxide
PAHs: Polycyclic aromatic hydrocarbons
PAM: Polyacrylamide
PCBs: Polychlorinated biphenyls
PE: Polyethylene
PP: Polypropylene
PS: Polystyrene
PVC: Polyvinyl chloride
SEM: Standard error of the mean
SMI: Sediment Microplastic Isolation Units
UPVC: unplasticised polyvinyl chloride
WWTW: Wastewater treatment works
$ZnCl_2$: Zinc chloride

Chapter 1: Literature Review

Movement and transformation of plastic debris through marine ecosystems

Plastic is a precious commodity, a fundamental component of our everyday lives and the running of the modern era. However, since the start of its mass production in the 1940s, the extensive use of plastic in today's society and the lack of appropriate disposal, has led to the contamination of marine habitats worldwide. In 2016, world plastic production stood at 335 million tonnes, a 36.7% increase on the 245 million tonnes produced only a decade earlier (Plastics Europe, 2008, 2017). While a proportion of this rising production is collected and recycled (an estimated 2.5% of the global production in 2016) (Plastics Europe, 2017), the majority of plastic debris is mismanaged and discarded, thereby contributing to the accumulation of plastic in our oceans (Schmidt *et al.,* 2017).

Out of the estimated 8,300 million metric tonnes of virgin plastics produced prior to 2017, 6,300 Mt is now plastic waste (Geyer *et al.,* 2017), highlighting that minimising releases into the environment and understanding negative impacts is essential. This review aims to highlight the ubiquitous nature of plastic debris in the marine ecosystem, examine the processes by which microplastics enter and move within the environment and to consider the risks that plastic pollution poses to marine organisms, particularly in benthic ecosystems.

1.1 Introduction to Microplastics

Microplastic is a widely-recognised term describing the heterogenous assortment of plastic particles, recently classified as 1 - 1000 μm in size (Hartmann *et al.,* 2019). Microplastic can be split into two categories: primary and secondary microplastics. Primary microplastics are manufactured to be of microscopic size, generally associated with cosmetics (Napper *et al.,* 2015), air-blasting media (Gregory, 1996), automotive tyre wear (Kole *et al.,* 2017), shedding from synthetic clothing (Napper & Thompson, 2016) and pre-production plastic pellets commonly known as nurdles (Karlsson *et al.,* 2018). These plastic particles can enter the marine environment inadvertently through wastewater outfalls (including domestic input e.g. from washing machines), sewage outlets, surface runoff and rivers amongst others (Siegfried *et al.,* 2017). Upon entering the marine environment, they are at a size already bioavailable to certain organisms (Farrell & Nelson, 2013; Setälä *et al.,* 2014).

In contrast, secondary microplastics result from the fragmentation and degradation of larger plastic debris where macro- (> 10 mm) and mesoplastics (1 - 10 mm) degrade into smaller microplastics (< 1000 µm). Common items that contribute to secondary microplastics include plastic bottles, food packaging, bags and fishing gear (GESAMP, 2015). There are numerous factors contributing to fragmentation of plastics in the marine environment including the physical stress from waves and currents (ter Halle et al., 2016), exposure to sunlight and oxidants and general weathering over time (Browne et al., 2007; Gewert et al., 2015). These degradation pathways make attributing microplastics to a single source problematic.

Diverse fragmenting mechanisms give rise to the varying microplastic shapes persisting in our oceans, some seemingly more prevalent than others. Certain studies have demonstrated the predominance of microfibres and fragments compared to microbeads, especially in the deep sea and seafloor sediments (Sanchez-Vidal *et al.,* 2018; Woodall *et al.,* 2014), fibres often accounting for 80 – 90% of microplastic counts (Gago *et al.,* 2018; Cesa *et al.,* 2017). Synthetic fibres are characterised by their threadlike appearance (below 5 mm in length but with a high relation length/radius) (Gago *et al.,* 2018). This makes them available for interaction within marine biota in different trophic levels. One study sampling the sub-surface waters of the Atlantic Ocean highlighted the dominance of fibres accounting for 94% of microplastics found between the Bay of Biscay and Cape Town, South Africa (Kanhai *et al.,* 2017). Additional studies supported this finding stressing again the high proportion of fibres in both the sediment and pelagic zones of the marine environment (Desforges *et al.,* 2016; Martin *et al.,* 2017; Nor & Obbard, 2014; Naji *et al.,* 2017). This discovery points to a previously underreported and unpublicised plastic fraction, now becoming a focus of current plastic research and media campaigns such as '#WhatsInMyWash?' (Restorick, 2018). It illustrates the plastic shape most likely to be exposed to biota in the natural environment, thereby highlighting the importance of environmentally realistic experimental designs involving microfibres as opposed to microbeads.

1.2 Sampling microplastics

Sampling efforts have frequently identified microplastics in the upper range of the microplastic size scale (Barrows *et al.,* 2018; Wessel *et al.,* 2016), with a lack of smaller particles amongst datasets, most likely due to sampling technique, not owing to an absence in the environment (Lenz *et al.,* 2016). Currently, the preferred surface microplastic sampling technique is through surface-trawling neuston nets with a mesh size typically around 333 µm (Law & Thompson, 2014; Zhao *et al.,* 2018). This means microplastic particles smaller than 333 µm quite simply 'fall through the net' and evade detection. The size of these smaller microplastics renders them available, to a wide range of organisms through ingestion, generating yet more potential for physical and toxicological harm to the smallest marine life forms (Cole *et al.,* 2013, 2015; Jeong *et al.,* 2017). Datasets of smaller microplastic abundance and in particular nanoplastic distribution in the ocean are lacking and should be the focus of future sampling studies.

Sampling techniques to predict total plastic abundance also include sediment and biological sampling, beach combing and observation surveys (Filella, 2015; Ryan *et al.,* 2009). Trade-offs exist within all sampling techniques; notably grab samples will capture the whole range of plastic particles but the small sample size can result in high levels of variability between replicate samples (Barrows *et al.,* 2017). On the other hand, trawling neuston nets sample greater volumes of water however the extraction efficiency of the smaller plastic particles is compromised.

Contamination control is an important process both in the field and in the laboratory. Airborne microplastics and microfibers are released readily from daily materials, contaminating both indoor and outdoor air (Dris *et al.,* 2016, 2017; Gasperi *et al.,* 2018). Levels at which these contaminants persist in the air vary and are dependent on air circulation and the clothes people are wearing, amongst other factors. Following protocols to limit airborne contamination is crucial, however without assessing contamination levels throughout experiments, scientists are now questioning the reliability of results (Prata *et al.,* 2020)

1.3 Global distribution of floating microplastics and sources

Early plastic research focussed on determining the abundance of microplastics floating on the surface of our seas. Sampling all 360 million square kilometres of global ocean would be logistically impossible and so several techniques are used to sample a sub-section of this vast area, whereupon one can employ modelling to predict plastic pollution abundance on a larger scale.

The temporal and spatial variability of plastics is not wholly understood; it moves horizontally and vertically and changes its shape and buoyancy as it ages, weathers and fouls. Computer modelling enables movements of microplastics, both horizontally and vertically, to be predicted. The surface drifting of buoyant particles such as polyethylene, polypropylene and expanded polystyrene is dominated by wind and wave conditions (Zhang, 2017). Both Stokes drift and Ekman transport mechanisms are key to the movement of surface-dwelling marine debris (Bi *et al.,* 2012), whereas those residing in the subsurface either due to increased density or Langmuir circulation (Teixeira, 2019) are primarily subjected to ocean currents. Physical characteristics of the plastics, such as density, shape and size determine the speed and trajectory at which these particles are transported (Zhang, 2017). Inputting this type of data into models enables predictions of the abundance and movements of microplastics in our oceans. Scientists are also using simulation backtracking of models to shed light on where the plastics obtained through fieldwork samples originated (Peeken *et al.,* 2018). Depending on the task, models can be incredibly useful, however wholly relying on them for microplastic fate and transport is risky as their credibility is based solely on how accurate the hydrodynamic input data is that underpins them. In using results from fieldwork, one can support (or reject) model predictions. If the models are deemed accurate, their results can act as prioritisation tools for cleaning up plastics.

A landmark oceanographic modelling paper by van Sebille *et al.,* estimated, using three different ocean circulation models combined, that the accumulated number of surface floating microplastic particles in 2014 ranged from 15 to 51 trillion particles, weighing between 93 and 236 thousand metric tons (van Sebille *et al.,* 2015). Here, even when taking the more conservative estimate of 93 thousand Mt of ‘small’ (nominally <200 mm) plastic debris into account, there is huge potential for ecological impact (Rochman *et al.,* 2016) especially considering microplastics are indiscriminate in terms of location.

While there is a lack of physical microplastic sampling amongst certain marine regions, oceanic circulation modelling has been used to predict the accumulation of microplastics in all five subtropical gyres (Lebreton *et al.,* 2012; Maximenko *et al.,* 2012). It appears microplastics are amassing in central convergence zones due to the actions of currents, wind and wave induced drift and turbulent vertical mixing (Zhang, 2017). High concentrations of floating plastic debris have been documented in the North Atlantic (Law *et al.,* 2010) and Pacific oceans (Eriksen *et al.,* 2013) amongst others. The presence of a so called 'garbage patch' was recorded in the central South Pacific with abundance and mass of surface plastic concentrations averaging 26,898 particles km^{-2} and 70.96 g km^{-2} respectively (Eriksen *et al.,* 2013). The North Pacific Subtropical Gyre otherwise known as the 'Great Pacific Garbage Patch', located halfway between Hawaii and California covers an estimated surface area of 1.6 million square kilometres and is said to contain 80,000 tonnes of floating plastic waste; a value four to 16 times the amount previously estimated (Lebreton *et al.,* 2018). Seasonal and interannual variabilities change the positioning of the 'garbage patch' (Goldstein *et al.,* 2013) and so models are relied upon to gauge the abundance of plastics at different times of the year.

Dependent on region, various currents play important roles in the distribution of microplastics, additionally influenced by anthropogenic inputs. A global model used to predict plastic emissions from rivers into the world's oceans highlighted that the 20 most polluting rivers, the majority of which are located in Asia, accounted for 67% of the total global plastic entering the oceans every year from rivers (Lebreton *et al.,* 2017). The positioning of those river mouths undoubtedly plays a part in the global distribution of microplastics. The largest of those contributing catchments is the Yangtze River discharging into the East China Sea, followed by the Ganges into the Bay of Bengal. The Kuroshio, a north-flowing ocean current in the Northwestern Pacific, is known to play an important role in the retention and distribution of microplastics from nearby terrestrial sources (Pan *et al.,* 2019). Eddies also influence the abundance and spatial distribution within smaller regions, such as that near Taiwan (Yuan *et al.,* 2014), however the overall flow direction north-eastward towards the Oyashio means microplastic circulation away from the Yangtze delta, across the East China sea and into the North Pacific is irrefutable. Similarly, the north equatorial current transports plastics from the Ganges away from the Bay of Bengal and over time, southwards into the Indian Ocean. Here we can see the highly influential impact of currents, themselves governed by wind patterns, temperature and salinity variations, transporting pelagic and surface dwelling microplastics horizontally away from coasts and across oceans.

Predictably, there are correlations between high levels of plastic pollution in areas neighbouring densely populated coastal regions (Jarvie *et al.,* 2000; Murphy *et al.,* 2016; Tibbetts *et al.,* 2018). Inadequate wastewater treatment works (WWTW), industrial manufactures and recreational and commercial fishing for example, each contribute considerably to coastal plastic pollution (Andrady, 2011; Cole *et al.,* 2011; Derraik, 2002). To illustrate, it is without doubt that the most densely populated coast on Earth, the eastern coast of the Asian continent accommodating one-third of the global coastal population (CIESIN, 2012), influences the high plastic load of the North Pacific Ocean. Results of a study identifying microplastic abundance in East Asian Seas in 2015 demonstrated concentrations 16 times greater than that of the North Pacific (Isobe *et al.,* 2015). This suggests proximity to densely populated coastal zones as well as the activities and infrastructure existing within certain regions play a significant role in microplastic accumulation (Beer *et al.,* 2018). Intriguingly however, there are numerous studies demonstrating substantial build-up in central convergence zones thousands of kilometres from land (Eriksen *et al.,* 2014; Kaiser, 2010; Yamashita & Tanimura, 2007). This indicates environmental factors such as currents, wave induced drift, tides and turbulent vertical mixing each play a part not only in the transport of microplastics vast distances but also in governing their fate (Kukulka *et al.,* 2012; Zhang, 2017).

It has come to light that even the remotest regions are also at risk of microplastic pollution (Hamid *et al.,* 2018). Concentrations, some of which exceed that in the North Pacific Subtropical Gyre, have been found in the remote Arctic (Barrows *et al.,* 2018; Cózar *et al.,* 2017a). This not only highlights the presence of a plastic hotspot kilometres away from densely populated regions, but also demonstrates their persistence and spatially dynamic nature. Looking at the global patterns of marine microplastics, one study found concentrations in the Arctic to be higher than any other ocean basin worldwide (Barrows *et al.,* 2018). A circumpolar survey of microplastics carried out in 2013 indicated that of all the plastic loads found in the Arctic Ocean, 95% is found in the Northeastern Atlantic sector, specifically the Greenland and Barents seas (Cózar *et al.,* 2017b). The use of long-term model simulations then helped to propose transportation of the plastics occurred via the Atlantic branch of the thermohaline circulation. On reaching these latitudes, plastics become incorporated into sea ice (Baztan *et al.,* 2017; Lusher *et al.,* 2015), consequently acting as a sink of such particles in the Arctic (Peeken *et al.,* 2018). This is again proof that proximity to plastic disposal is not the only determinant in regard to microplastic distribution in our oceans.

1.4 Benthic microplastics and downward flux

Whilst early microplastic studies concentrated on the floating contingent, there is now increasing evidence that there is a vast discrepancy between the volume of plastic entering our oceans and the amount recorded at the sea-surface level (Cózar *et al.,* 2017b). An estimated 93 to 236 thousand metric tonnes are found at the surface but 12.7 million metric tonnes of plastic is thought to have entered the oceans (Jambeck *et al.,* 2015; van Sebille *et al.,* 2015). There are two possible reasons for this discrepancy; either our inability to successfully quantify floating microplastic has meant we are vastly underestimating its occurrence or alternatively, microplastics are being transported vertically away from the surface to deep water and/or benthic sediments. Only through the use of the aforementioned sampling techniques can scientists ascertain the fate of the ‘missing plastic’ in our seas.

The presence of microplastic particles in the ocean benthos rather than solely surface and pelagic zones is becoming more apparent (Courtene-Jones *et al.,* 2017; Munari *et al.,* 2017; Peng *et al.,* 2017; Van Cauwenberghe *et al.,* 2013; van Sebille *et al.,* 2015; Woodall *et al.,* 2014). Evidence from Woodall *et al.,* (2014) reveals that of the regions sampled (Atlantic Ocean, Mediterranean Sea and Indian Ocean), microfibres were up to four times more concentrated in the deep-sea sediments than in the contaminated sea-surface waters. Moreover, if the most conservative estimates of microfibre abundance are extrapolated (1.4 to 40 pieces per 50 ml), they estimate 4 billion microfibres per km^2 to be present in the Indian Ocean seamount sediment alone.

Predicting the vertical movement of microplastics and the factors that contribute to those pathways is still very much an active area of research. Scientists are using mesocosm-scale experiments in the laboratory, in conjunction with modelling, to understand these complex transport mechanisms.

It is known that varying microplastic densities, altered further by the development of biofilms and weathering on the microplastic surface, contribute to their levelling out in different depths of the water column and benthos (Andrady, 2011) (Figure 1.1). Weathering works by reducing the microplastic mass thereby reducing the particles propensity to sink, whereas biofilms increase the mass of the plastic and increase sinking propensity. When the density of the particle with the added organic material exceeds that of the seawater, it starts to sink (Ye & Andrady, 1991; Long *et al.*, 2015). This is a mechanism by which floating plastic can be vertically transported away from the surface waters, down towards the sediment, subsequently thereby altering their fate and bioavailability to other organisms. Processes such as biofilm formation, otherwise known as biofouling (Long *et al.*, 2015; Kooi *et al.*, 2016; Kaiser *et al.,* 2017) as well as incorporation into marine aggregates such as marine snow (Porter *et al.,* 2018) and faecal pellets (Cole *et al.,* 2016) all contribute by increasing the ballasting effect on microplastics over time. Biofouling enhances microplastic deposition to marine sediments, albeit with a velocity somewhat dependent on plastic type and water salinity (Kaiser *et al.,* 2017). Of the two plastics tested (negatively buoyant polystyrene and positively buoyant polyethylene), biofouling enhanced their sinking velocity compared to virgin particles, offsetting differences in polymer density. Additional factors further affect sinking velocities such as biofilm thickness and composition, temperature and light availability.

Additionally, the ingestion and egestion of microplastics in surface waters by pelagic species such as copepods are likely to alter the surface properties and sinking behaviours of particles (Cole *et al.*, 2013). Post-ingestion, copepods egest faecal pellets laden with microplastics. Depending on the buoyancy of these pellets, they can act as a potential route for microplastic transport to the benthos. The role of microplastics incorporated into marine snow was noted as a further potential transport link from the surface to the benthos (Porter *et al.,* 2018). Using laboratory-manufactured marine snow, Porter *et al.,* (2018) demonstrated how sinking rates of all tested microplastics increased when incorporated into snows, with large changes observed for the buoyant polymer polyethylene, a polymer ordinarily expected to remain in the surface-waters due to its positive buoyancy. Incorporation into snows also increased microplastic bioavailability for mussels, where uptake increased from 0 to 340 microplastics individual^{-1} for free microplastics to up to 1.6 x10^{5} microplastics individual^{-1} when incorporated into snows.

New theories concerning the biological processes that may be key to the downwards movement of microplastics are emerging. Pelagic and benthic environments are linked by a range of surface-dwelling filter-feeding species (Griffiths *et al.,* 2017), ordinarily removing large quantities of suspended organic matter from the water by filter-feeding, and through the production of faeces and pseudofaeces (Ward & Shumway, 2004); a process known as bentho-pelagic coupling. One such organism capable of processing large volumes of water is the blue mussel, *Mytilus edulis* with a study indicating how a 21.5 mm sized blue mussel will filter an average of 15 mL min^{-1} under optimal algae conditions (Riisgård *et al.,* 2011). As the size range of certain microplastics overlaps that of mussel prey (Galloway *et al.,* 2017), mussels have been shown to facilitate the drawdown of microplastics to the sediment (Piarulli & Airoldi, 2020). In this study, commercially available microplastics of varying densities (polyamide = 1.15 g cm^{-2} and polypropylene = 0.92 g cm^{-2}) were ingested by mussels. The incorporation of microplastics into the faecal pellets increased the particles' sinking velocity by 3 to 4 orders of magnitude, providing evidence that detrital pathways are a transfer route of microplastics across benthic regions.

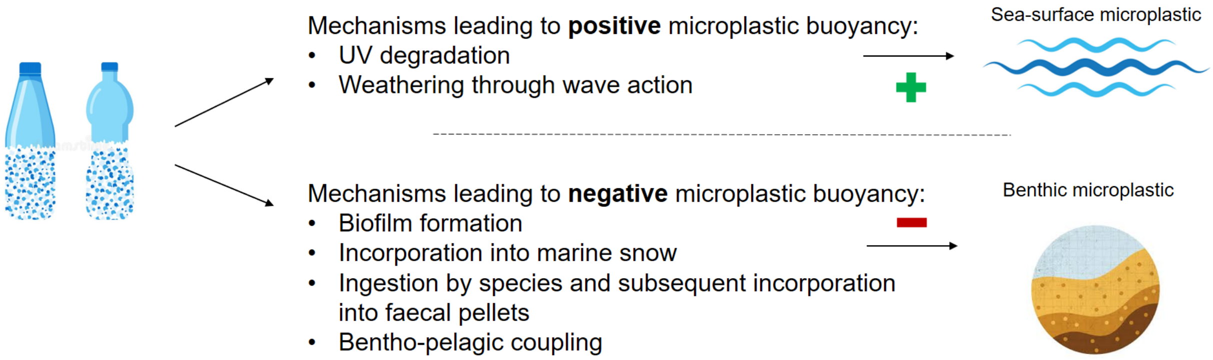


Figure 1.1: Schematic illustrating the evidence-based mechanisms by which microplastic buoyancy is altered resulting in the transport of microplastics to the sea-surface or to the ocean benthos.

Comprehensive knowledge on accumulation or redistribution that might occur by biological communities once microplastics have reached the benthos is lacking. However, studies assessing the abilities of organisms to fragment and re-partition plastic are gaining momentum. Such organisms are termed ‘bioeroders’ as they are known for their ability to erode and weaken hard substrates either through mechanical or chemical boring into substrates or through grazing whereby organisms scrape off the surface of the substrate while feeding on the microflora present. Feeding rate, mobility and propensity to settle on hard substrates are all qualities that make particular organisms’ efficient bioeroders and hence have the capacity to be good bioeroders of plastic. Numerous marine organisms are responsible for bioerosion and as a result, the fragmenting action of such organisms has huge potential to influence the distribution and risk of plastic pollution (Davidson *et al.*, 2018; Jang *et al.*, 2018). Sea urchins have been shown to be effective in generating microplastics from large macroplastic crates. Urchins feed using what is known as an Aristotle’s lantern; a mouth made up of five calcium carbonate plates enabling them to scrape hard substrates clean. This feeding mechanism is highly efficient but also acts as an eroding tool to the hard substrate itself. In one study, this feeding action produced on average 85.67 microplastics per urchin over a 9 day tank exposure (Porter *et al.,* 2019). These results point to a previously overlooked area, highlighting the ability of bioeroders to transform relatively low risk macroplastic items into high-risk smaller items, re-partitioning microplastics as a result.

Considering the ever-increasing number of species known to be contaminated with marine microplastics, in-depth understanding of processes key to the fate of microplastics is of critical importance if we are to effectively assess the risks of this pervasive pollutant.

1.5 Biological and Ecological risks of microplastics

The magnitude at which we have produced and discarded plastics has come at a great cost to our ocean's inhabitants. Both macro- and microplastics pose direct threat to marine organisms with the biological consequences of the former including entanglement, lacerations, starvation and suffocation (Browne *et al.,* 2007) and the latter, compromised physiological performance and digestive tract obstruction amongst others (Murray & Cowie, 2011; Wright *et al.*, 2013). With a size range that overlaps that of plankton and natural sediment grains, it is not surprising that there is concern over the environmental impact microplastics have in regard to ingestion by marine organisms. Microplastics are bioavailable to a wide range of marine taxa, including both vertebrates (Neelam & Ishteyaque, 2018; Simmonds, 2012) and invertebrates (Browne *et al.,* 2007) and can be bioavailable either directly through ingestion or indirectly through trophic transfer from contaminated prey (Nelms *et al.,* 2018).

The ubiquity of microplastics in sea-surface and pelagic zones mean they are a potential threat to a multitude of pelagic-dwelling organisms. A study carried out in 2018 found that 73% of 233 deep water fish from the Northwest Atlantic had plastics within their gut contents illustrating a much higher occurrence of microplastic fragments (mainly polyethylene fibres) in mesopelagic fish than previously reported (Wieczorek *et al.,* 2018). However, what is currently unclear is whether the exponential increase in global plastic production has meant marine organisms now are more at risk compared to decades ago. A study looking into the internal microplastic concentrations of two foraging fish and plankton species in the Baltic Sea found no significant increase in those sampled over the past three decades (Beer *et al.,* 2018). This not only suggests microplastic ingestion by these species may be less common than initially assumed but also highlights the complexity of assuming a simple correlation, i.e., an increase in surrounding plastic pollution causes an increase in uptake. This indicates the need for greater understanding of how plastic is cycled through marine ecosystems.

There is now a wealth of evidence to suggest microplastics are being ingested by a wide array of benthic marine biota (Taylor *et al.,* 2016) for example arthropods (Watts *et al.,* 2014), echinoderms (Graham & Thompson, 2009; Messinetti *et al.,* 2018), molluscs (Sussarellu *et al.,* 2016; von Moos *et al.,* 2012) and polychaetes (Browne *et al.,* 2013; Wright *et al.,* 2013) amongst others. Li *et al.* investigated microplastic pollution in the benthic common mussel (*Mytilus edulis*) along the coastlines of China (Li *et al.,* 2016). They described results varying between 0.9 to 4.6 items/g and from 1.5 to 7.6 items/individual demonstrating a substantial prevalence of microplastics in benthic filter-feeders. Impacts on health include compromised physiological performance (Wright *et al.,* 2013), digestive tract obstruction (Murray & Cowie, 2011), altered feeding behaviour (Besseling *et al.,* 2013; Cole *et al.,* 2015), accumulation in tissues (Watts *et al.,* 2014) and translocation to the circulatory system (Browne *et al.,* 2008).

Chemical additives used in the plastic manufacturing process may increase the toxicity of the plastic itself, including plasticizers, flame retardants and antioxidants (Lahimer *et al.,* 2017; Groh *et al.,* 2019). Microplastics are also potential substrates for biological and chemical contaminants, acting as vectors for a multitude of contaminants including polychlorinated biphenyls (PCBs), Polycyclic aromatic hydrocarbons (PAHs) and even pollutants such as the insecticide dichlorodiphenyl-trichloroethane (DDT) (Caruso, 2019; Engler, 2012; Mai *et al.,* 2018; Van *et al.,* 2012). These plastics not only have the ability to release these toxic chemicals into the marine environment, but also have the potential to transfer such pollutants to organisms ingesting the plastics. Under experimental conditions, the transfer of such pollutants from microplastics to marine organisms has been confirmed (Li *et al.,* 2016; Pittura *et al.,* 2018) with one study showing how uptake of microplastics laden with additive chemicals (Triclosan and PFDE-47) and pollutants (nonylphenol and phenanthrene) damages ecophysiological functions within the deposit-feeding lugworm, *Arenicola marina* (Browne *et al.,* 2013). Microplastics transferred pollutants and additive chemicals to the lugworm, with uptake of Triclosan and PVC diminishing the ability of worms to engineer sediments. Similar findings are yet to be replicated in the field due to the issues associated with measuring sub-lethal effects of chemicals in the wild, especially considering the many other stressors present.

In colonized soft bottom habitats, organisms alter their habitats by influencing the sediment structure in a process called bioturbation (Kristensen *et al.,* 2012). It covers all actions of benthic fauna such as burrowing, ingestion, defecation and ventilation and has even been shown to transport microplastics deeper within marine sediments (Näkki *et al.,* 2017). With the diminishing ability of worms to engineer sediments highlighted (Browne *et al.,* 2013), the influence of microplastics on bioturbation intensity could be at risk. Other than altering the structure of sediment, bioturbation influences microbial activities and biogeochemical processes in sediments by modifying water and sediment fluxes at the water-sediment interface (Barbanti *et al.,* 1992; Laverock *et al.,* 2011; Mermillod-Blondin & Rosenberg, 2006). Future research must focus on the role microplastics play indirectly on these processes.

Recent studies are now highlighting the ability of microplastics to act as novel vectors of disease. *Aeromonas salmonicida,* a bacterial fish pathogen (Viršek *et al.,* 2017) has been found in 'plastisphere' communities on microplastic surfaces. The pathogenic bacteria, *Vibrio cholerae* was discovered within biofilms accumulating on the surface of polystyrene microplastics in the North and Baltic Sea (Kirstein *et al.,* 2016). This threatens not only susceptible marine organisms but humans too, and with seawater temperature rise identified as a key factor influencing the presence of *Vibrio spp.,* scientists are rightly considering the likelihood of an increased prevalence of *Vibrio* diseases in the coming decades (Sobrinho *et al.,* 2010). Further research is crucial to ascertain whether microplastic pollution is responsible for the spreading of more diseases (Naik *et al.,* 2019).

The varying sizes, shapes, concentrations and composites of these plastics add to the importance of determining their origins and the impact each type has on marine ecosystems. Lehtiniemi *et al.,* demonstrated that size of the plastics more than shape is a crucial nominator influencing ingestion in the two planktivores; the mysid shrimp (*Praunus* spp.) and three-spined stickleback (*Gasterosteus aculeatus*) (Lehtiniemi *et al.,* 2018). Ziajahromi *et al.,* highlighted the increased toxicological risk to the waterflea, *Ceriodaphnia dubia* from microfibers compared to microbeads, in this case manifesting as reduced reproductive output (Ziajahromi *et al.,* 2017). These two studies alone show that there are a number of parameters influencing the impact different types of plastics have on marine organisms. We are yet to fully comprehend many of these contributing factors.

Knowledge of the detrimental effects plastic pollution has on marine organisms is forever increasing and failure to limit the rise in plastic production and cut back on its disposal simply increases the chances of more marine organisms being affected by detrimental health effects. Improving our understanding of the processes key to the transport of plastic debris, while identifying hotspots is crucial in our overall understanding of the risks of plastic pollution.

1.6 Aims of this thesis

This thesis sets out to investigate microplastic distribution and composition in benthic habitats through characterising particle uptake in natural populations of the benthic polychaete worm, *Hediste diversicolor* at multiple sites across South Devon, UK. In **Chapter 2**, this is investigated by addressing the following questions:

1. How abundant is microplastic within estuarine benthic sediments and what are the particle characteristics present?
2. Is there uptake up microplastic into the benthic polychaete, *Hediste diversicolor* and if so, what are the particle characteristics present.

Additionally, it seeks to increase our understanding of the interactions benthic invertebrates have with plastic litter, particularly in the fragmentation and movement of subsequent particles. To do this, an experimental mesocosm exposure comprising of organisms from three different functional groups was set-up. This provides insight into how plastics move and accumulate within a benthic marine ecosystem. In **Chapter 3**, this is investigated by addressing the following questions:

1. Does the feeding activity of a benthic grazer in the presence of macroplastic litter generate microplastics into the benthos via fragmentation?
2. Does the presence of food influence the number of plastic particles generated by the benthic grazer?
3. Does the presence of a benthic filter-feeder alter the downward flux of microplastics to the sediment?
4. Does the presence of a filter-feeder influence the uptake of microplastics in a benthic sediment-dweller?

Chapter 2: Evidence of synthetic particle ingestion in natural populations of the benthic polychaete worm, *Hediste diversicolor*

2.1 Introduction

Whilst the early studies into marine microplastic pollution focused on plastics residing in the sea-surface waters and the oceanic gyres, it is increasingly being recognised that, despite having different densities including many buoyant polymers, in time the majority of microplastic will eventually sink and accumulate in the benthos (Courtene-Jones *et al.,* 2017; Munari *et al.,* 2017; Peng *et al.,* 2017; Van Cauwenberghe *et al.,* 2013; van Sebille *et al.,* 2015; Woodall *et al.,* 2014). As such, benthic sediments are now considered a major sink for plastic debris being reported in almost all benthic habitats such as beaches, estuaries, salt marshes, mangroves and the seafloor (Barasarathi *et al.,* 2014; Nor & Obbard, 2014; Stolte *et al.,* 2015; Weinstein *et al.,* 2016; Willis *et al.,* 2017) Evidence from Woodall *et al.,* (2014) reveals that of the regions sampled (Atlantic Ocean, Mediterranean Sea and Indian Ocean), microfibres were up to four times more concentrated in the deep-sea sediments than in the contaminated sea-surface waters and with comparatively little known on the effect of microplastics residing in sediments compared to surface-waters, this large volume of plastic could lead to impacts beyond our current comprehension.

With a size range overlapping that of plankton in addition to a prevalence in many marine sediment habitats, it is not surprising that there is concern over the microplastics availability to an array of benthic marine biota. While there is now a wealth of laboratory-based exposure studies highlighting the ingestion of microplastics amongst invertebrates (Taylor *et al.,* 2016), the majority of environmental data on microplastic ingestion tends to focus on pelagic species such as zooplankton (Desforges *et al.,* 2015; Sun *et al.,* 2017), fish (Jabeen *et al.,* 2017; Lusher *et al.,* 2013; Nadal *et al.,* 2016) and marine mammals (Hernandez-Gonzalez *et al.,* 2018; Nelms *et al.,* 2018). Evidence of microplastic ingestion by wild benthic species is scarce although a study focussing on wild and cultured Manila clams (*Venerupis philippinarum*) in British Columbia documented microplastic concentrations ranging between 0.07 to 5.47 particles/g with fibres being the dominant microplastic shape observed (Davidson & Dudas, 2016).

The harbour ragworm, *Hediste diversicolor*, is an intertidal burrowing polychaete inhabiting the shallow marine and brackish waters in the North Temperate Zone of the Atlantic (Scaps, 2002). This infaunal species builds U or Y shaped burrows in the sediments, is omnivorous and displays two feeding modes. The first as a deposit feeder consuming both the flora and fauna from surface sediments and also suspension feed via a mucus web secreted by the worm itself. As one of the most common intertidal polychaetes, they are an important prey item for wading birds and several species of flatfish. Recent studies have focussed on their potential to contribute to aquaculture diversification (Pombo *et al.,* 2020). *H. diversicolor* could be characterized as a keystone species due to its characteristic sediment reworking activity and bio-irrigation behaviour. As such, they have predominant impacts on sedimentary processes and on ecosystem functioning (Moreira *et al.,* 2006).

It has been highlighted that certain benthic deposit feeders are known to exhibit selectivity in terms of particle uptake. For example, three polychaete species; *Spiochaetopterus oculatus*, *Spio setos* and *Marenzelleria viridis* all showed elements of particle-size selectivity (Bock & Miller, 1999) whereas the bivalve *Nucula annulate* selectively ingested the organic and bacteria-rich sediment fraction (Lopez & Cheng, 1983). What is not clear however is whether these benthic species can differentiate sediment from the contaminated plastic and whether they are more likely to ingest certain plastics over others. Only two studies have published research on the impact of microplastic ingestion on *H. diversicolor* (Gomiero *et al.,* 2018; Revel *et al.,* 2020)*,* yet despite being an ecologically important species, the ingestion of microplastics is not yet determined in wild populations.

Here a field sampling campaign was conducted to assess the abundance and characteristics of synthetic particles within the sediment, as well as the uptake of these particles in natural populations of the benthic polychaete worm *H. diversicolor* at multiple sites across South Devon, UK. It was hypothesised that:

(1) there will be synthetic particle contamination at each of the three sites both within the sediments and the worms. (2) There will be site specific differences in particle contamination in the sediment and in the worms. (3) There will be a relationship between worm size (defined by their wet mass) and the number of particles present within the worm. (4) There will be more fibres than other particle shapes found in the sediment and worms. Characteristics in terms of shape, size, colour and composition of the ingested particles were examined.

2.2 Materials and Methods

2.2.1 Site selection

Three sites across South Devon, UK were chosen for this study. Sites were selected to represent different levels of 'expected' contamination (high, medium and low), ease of access and established *Hediste diversicolor* populations.

Levels of likely contamination were assessed by proximity to wastewater treatment works (WWTW) discharge into the estuary as WWTWs have been shown to act as a source of microplastics to estuaries (Estahbanati & Fahrenfeld, 2016; Conley *et al.,* 2019), the human population of their associated catchment areas, surrounding industrial infrastructure, human settlement and litter observations (Table 2.1). The Plym Estuary, Plymouth (50°22'22.3" N 4°06'10.6" W) was classed as the high- level site, the Exe Estuary, Exton (50°40'03.1" N 3°26'39.2" W) was classed as the medium site and Kingsbridge Estuary, Bowcombe Creek, Salcombe (50°16'36.9" N 3°45'37.8"W) was classed as the site with the lowest levels of contamination (Figure 2.1).

Sample Site	**WWTW**	**Population of catchment area**
Plym Estuary	Marsh Mills	59,245
	Central	107,931
		Total: 167,176
Exe Estuary	Countess Wear	137,000
	Lympston	1,496
		Total: 138,496
Kingsbridge Estuary	Kingsbridge	6,669
	East Portlemouth	31
	West Charleton	441
	Malborough	2,578
		Total: 9719

Table 2.1: Waste Water Treatment Works (WWTW) discharging into sample sites; Plym Estuary, Exe Estuary and Kingsbridge Estuary with the associated catchment area population. (Data taken from South West Water, 2019)

2.2.2 *Hediste diversicolor* and sediment collection

Using a garden fork, 30 *H. diversicolor* individuals were collected from each sample site within the mid-shore zone at low tide during Jan/Feb 2019. Each worm was washed thoroughly *in situ* using milli-Q water and transferred into individual 25 ml falcon tubes. Upon arrival at the laboratory, each worm was weighed, snap frozen using liquid nitrogen, weighed again and stored at -20 °C until digested.

Using a 50 ml falcon tube, 10 sediment cores were collected adjacent to the first 10 *H. diversicolor* collected. Having removed the lid, the falcon tube was upturned and pressed down into the top 10 cm of sediment. Having ensured the falcon tube was filled, it was removed from the sediment, turned upright and the lid was replaced immediately.

2.2.3 Tissue digestion and density separation

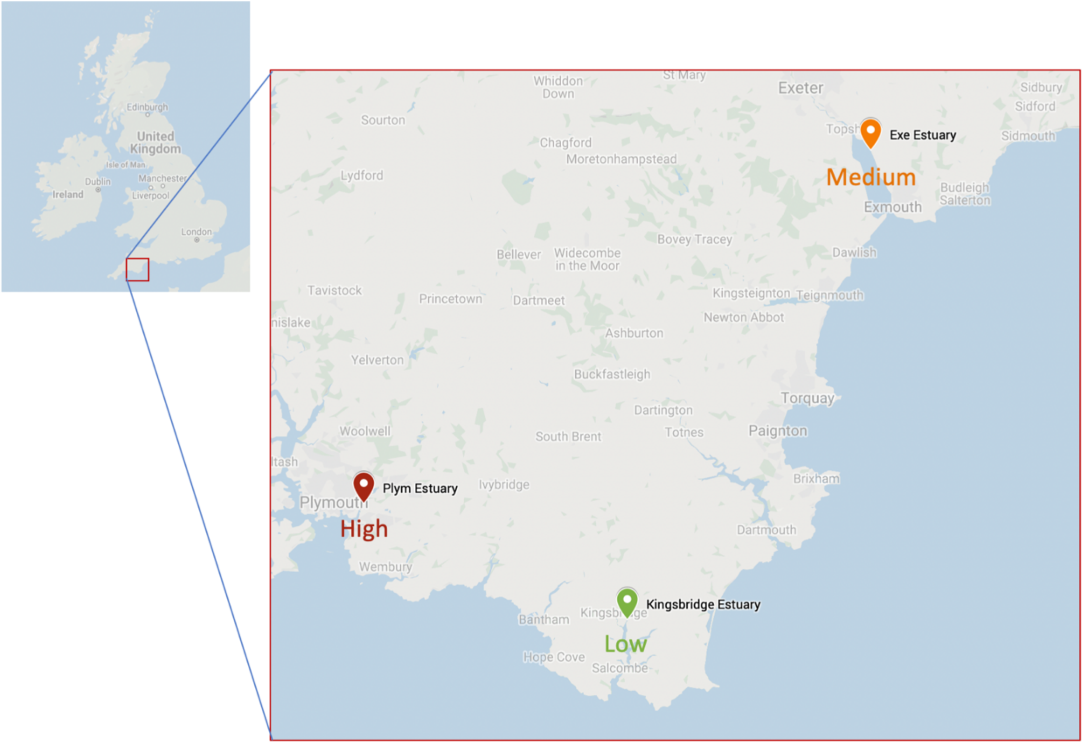


Figure 2.1: Map showing the location of sample sites: Exe Estuary, Plym Estuary and Kingsbridge Estuary in South Devon, UK. Map images were taken from Google Maps with accompanying contamination categories overlaid (high, medium and low).

Twenty-five millilitres of 10 % filtered potassium hydroxide (KOH) solution was added to the falcon tubes, each containing an individual *H. diversicolor*. Each tube was left to digest at 60 °C for 24 hrs after which the digested worms were moved to a laminar flow hood where all subsequent steps were conducted. The digested worms were vacuum filtered through 10 μm Cyclopore polycarbonate membrane filters. Filter papers were then transferred into glass petri dishes and sealed with a parafilm until analysed.

Defrosted sediment from each falcon tube was placed into individual 500 ml beakers, mixed using a metal spatula and left to dry in an oven at 60 °C for 12 hrs. From each of these samples (ten per site), 50 g of dry sediment was isolated for the identification of potential microplastics, resulting in a total of 500 g of analysed sediment per sample site. Isolating this volume of sediment per sample allows for the use of Sediment-Microplastic Isolation (SMI) units, a custom-built piece of apparatus replicating the design and methods developed by Coppock *et al.,* (2017). This method uses the principle of density floatation to separate plastic-like particles from differing types of sediment with a high recovery mean efficiency (95.8 %). A double-filtered (50 μm) solution of zinc chloride ($ZnCl_2$) at an optimal density of 1.5 gcm^{-2} was chosen as the floatation media as it balances the requirements for fine sediment to settle, whilst still being dense enough to enable the floatation and subsequent recovery of denser polymers. This procedure was carried out in a fume hood where after the $ZnCl_2$ sediment solution was vacuum filtered through 10 μm Cyclopore polycarbonate membrane filters. Procedural blanks were used to account for any atmospheric contamination. Filter papers were then transferred into petri dishes where they were sealed with a parafilm until analysed.

2.2.4 Analysis of filters and FTIR analysis

Filtered material from each worm and sediment sample was analysed visually using an Olympus SZX16 dissecting microscope. Particles synthetic in appearance were identified by scanning the filter papers at 3.2 and 1.6 magnification for 5 minutes each. These particles were photographed using an Olympus XC10 camera, counted, classified by shape (fibre, fragment or film) and colour and stored for Fourier-Transform Infrared (FTIR) analysis on a separate virgin filter paper. Each of the procedural blanks from previous stages underwent the same processing producing only fibrous particles. Worm procedural blanks contained on average 0.56 ± 0.22 (SE) black fibres and 1.12 ± 0.32 (SE) colourless fibres. Sediment procedural blanks contained on average 1.42 ± 0.15 (SE) black fibres, 0.32 ± 0.05 (SE) colourless fibres and 0.22 ± 0.06 (SE) blue fibres. The mean number of particles for each particle category (colour and shape) across all the blanks was subtracted from all data prior to further data analysis and is not included in any data presented.

Images of particles taken using the Olympus XC10 were uploaded onto ImageJ 1.47v where both lengths and diameters of synthetic particles were measured. Anthropogenic highly modified cellulose polymers in addition to petrochemical based plastics are included in our definition of synthetic particles.

Potential synthetic particles were visualised on an Olympus MVX10 before being transferred onto a Sterlitech 5.0 μm silver membrane filter for analysis. All particles were analysed using a PerkinElmer Frontier Fourier-transform infrared (FTIR) spectrometer. An attenuated total reflection (ATR) universal diamond attachment was used for the larger (easier to handle) particles however the majority of particles were analysed without the attachment. Spectra were obtained using a PerkinElmer Spotlight 400 μFTIR Imaging System (MCT detector, KBr window) operating in reflectance mode and with a wavenumber resolution of 4 cm^{-1}. A total of 16 scans were collected, across a wavenumber range from 4000 to 650 cm^{-1}. Normalisation of the spectra data and base-line correction tools were utilised using the Perkin-Elmer's Spectrum$^{\text{TM}}$ 10 software (version 10.5.4.738). The software automatically matches the obtained spectra to commercially available spectral libraries, including Perkin-Elmer's standard Polymers Library. It is important to note however, that the match quality reading is only as accurate as the number of spectral libraries within the database. Therefore, with the amount of spectral data increasing with time, more confident matches are likely to be found. The top 10 closest matches were analysed visually to improve confidence in the results. Only match qualities > 60 % were accepted, with a mean match quality of sediment samples of 78 % and worm samples of 71 % (with a combined match quality of 76% for all sediment and worm samples). Of the 271 suspected synthetic particles, 25 % (68 particles) were disregarded after FTIR analysis due to identification as natural polymers or weak library spectral matches (< 60 %).

2.2.5 Contamination Control

Specific precautions were implemented in order to minimise sample contamination in the field and within the laboratory.

In the field, nitrile gloves and cotton clothing were worn to minimise contamination. Worms were washed thoroughly using milli-Q and transferred into virgin falcon tubes before being transported to the laboratory. To ensure there was no source of contamination from field sampling equipment, five procedural blanks per sampling site were obtained with clean falcon tubes containing no samples filled with milli-Q water, filtered and visually assessed using FTIR as per the falcon tubes containing samples. Potential airborne contamination was controlled for by leaving exposed dampened blank filter papers held within glass petri dishes near the place of collection. Data was corrected for any fibres found in the procedural blanks and damp filter papers.

In the laboratory, nitrile gloves, lab coats and cotton clothing were worn throughout to limit external synthetic fibre contamination. All equipment used was acid washed and instruments cleaned using ethanol and Milli-Q water to prevent potential cross-contamination of plastics before each processing step. Lab surfaces, including fume hoods, were thoroughly cleaned with ethanol or Milli-Q again before each step. Metal and glass materials were used in favour of plastics where possible and feasible. The extraction, tissue digestion and density separation steps were carried out in fume hoods to minimise airborne contamination. All samples and containers were covered whenever possible by aluminium foil and the membranes kept individually in clean glass petri dishes sealed by paraffin. Procedural blanks were undertaken for all steps whereby sample-free $ZnCl_2$ and KOH solutions were filtered and the filter papers visually assessed individually using FTIR to look for synthetic particles, ensuring there was no source of plastic contamination from chemicals used. These controls were taken before starting and after every five samples in the same conditions. Airborne contamination controls comprising of dampened blank filter papers in petri dishes were placed in the oven and then fume hood to control for atmospheric plastic contamination during digestions and filtering. See section 2.2.4 for details revealing minor evidence of contamination.

2.2.6 Data analysis

All data presented is based on the confirmed particles following FTIR analysis corrected for procedural blanks. SPSS Statistics 27 was used for statistical analyses. All data were tested for normality using Shapiro-Wilk test and for equal variances using Bartlett's test if normally distributed and a Levene's test if not normally distributed. Data is reported as mean ± standard error.

To test for differences in microparticle abundance within sediments by site, a One-way ANOVA was conducted followed by a Tukey's post-hoc test to test between sites. The explanatory variable used was site and the response variable was amount of synthetic particle present.

To test for differences in microparticle abundances within *H. diversicolor*, where the data were not normally distributed a Kruskal-Wallis test was performed to test between sites. In these tests, the explanatory variable used was site and the response variable was amount of synthetic particle present.

To test whether particle uptake scales with worm mass, a linear regression was undertaken to look for correlation between worm wet mass and particle numbers present within individuals. The explanatory variable here was worm wet mass, with number of synthetic particles present within the worms as the response variable.

To test whether more fibres than other particle shapes are found in the sediment and worms, two chi-squared tests (one for sediment and one for worms) were conducted to assess whether site had an impact on particle shape. Due to unequal sample sizes of particles observed, it was not possible to test between sites. The explanatory variable used was site and the response variable was number of different synthetic particle shapes observed.

2.3 Results

2.3.1 Synthetic particle abundance, worm mass and particle shape

Synthetic particles were present in every sediment sample analysed, but with significant differences in particle counts amongst the three different sites (One-way ANOVA; $F_{2,27}$ = 3.854, P = 0.034), as predicted based on their proximity to WWTW. The Plym Estuary classed the high-level site with 116 ± 18.09 (SE) particles per kg, the Exe Estuary as the medium site with 98 ± 12.01 (SE) particles per kg and Kingsbridge Estuary classed as the site with the lowest levels of contamination of 62 ± 13.55 (SE) particles per kg (Figure 2.2A). A post-hoc Tukey test confirmed that the mean particle load per kg sediment at the Plym Estuary were significantly higher than at the Kingsbridge Estuary (P < 0.001).

Of the 90 worms sampled across the three sites, 48 % (n = 43) were found to contain synthetic particles. Unlike in the sediment samples, no significant difference in the number of particles per individual worm was observed between the three sites (Shapiro-Wilks test for non-normal data; (H(2) = 2.38, P = 0.30), with an average of 0.73 ± 0.15 (SE) particles per worm (Figure 2.2B). Even so, Kingsbridge worms did contain the lowest mean particle load (0.37 ± 0.10 particles per individual) with both Plym and Exton displaying higher synthetic particle counts (Exton; 0.97 ± 0.20 and Plym 0.87 ± 0.15 counts per individual).

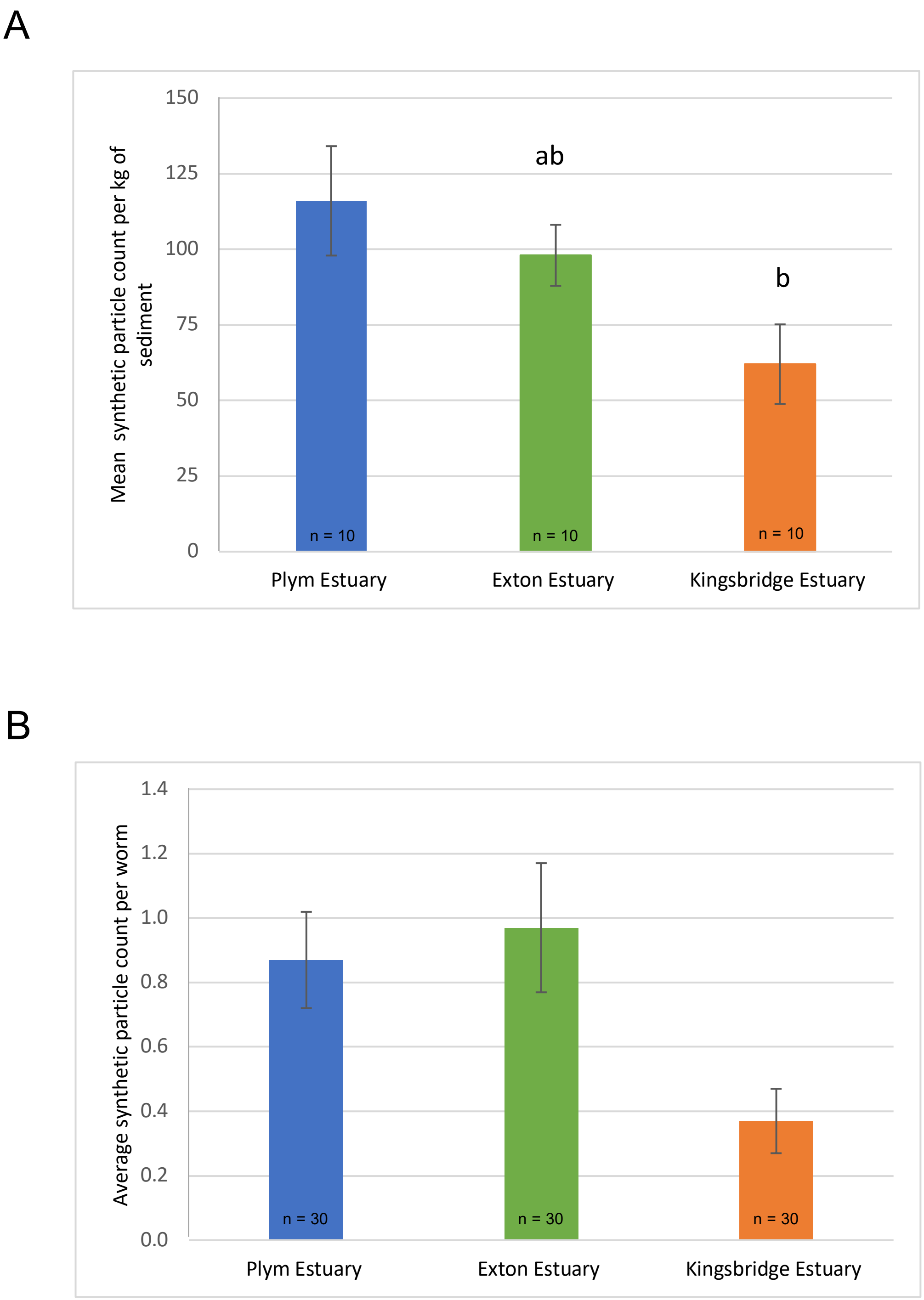


Figure 2.2: (A) Mean particle abundance (± SE) per kg of sediment (N = 30) and **(B)** an individual *Hediste diversicolor* (N = 90) sampled from three sites across South Devon. Note differing scales for average synthetic particle counts. Results include all FTIR analysed particles identified as 'synthetic'. Bars that do not share a letter are significantly different (Tukey's pairwise comparison)

No linear relationship between worm mass and the number of synthetic particles present within individual worms was observed (linear regression analysis; R_2 = 0.003; $F_{1,28}$ = 0.10; P = 0.74) (Figure 2.3). Therefore, all data is presented here as synthetic particles per individual rather than normalised to wet mass.

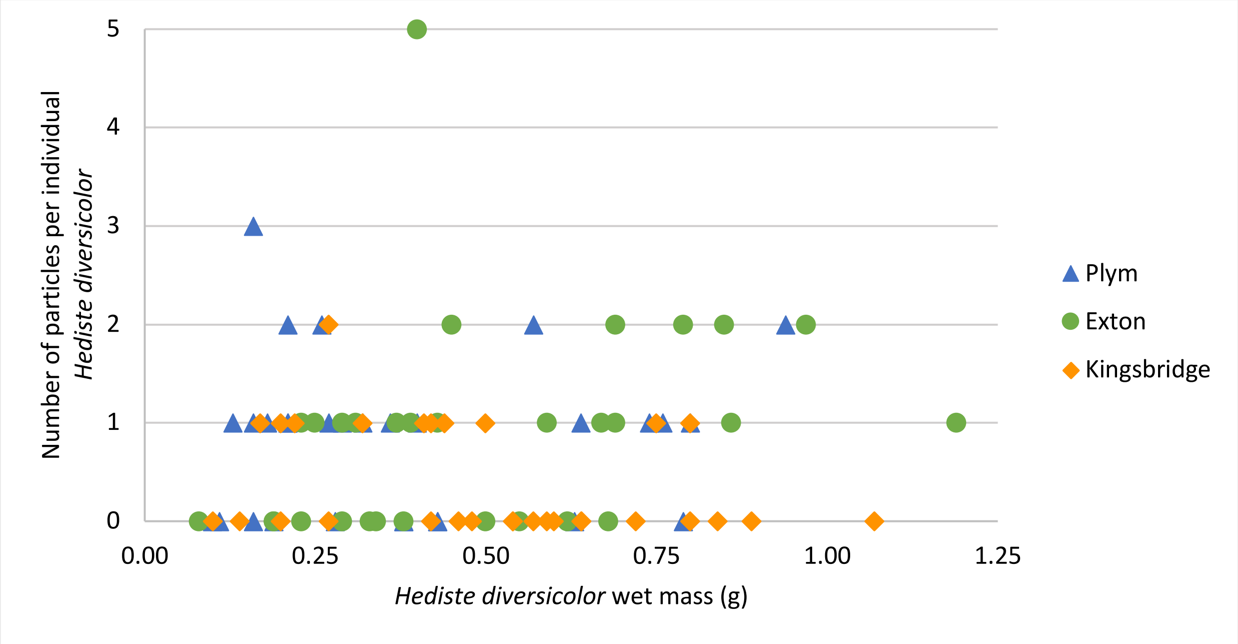


Figure 2.3: Relationship between the number of particles present within a *Hediste diversicolor* individual vs *Hediste diversicolor* wet mass (g) from the three different sample locations; Plym Estuary, Exton Estuary and Kingsbridge Estuary (N = 90).

Fibres made up 50 % of particle shapes found across sediment samples, with 42 % fragments and 7 % film. Fragments were the second most common shape identified, with the exception of Kingsbridge sediment where a majority of fragments were found (48 %). There is no significant difference in the distribution of shapes in the sediment across sites (X^2 (4, N = 67) = 7.75, P = 0.10). This is evident in Figure 2.4A due to the mix of particles found across all sites. In all cases, film made up the minority of particle shapes, none being present in Exton sediment.

Of the total synthetic particles found in the worms, 54 % were fibres, 34 % were fragments whilst 12 % were film. A significant difference in the distribution of shapes between the three sites was observed (X^2 (4, N = 21) = 12.95, P = 0.012) (Figure 2.4B). There were found to be different trends of particle uptake in the worms at each site, the Plym site having the highest proportion of film, Exton being dominated by fibres and Kingsbridge having a mix of fibres and fragments. Again, in all cases, film made up the minority of particle shapes, none bring present in Kingsbridge worms.

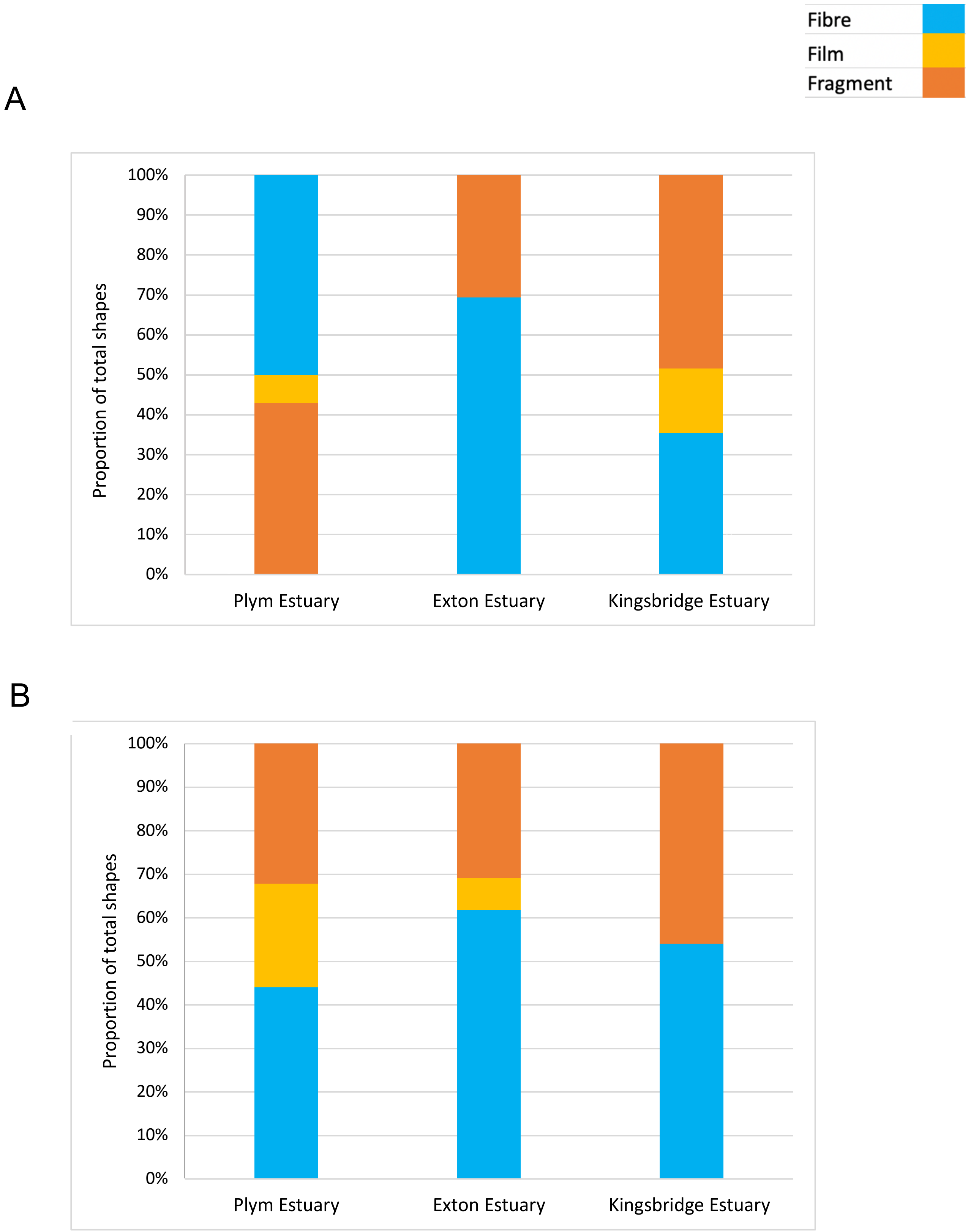


Figure 2.4: Proportion of different particle shapes (Fibre, Film or Fragment) in (**A**) 50 g sediment samples collected (N = 30) and (**B**) across all individual *Hediste diversicolor* samples (N = 90) from the three different sample locations; Plym Estuary, Exton Estuary and Kingsbridge Estuary**.**

2.3.2 Particle characteristics

Particle length

The mean length of synthetic particles in the sediment was approximately twice that of the synthetic particles found within the worms. The range of particle sizes found amongst the sediment samples was 112.7 µm to 67088.1 µm, with a mean of 2451.9 µm ± 5958.2 µm (Figure 2.5A). The range of particle sizes found within the worms was 97.7 µm to 4429.7 µm, with a mean of 1024.8 µm ± 935.1 µm (Figure 2.5B).

The longest measured particles were found amongst the fibres (with the exception of large fragments found in the Kingsbridge sediment (5152.4 µm ± 4425.3)). Mean lengths of the fibres obtained from sediment in each site were; Plym (3278.3 µm ± 667.4), Exton (2821.7 µm ± 358.7) and Kingsbridge (2829.7 µm ± 638.5) with the mean lengths of sediment fibres across all sites being 2976.5 µm ± 554.8. Fibres accounted for the longest particle shape present within the worms in each site and also in the Plym and Exton sites. However, there was a very large fragment found in the sediment at Kingsbridge (67 mm) (Figure 2.5A).

Mean lengths of fibres obtained from the worms within each site were; Plym (1580.8 µm ± 400.4), Exton (1377.8 µm ± 186.5) and Kingsbridge (1681.8 µm ± 392.7). When present, film particle mean lengths tended to exceed that of the fragments (with the exception of Exton film particles obtained from the worms). Film particles however, were not present in both Exton sediment and Kingsbridge worm samples, only accounting for 12 % of all particles measured.

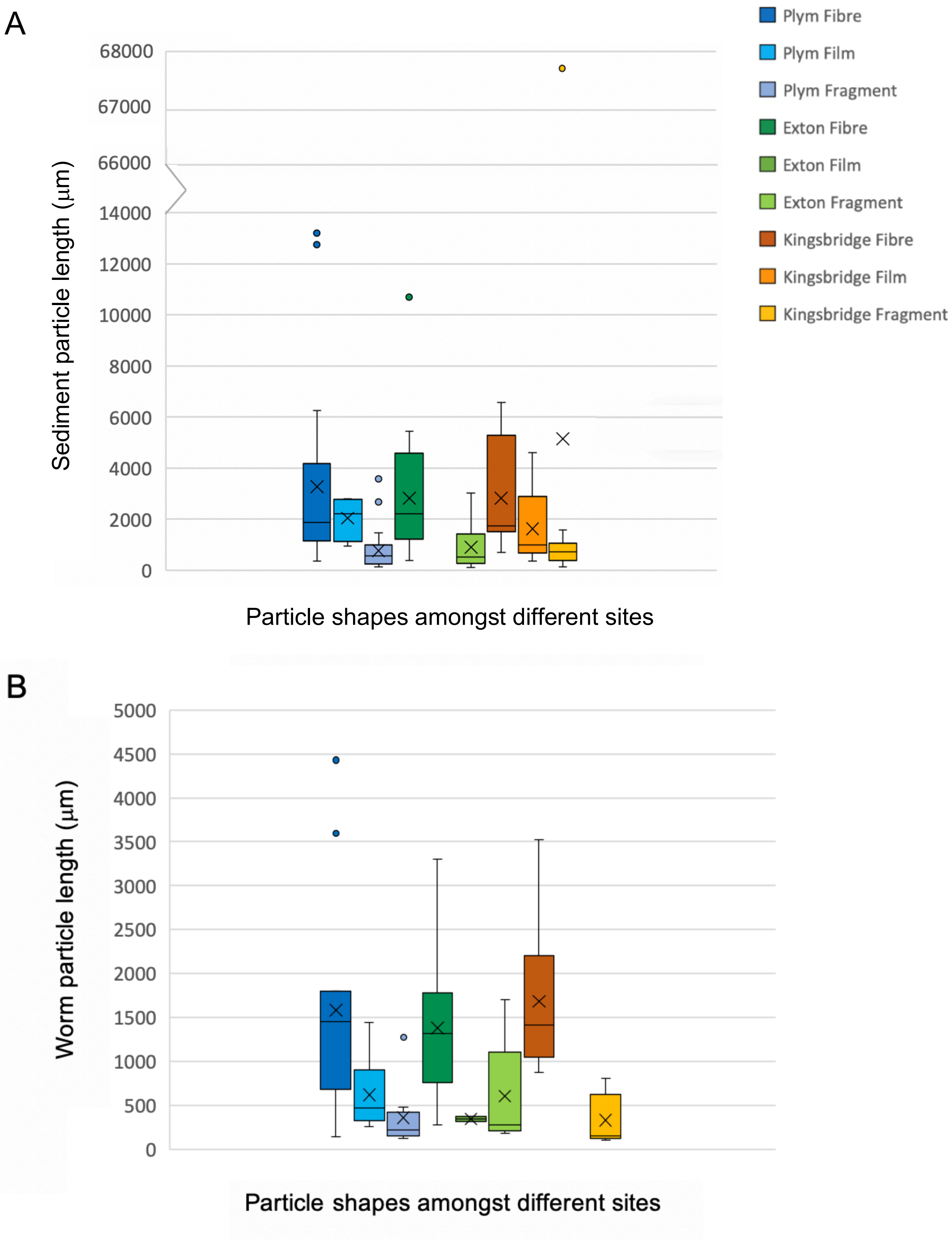


Figure 2.5: Mean lengths amongst different shapes of particle (Fibre, Film and Fragment) (± SE) within **(A)** 50 g sediment samples collected (N = 30) and **(B)** across all individual *Hediste diversicolor* samples (N = 90). Note broken y-axis and differing scales for particle lengths. On the graph, 'x' is the mean, the horizontal line is the median, the coloured box represents the interquartile range associated with the median and the whiskers represent the upper and lower extremes.

Particle colour

Particles were dominated by blue, black and colourless particles. Amongst all the sediment fibres; Plym comprised of 25 % blue, 12 % black and 25 % colourless; Exton had 18 % blue, 26 % black and 38 % colourless; Kingsbridge had 46 % blue, 18 % black and 27 % colourless (Figure 2.6A). Red, green, orange and yellow fibres made up the deficit with red being the fourth most predominant colour (albeit a small proportion compared to blue, black and colourless). It was also the only other fibre colour to be found in each site (albeit a small percentage). A similar pattern was found amongst the worm fibre samples; Plym comprised of 37 % blue, 27 % black and 36 % colourless; Exton had 50 % blue, 17 % black and 28 % colourless; Kingsbridge had 67 % blue, 16 % black and 17 % colourless (Figure 2.6B). Although blue, black and colourless fibres comprised the vast majority of fibre colour, red again was the fourth most prevalent fibre colour, although in this case only found in the Exton estuary site.

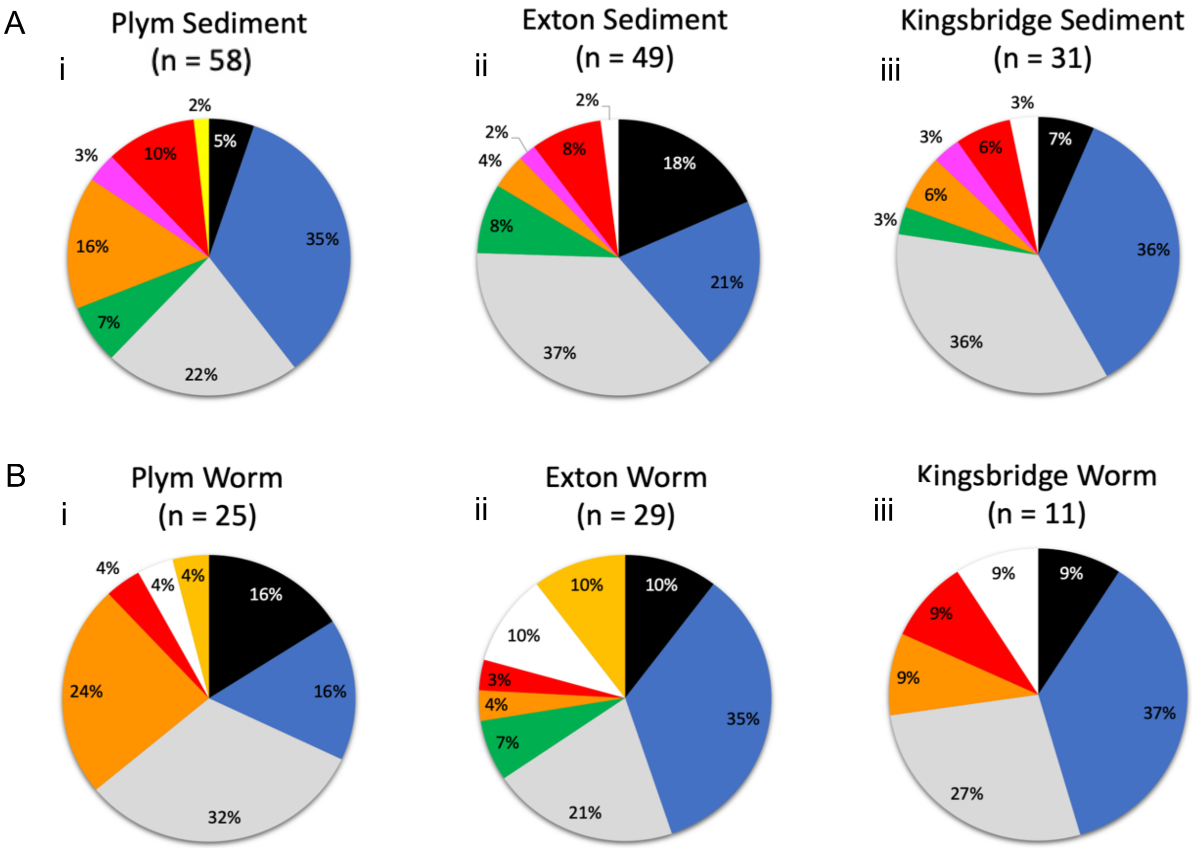


Figure 2.6: Particle colours in **(A)** 50 g sediment samples (N = 30) and **(B)** across all individual *Hediste diversicolor* samples (N = 90) from **(i)** Plym Estuary, **(ii)** Exton Estuary and **(iii)** Kingsbridge Estuary**.**

Polymer composition

All particles included in this data were verified by FTIR (n = 203). An additional protocol focussing on the characteristic wavelength band at 1105 cm^{-1} was used to distinguish natural from semi-synthetic cellulose (Cai *et al.,* 2019). The presence of the band only existed in natural fibres.

Of the particle compositions identified, polypropylene (PP) accounts for the highest proportion of synthetic particles found within the sediment, closely followed by either polyacrylamide (PAM), polyvinyl chloride (PVC), polystyrene (PS) or semi-synthetic cellulose (CE) depending on the site (Figure 2.7A). Within the worm particle composition, semi-synthetic CE accounted for the highest proportion of particles in Plym and Exton sites, whereas PS was dominant within the Kingsbridge site (Figure 2.7B). Polymers of PVC, PP and PAM made up a high proportion of the other polymers identified.

Of the cellulosic particles found in both the sediment and worms, a vast majority of these were fibrous in shape (77.6 %). Of those fibres 36.8 % were colourless, 28.9 % were blue, 23.7 % black, 7.9% green and 2.7 % pink.

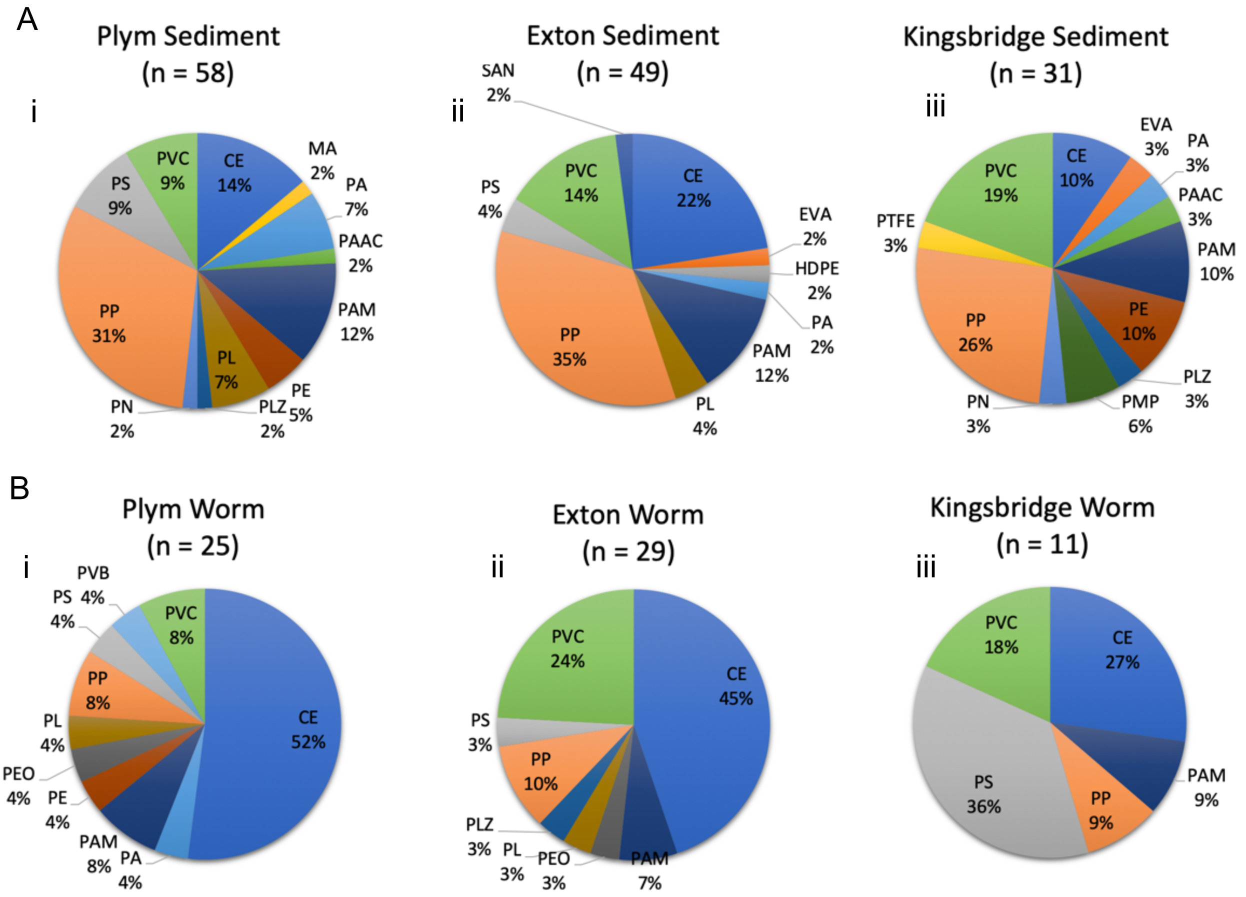


Figure 2.7: Type distribution of synthetic particles identified in **(A)** 50 g sediment samples (N = 30) and **(B)** across all individual *Hediste diversicolor* samples (N = 90) from **(i)** Plym estuary, **(ii)** Exton estuary and **(iii)** Kingsbridge estuary. Particle compositions (20 total): EVA: Ethylene/Vinyl Acetate copolymer, HDPE: High Density Polyethylene, PA: Polyamide, PAM: Polyacrylamide, PE: Polyethylene, PEO: Poly(ethylene oxide), PL: Polyester, PLZ: Plasticizer, PMP: Polymethylpentene, PN: Poly(norbornene), PP: Polypropylene, PS: Polystyrene, PTFE: Polytetrafluoroethylene, PVB: Poly(vinyl butyral), PVC: Polyvinyl chloride, SAN: Styrene/Acrylonitrile copolymer, TEP: Triethyl Phosphate, MA: Modacrylic, CE: semi-synthetic Cellulose, PAAC: Poly(acrylic acid).

2.4 Discussion

In this study, synthetic particle contamination of sediments and individuals of the polychaete *Hediste diversicolor* was observed at all three sites in South Devon sampled. This shows that particles in the marine sediments are available for uptake into marine worms and are present in the marine food web. The mean length of particles in the sediment was approximately twice the mean length of particles taken up by the worms suggesting there are some sort of selective mechanisms determining uptake into worms. These particles included a range of polymer types, including those that are buoyant as virgin particles such as polyethylene and polypropylene, adding to the growing weight of evidence that microplastics eventually make their way to the benthos and are not just a sea-surface pollutant.

Sediments are becoming increasingly recognised as important sinks for microplastics to accumulate, often observing higher levels in the sediment than at the sea surface (Courtene-Jones *et al.,* 2017; Munari *et al.,* 2017; Peng *et al.,* 2017; Van Cauwenberghe *et al.,* 2013; van Sebille *et al.,* 2015; Woodall *et al.,* 2014). Some of the highest levels recorded thus far come from the deep-seafloor of the Tyrrhenian Sea (part of the Mediterranean Sea), where an average of 190 pieces per 50 grams of sediment were recorded (Kane *et al.,* 2020) and sea ice cores from the Arctic Central Basin harbouring 2-17 particles L $^{-1}$ (Kanhai *et al.,* 2017). The abundance of synthetic particles found in this study within the South Devon sediments analysed was 62-116 synthetic items kg^{-1} which, compared to the Mediterranean seafloor, is a fortytwo-fold decrease.

When comparing results to the few studies carried out elsewhere in the UK, there was less synthetic particle contamination in Devon compared to four areas analysed within the Thames river basin (locations selected based on average percentage effluent present and population density) (Horton *et al.,* 2017). These results (185 – 332 items kg^{-1}) exceeded the abundance of synthetic particles found in South Devon three-fold. While urban areas generally have a greater abundance of microplastics as compared with rural (Jarvie *et al.,* 2000; Murphy *et al.,* 2016), the greatest concentrations of microplastics tend to be on the floodplains and low velocity environments, rather than in the centre of the urbanised area itself (Tibbetts *et al.,* 2018). As such, it is perhaps unsurprising that the Thames river basin contained higher levels of contamination compared to that of the more rural estuaries in Devon. The abundance of synthetic particles found within this study is similar to that of contaminant levels found in Belgian marine sediments in coastal harbours, beaches and sublittoral areas (91.9 – 166.7 items kg^{-1}), each with varying industrial and human population levels (Claessens *et al.,* 2011). As expected, the region in this study with the least sediment contamination came from Kingsbridge, the area with the lowest population and industrial activities of the three locations chosen. Amongst the three sites being chosen to represent a range of different urban and rural scenarios, synthetic particle count in the sediments at these three locations varied with a twofold difference between Plym estuary and Kingsbridge.

The main sources of the fibrous contaminants are thought to be fishing equipment (Xue *et al.,* 2020), shedding from synthetic clothing (Napper & Thompson, 2016) or abrasion of tyres (Kole *et al.,* 2017) which are then washed into rivers and seas. Sources of fragments and films tend to be from the fragmentation of macroplastic items such as plastic bottles, food packaging and bags (GESAMP, 2015).

Twenty different synthetic particles were identified across all three sites. The two most commonly found synthetic particles in the sediments were polypropylene (PP) (fragments) and semi-synthetic cellulose (CE) (fibres). Polypropylene is an abundantly manufactured polymer commonly used in the plastic industry (Maddah, 2016) and contributed to the second highest proportion of polymers (16.5 %) collected from sea surface samples in the Northwestern and Southwestern Mediterranean sea (highest proportion attributed to polyethylene (PE) accounting for 54.5 % of total microplastics sampled) (de Haan *et al.,* 2019). Polypropylene also contributed to a high proportion of microplastics in seabed sediments sampled in four sites along the eastern shores of Hong Kong, accounting for 13.8 % of the total collected microplastic items (highest proportion was again attributed to PE accounting for 46.9 %) (Cheang *et al.,* 2018). The high prevalence of this polymer in the present study is therefore not surprising given the frequent reporting in various regions sampled globally.

Types of semi-synthetic cellulose, for example cellophane, rayon and cellulose acetate also show high prevalence in microplastic studies when they are reported (Cai *et al.,* 2019). Applications of semi-synthetic cellulose polymers include apparel, upholstery, hoses and linings for a variety of products (Kauffman, 1993). High proportions of such polymers have been reported in sediment samples from Southern Portuguese shelf waters with 21 out of 31 sediment samples identified as a semi-synthetic cellulose polymer, commonly known as rayon. Although the proportion of synthetic CE found in the sediment analysed in this study is less than that of the Portuguese sediments, the presence of semi-synthetic CE in sediments is undeniable. Semi-synthetic CE also comprised the majority of polymers present within the worms from two sites (the other being polystyrene). Evidence that *H. diversicolor* ingests high levels of such polymer is replicated in a study assessing the bioaccumulation and biological effects of cigarette litter in *H. diversicolor*, a major component of which is the semi-synthetic CE, cellulose acetate (Wright *et al., 2*015). Polyvinyl chloride (PVC) was reported in all sediment and worm samples (when synthetic particles were present) although to a lesser extent compared to PP and CE. It is used in a variety of applications including health care, electronics, construction and building components (Andrady & Neal, 2009). These findings highlight the pervasive nature of a wide range of microplastic types found in marine ecosystems. It is worth noting that the affects synthetic particles have on organisms are likely to range depending on the chemical composition (Beiras *et al.,* 2021), leacheates present (Silva *et at.,* 2016; Schiavo *et al.,* 2020) and bioavailability to the organism itself (Botterell *et al.,* 2019). It is important to assess therefore the impacts that the numerous different types of bioavailable plastics have on organisms, rather than see all plastics as 'equal risk' (Koelmans *et al.,* 2022).

Whilst the vertical position of synthetic particles in the water column is considered to be mainly dependent on the item's density and composition (Andrady, 2011; Reisser *et al.,* 2015), both positively and negatively buoyant synthetic particles were found within the study benthic sediments. Polypropylene for example is buoyant in seawater with a specific gravity of 0.83-0.85 g cm^{-3} whereas polyvinyl PVC is denser than seawater with a specific gravity of 1.38 g cm^{-3} meaning it has a greater propensity to sink. Finding these buoyant polymers in the sediment is clear evidence that they have been transformed by their presence within the marine environment in some way which has altered the particle density, causing them to sink to the seafloor. There are numerous biological processes thought to contribute to the ballasting effect of synthetic particles overtime. For example, biofilm formation on the microplastics themselves, otherwise known as biofouling which affects the hydrophobicity and buoyancy of plastic (Kooi *et al.,* 2017). When the density of the particle with the added biofilm exceeds that of seawater, it starts to sink (Long *et al.,* 2015; Ye & Andrady, 1991). The incorporation of microplastics into marine aggregates such as marine snow (Porter *et al.,* 2018) and faecal pellets (Cole *et al.,* 2016) also represents mechanisms by which floating plastics can be vertically transported away from surface waters down towards the sediment, thereby contributing to the increased availability of microplastics to benthic organisms.

Whilst a number of laboratory based exposures have now provided clear proof of principle that benthic organisms can readily ingest a range of microplastic particles (Davidson & Dudas, 2016; Van Cauwenberghe *et al.,* 2015b), only few studies have investigated the presence of synthetic particles in benthic worms within their natural habitat. One such study that looked at microplastic uptake in benthic organisms examined two bivalve species, *Astarte crenata* and *Macoma tokyoensis* from the Arctic and sub-Arctic regions (Fang *et al.,* 2018), finding a range of 0.04 – 1.67 items individual^{-1} in *A. crenata* (n = 28) and 0.25 – 1.12 in *M. tokyoensis* (n = 29). Although bivalves were analysed, both bivalve species and *H. diversicolor* adopt filter-feeding as a feeding strategy. These mean body burdens of synthetic particles reported in bivalves by Fang *et al.,* (2018) are similar to those reported here for *H. diversicolor* (0.37 ± 0.10 to 0.97 ± 0.20 items individual^{-1}). When comparing results however, it is important to compare not only like-for-like species but also studies assessing a similar size range of microplastics, thereby allowing for accurate comparison of particle ingestion rates. Missawi *et al.* (2020*),* researched the abundance and potential toxic effects of small (≤ 3 μm) environmental microplastics on *H. diversicolor* among eight sites along the Tunisian coast. While microplastic ingestion in the worms was similar to that found in the present study (0.19 – 1.42 items individual^{-1}), this method investigated only the smaller size range of microplastics (≤ 3 μm). One could therefore assume that had they investigated the larger size range of microplastics in alignment with the current study, more ingested items per individual would be found. This highlights the difficulty when comparing the microplastic uptake concentrations between studies with varying protocols and study species.

It has been highlighted that certain benthic deposit feeders are known to exhibit selectivity in terms of particle uptake, however the general consensus is that the preference may be due to either the size (Lehtiniemi *et al.,* 2018), colour (Güven *et al.,* 2017; Ory *et al.,* 2017) or the presence of bacteria-rich substrates on the particle (Rodriguez *et al.*, 2001) rather than the polymer composition itself. One such study suggesting colour influences particle uptake found the Amberstripe scad, *Decapterus muroadsi* fish to ingest proportionally more blue particles compared to other colours, supposedly due to the close resemblance to their copepod prey (Ory *et al.,* 2017). Particle discrimination on the basis of colour however was not observed in this study

The relationship between sediment concentrations of microplastics and the abundance of particles found within the guts of benthic biota is not well understood. As concentrations of synthetic particles in the sediment were higher than those in the worms, it suggests that a parameter other than contaminant availability in the sediment impacts the number of synthetic particles ingested by worms. This is likely to be influenced by shape and size of particles and the various feeding modes of benthic species.

The mean length and range of synthetic particles taken up by worms was on average half that of the mean length and range of particles present within the sediments, suggesting not all sizes are bioavailable to be ingested. This finding may be down to worm mouth size as well as feeding mode of the species. *H. diversicolor* uses diverse feeding modes but usually behaves as a filter and deposit feeder, scavenging for organic material and detritus on the sediment surface (Olivier *et al.,* 1997). This species has been reported to ingest particles ranging from 63 µm to 3000 µm (Moreira *et al.,* 2006) similar to the sizes found in this study, suggesting only the size range of smaller synthetic particles in the sediment are bioavailable to worms.

Exposing Pacific oyster, *Crassostrea gigas* larvae to polystyrene particles of varying size demonstrated that as the larvae got bigger, so did the range of particle sizes they could ingest (Cole & Galloway, 2015). Here there is clear particle discrimination on the basis of size showing only when larvae grow bigger, can they ingest larger particles. Another study conducted in 2018 found size to be more important than shape when exposing small predators to naturally occurring secondary microplastics (Lehtiniemi *et al.*, 2018). It is important to note however that their results solely focussed on different shapes of fragment and beads, rather than fibres or film. All the same, they found that size of the fragments more than the shape is a crucial nominator influencing the numbers of plastics ingested.

Of the cellulosic and polypropylene particles found, 77.6 % of cellulosic and 50 % of the polypropylene particles were fibrous in shape. Had the majority of other particle compositions also been fibrous in form, one could have deduced whether shape or chemical composition played a larger role when influencing worm ingestion. As the shapes of the other particle compositions were varied however, one cannot presume this.

These studies suggest that ragworms are unable to preferentially distinguish between sediment and contaminated material, inferring these species ingest contaminants just as readily as they would normal sediment. Studies on the deposit feeding lugworm, *Arenicola marina* also showed an inability to differentiate between plastics and sediment (Browne *et al.,* 2013; Van Cauwenberghe *et al.,* 2015a). This would support the reasoning that the more contaminated a region, the more contaminants ingested by marine worms. This principle however was not seen in the results of this study, with no significant difference in worm uptake of synthetic particles despite significant variation in the synthetic particle abundance of sediments between sites.

Identifying fibres as the most commonly found particle shapes across the majority of sediment sites and all ingested worm samples is supported by other studies assessing the shapes of particles found in benthic-dwelling biota (Barrows *et al.,* 2018; Courtene-Jones *et al.,* 2017; Kanhai *et al.,* 2017; Taylor *et al.,* 2016). However, there is some discussion as to whether this is because of the mesh size used in experiments (Lindeque *et al.,* 2020). In said study, the use of finer nets resulted in the collection of significantly thinner and shorter microplastic fibres, also retaining a high proportion of larger fragments in the mesh. While this is the case, a balance between efficiency and accuracy must be met in all experiments. Those with smaller mesh sizes will give a better representation of microplastic concentrations in the natural environment, thereby enabling more reliable estimates of microplastic budgets.

All ingested fibres were monofilament in nature and accounted for the longest particles ingested by worms even through larger fragments were present in the sediment. This finding adds to the previous evidence suggesting fibres are easier to ingest in comparison to other shapes (Lehtiniemi *et al.*, 2018), likely due to their width being within the size range of particles they can ingest.

Extensive knotting of fibres has been reported in the decapod crustacean, *Nephrops norvegicus*, with tangled balls of fibres found within 62 % of the animals studied (Murray & Cowie, 2011). Whether the lobsters themselves coil the fibres or whether they were tangled pre-ingestion is yet to be understood. In the present study, fibres were measured by tracking the outside edge all along the length of the particle. The majority of fibres observed were uncoiled, yet those that were knotted were measured in the same way. It is unclear whether the extraction and analysis of these fibres gave rise to a change in appearance post extraction, whether the worms knotted the fibres themselves or indeed whether the fibres were knotted prior to ingestion. If the latter is the case, knotted fibres would give the impression of being smaller to the worms than the lengths provided in the results thereby overestimating the sizes of fibres ingested by the worms. It is essential that research be carried out on the impact different extraction protocols have on the appearance of fibres, especially considering their occurrence in the marine environment outweighs that of any other shape (Gago *et al.,* 2018; Suaria *et al.,* 2020).

Our understanding of synthetic particle ingestion by benthic deposit feeders and the physical impacts they incur is mainly based on a limited number of controlled laboratory studies, some of which have been shorter term with unrealistically high concentrations. One study showed how sediments contaminated with plastic particles (microscopic unplasticised polyvinyl chloride (UPVC) at concentrations overlapping those in the environment) take longer to pass through the gut of the polychaete worms than normal food, but more importantly that feeding activity reduced as well as growth (Wright *et al.,* 2013). This study focussed on *Arenicola marina* which has different feeding strategies and habitat preferences to the *H. diversicolor* used in this research. Therefore, we cannot directly infer a similar response in our study species, the Ragworm. However, a more recent study compared the effects of ingested PVC microplastics at a size range similar to what was ingested (with and without adsorbed benzo(a)pyrene) on *H. diversicolor* (Gomiero *et al.,* 2018). Within both treatments (with and without the added organic pollutant) permanent effects of oxidative stress were observed at tissue level and in the added pollutant treatment, there was evidence of toxicity mainly targeting impairment of cellular functioning and genotoxicity in coelomocytes. Oxidative stress, while contributing to a range of health conditions, is an important component of the stress response in marine organisms exposed to changes in conditions, such as thermal stress, exposure to ultraviolet radiation and pollution (Lesser, 2006). An inability to combat these changes in the environment can lead to a reduction in energy reserves and feeding ability, subsequently meaning organisms are smaller as a result. As the concentrations of PVC particles here are not in alignment with the levels found in the current study, we cannot infer the same levels of oxidative stress and subsequent reduced feeding in the *H. diversicolor* populations studied, however a decline in physical health to a small extent can be presumed. Research into the impact of other synthetic particles on health is lacking and demonstrates the need to investigate possible oxidative stress caused by the numerous different synthetic particles found within marine worms. This may in turn alter the feeding habits of worms and hence impact individual fitness.

The work in this chapter has demonstrated the presence of a wide range of microplastic particles present with benthic sediments and a sediment dwelling organism, but the mechanisms by which these particles, many of which were secondary fragments and buoyant polymers, reach benthic sediments remain poorly understood. The next chapter explores a novel route by which large benthic macroplastic items might become fragmented into smaller particles and enter a benthic ecosystem.

Chapter 3: Interactions with biota influence the size, uptake and environmental fate of plastic debris in benthic ecosystems

3.1 Introduction

It is now clear that regions contaminated with plastic debris are not constrained to specific ocean depths. Whilst early plastic studies concentrated on the floating proportion of microplastics, there is now a wealth of evidence to suggest that microplastics (1 to <1000 μm, Hartmann *et al.,* 2019) are being recorded in the ocean benthos (Van Cauwenberghe *et al.*, 2013; Woodall *et al.*, 2014; van Sebille *et al.*, 2015; Courtene-Jones *et al.*, 2017; Munari *et al.*, 2017; Peng *et al.*, 2017). Macroplastic litter items (1 cm and larger) will also foul and sink to the seabed (Woodall *et al.,* 2014). Once on the seafloor fragmentation processes will occur, however very little is understood about these processes. The size, shape, charge and other properties of microplastic are constantly changing, altering its biological fate and bioavailability. It has also been suggested that interactions between plastics and marine species might also play a role in the fragmentation, transport, and fate of plastic particles (Galloway *et al.,* 2017).

The susceptibility of plastics to further fragment while on the seafloor is influenced by a suite of physical and biological interactions. Exposure of plastic objects on the surface to solar radiation results in their photodegradation, embrittlement and fragmentation by wave action (Andrady, 2011). Photodegradation has been shown to increase the capability of plastics to biofoul (Kerr & Cowling, 2003), while colonising organisms such as microbes (Zettler *et al.,* 2013), polychaetes (Jang *et al.*, 2018), crustaceans (Davidson, 2012) and echinoderms (Porter *et al.,* 2019) contribute to the fragmentation of plastic acting as bioeroders. Bioerosion has been suggested to play a major role in the fragmentation of macroplastics and is a process where substrates are broken down by living organisms, thereby weakening the overall structure and increasing the likelihood of weathering (Glynn & Manzello, 2015; Neumann, 1966). As a result, the fragmenting action of bioeroders has huge potential to influence the distribution and risk of plastic pollution (Davidson *et al.*, 2018; Jang *et al.*, 2018).

Sea urchins are abundant herbivores in many benthic habitats (Sheppard-Brennand *et al.,* 2017), known to alter the physical structure of their substrates in the natural environment via their feeding activity which uses a beak-like structure of five hard plates, known as Aristotle's lantern to scrape organic matter off substrates (Boudouresque & Verlaque, 2007; Porter *et al.,* 2019). Given that macroplastic litter readily biofouls in marine environments, there is the potential for sea urchins to graze on these surfaces and bioerode macroplastic items, if these items are encountered in their benthic habitat. The purple sea urchin, *Paracentrotus lividus* was effective in generating microplastics from large macroplastic polyethylene crates (hereafter referred to as 'plastic crate(s)') and produced on average 85.67 microplastics per urchin over a 9 day tank exposure (Porter *et al.,* 2019). These particles generated by the feeding activity of the urchins varied in shape and size and included freely released particles that floated together with particles incorporated into faecal material that sunk. This raises the question as to whether the feeding behaviour of urchins might play a role not only in the fragmentation of benthic plastic debris, but also in the movement and fate of plastics within marine ecosystems.

Pelagic and benthic environments are linked by a range of surface-dwelling filter-feeding species (Griffiths *et al.,* 2017), ordinarily removing large quantities of suspended organic matter from the water by filter-feeding, and through the production of faeces and pseudofaeces (Ward & Shumway, 2004). Riisgård *et al.,* (2011) highlighted the ability of the blue mussel, *Mytilus edulis* to process large volumes of water with a 21.5 mm sized mussel filtering an average of 15 mL min^{-1} under optimal algae conditions. With the size range of microplastics overlapping that of mussel prey, it is not surprising that ingestion of microplastics by mussels has been widely documented, both in lab studies and with field collections of natural populations (Avio *et al.,* 2015; Browne *et al.,* 2008; Li *et al.,* 2016; Scott *et al.,* 2019). The egestion of microplastics in pseudofaeces has also been documented (Woods *et al., 2*018) and represents an important food source for benthic-dwelling organisms (Ysebaert *et al.,* 2009). Given the key functional role that mussels play in bentho-pelagic coupling, and the fact that they readily ingest any microplastic present in their surrounding water column, it could be hypothesised that the filter feeding activity of mussels might act to drawdown of plastic particles from the water column to the benthos.

The king ragworm, *Alitta virens* is a key bioturbator influencing not only the structure of benthic sediments through burrowing, ingestion, defecation and ventilation (Kristensen *et al.,* 2012), but also ecosystem functioning (Biles *et al.,* 2002; Herringshaw & Solan, 2008). Behavioural differences between infaunal species cause different modes and rates of bioturbation, and this has been shown to impact nutrient release and the amount of sediment suspended in the water column (Biles *et al.,* 2002). Research into how organic matter and various pollutants are affected by bioturbation is ongoing (Aller & Cochran, 2019, Solan *et al*., 2019) with crab-bioturbation for example, shown to affect organic matter at intertidal soft bottom areas, enhancing benthic metabolism and benthic fluxes of organic matter (Fanjul *et al.,* 2015). Polychaete worms have now been demonstrated to readily ingest microplastic particles both in a number of laboratory-based exposure studies (e.g., Wright *et al.,* 2013, Besseling et al., 2013, Revel *et al.,* 2020) and from field-based studies on natural populations (e.g., Knutsen *et al.,* 2020, Hamzah *et al.,* 2021) including my own work in Chapter 2. Although microplastic ingestion in marine worms is prevalent, it is not clear whether the activity of bioturbation may act to move microplastics downwards, 'locking' them away or whether they re-suspend them.

The aim of this study was to investigate the interactions of benthic organisms with plastic debris to determine whether their feeding and behavioural activities might play a role in the fragmentation, movement and fate of plastic in the benthos. Work by Porter *et al.,* (2019) showed that urchins have the capacity to generate microplastics from macroplastic items. Subsequently, the question whether these microplastics are bioavailable to the rest of the benthic food web is warranted and may act as a potential (so far undescribed) pathway of microplastics to enter benthic ecosystems. Here, I use a mesocosm experiment to look at the generation and subsequent movement of microplastics within a test benthic ecosystem comprising three different functional groups; the grazing echinoderm *Paracentrotus lividus*, the filter feeding bivalve *Mytilus edulis* and the sediment-dwelling bioturbator *Alitta virens.* This allows us to determine the role of each species in the fate and movement of plastic.

3.2 Materials and Methods

3.2.1 Animal collection and maintenance

The purple sea urchin, *Paracentrotus lividus* (mass: 52.2 g ± 8.75 g) was collected from Roscoff Marine Station, France, in December 2018. Upon return to the laboratory (Aquatic Resources Centre, Biosciences, University of Exeter) they underwent three water changes in an aquarium setting to allow them to depurate, encouraging the expulsion of biological contaminants and physical impurities such as sand and silt. They were subsequently transferred to 500 L tanks on a recirculating system (35 ppt, 15 °C, artificial seawater [ASW]), allowing them to acclimate for five days before being added to any exposure. The urchins remained in these tanks for the duration of the experiment. Urchins were fed 10g of *Saccharina latissima ad libitum* three times per week. Three urchins were weighed and added to each of the four treatment tanks for the exposure experiment.

The blue mussel, *Mytilus edulis* (mass: 28.1 g ± 9.75 g) was collected from a local source at Starcross, Devon (Lat: 50.618945, Long: -3.4462054). Their shells were scrubbed to remove organisms and underwent two water changes in an aquarium setting to allow them to depurate. Mussels were transferred to 500 L tanks on a recirculating system (35 ppt, 15 °C, ASW), allowing them to acclimate for five days before being added to any exposure. They were fed a concentrated blend of microalgae (Shellfish Diet 1800, Reed Mariculture) every three days. Mussels were starved for 12 hours before exposure to ensure maximum feeding during the exposure and removed from the tanks 1 hour pre exposure to ensure optimum ventilation. Five mussels were weighed and added to three of the four treatment tanks for the exposure experiment.

The king ragworm, *Alitta virens* (previously *Nereis virens*) (mass: 3.8 g ± 1.35 g) was collected from a local bait shop (but originally sourced from Holland). Upon arrival at the laboratory the ragworms underwent two water changes in an aquarium setting to allow them to depurate. They were subsequently transferred to 20 L static opaque tanks with lids (35 ppt, 15 °C, ASW), allowing them to acclimate for four days before being added to any exposure. Small amounts of blue roll were added to the 20 L tank to allow ragworms to burrow into. Ragworms were fed *Saccharina latissima ad libitum* twice before being introduced to the exposure tanks. Five ragworms were weighed and added to each of the four treatment tanks for the exposure experiment.

3.2.2 Experimental Design

This experiment was designed to assess the following hypotheses:

(1) The fragmentation of plastic crates into plastic fragments by the Purple sea urchin, *Paracentrotus lividus,* will lead to uptake of plastic fragments by the Blue mussel, *Mytilus edulis,* and the King ragworm, *Alitta virens* within a mesocosm environment.
(2) Urchins will generate more plastic fragments in the presence of food.
(3) The presence of Blue mussels will increase the amount of plastic fragments in the sediment.
(4) The King ragworm will have more plastic fragments available to it and ingested in the presence of Blue mussels.

To address these hypotheses, four mesocosm treatments (Figure 3.1) were set up and left to run for 13 days. The experiment was repeated three times, resulting in data for three replicate tanks per treatment.

Treatment 1: a 'no plastic' control containing three urchins, five mussels and five ragworms. This is to confirm any plastics in later treatments originated from the plastic crate.

Treatment 2: a treatment containing half a biofouled plastic crate with all organisms present (three urchins, five mussels and five ragworms). Macroalgae was absent. This addresses Hypothesis 1 and 2 in testing the influence a benthic grazer has on the environmental partitioning of plastic litter and the effect of macroalgae absence.

Treatment 3: a treatment containing half a biofouled plastic crate with all organisms present (three urchins, five mussels and five ragworms). Macroalgae was present. This addresses Hypothesis 1 and 2 in allowing us not only to test the influence a benthic grazer has on the partitioning of plastic litter in the presence of food, but also ascertaining whether the presence of food for the urchins alters the abundance of plastic fragments generated by the urchins and whether these are available to the King ragworm and Blue mussel. Treatment 3 also addresses Hypothesis 3 testing the ability of mussels to increase the number of plastic fragments in the sediment.

Treatment 4: a treatment containing half a biofouled plastic crate, three urchins, five ragworms and no mussels. Macroalgae was present. This addresses Hypothesis 3 and 4 examining whether the presence of mussels influences the amount of plastic in the sediment and the subsequent uptake of microplastics in the ragworms.

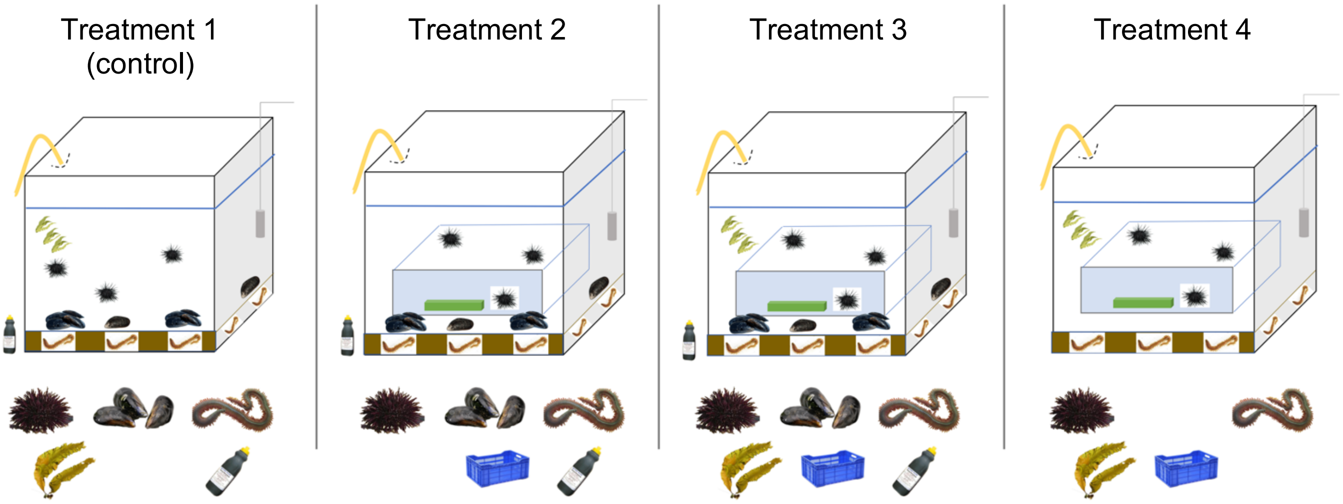


Figure 3.1: Exposure treatment scenarios. Diagram showing contents of each of the four mesocosm treatment tanks (n = 3 replicates per treatment). Three individual *Paracentrotus lividus* per tank (Treatments 1, 2, 3 & 4), five *Mytilus edulis* (Treatments 1, 2 & 3) and five *Alitta virens* (Treatments 1, 2, 3 & 4) were used. Yellow tubing illustrates a siphoning hose with mesh attached for water changes, the blue box the urchins are on represents the biofouled half plastic crate, grey cylinder illustrates constant air input and the green block illustrates plant weight used to keep the plastic crate from floating to the surface. The kelp frond symbol signifies food for the urchins and the blue bottle symbol signifies microalgae feed for the mussels.

3.2.3 Sediment and plastic crate preparation

One week before exposure, sediment was collected from Exton, Devon (Lat: 50.667623, Long: -3.444380) at the mid-shore zone at low tide and brought back to the laboratory where it was sieved to 500 μm to get rid of small stones, shells and other macrofauna. Three 50 ml falcon tube cores were then taken and a method developed by Coppock *et al.*, (2017) using the principle of density floatation to separate plastic-like particles from sediment was employed to isolate any potential environmental microplastics present. Few plastic-like fibres and fragments were found on each occasion however none of which were blue fragments so no further sieving to remove plastics was deemed necessary. Filtered ASW (35 ppt at 15 °C) was added to the sieved sediment to bring the sediment salinity in line with that of the exposure experiment. Approximately 8500 cm^3 of sieved sediment was added to each of the four 40 L exposure tanks as this was deemed an adequate volume and depth of sediment (6 cm) for *Alitta virens* to survive (Herringshaw *et al.*, 2009).

Plastic crates were used as the sole input of plastic in the exposure experiment. These were 40 cm by 30 cm by 11 cm blue polyethylene crates (see Figure 3.2) commonly used in the transport and storage of food produce. Previous research by Porter *et al.*, (2019) highlighted the fragmentation of plastic crates by urchins. In this experiment, urchins produced negatively buoyant fragments irrespective of the presence of additional food. We therefore wanted to test whether these urchins produced fragments that are bioavailable to other benthic organisms, such as *Alitta virens and Mytilus edulis.*

Three of the four exposure treatments contained half a biofouled plastic crate. Crates were biofouled to mimic time spent as litter on the seabed to best stimulate the conditions in which macroplastic would be found by urchins in the natural marine environment. To foul the trays, permission was granted by Queen Anne's Battery Marina, Plymouth to tie six plastic crates beneath one of the jetties (thereby ensuring the crates would be submerged for the duration) for 2.5 months between January and March 2019. The crates were primarily fouled with green filamentous algae. Upon return to the laboratory the crates were kept in a 500 L static tank where the biofouled organisms remained and grew on the crates for the duration of the experiment. Plastic crates were cut in half before being weighed and placed on top of the sediment in three of the four treatments. They were weighted down using aquarium plant weights to ensure negative buoyancy. A ‘crate only’ control was not included based on previous pilot work demonstrating the plastic crate does not fragment in a tank within the 2 week period of these experiments.

3.2.4 Experimental Set-up

Each tank (for all treatments) contained sediment at a depth of 6 cm and 20 L of ASW (35 ppt, 15 °C and filtered to 0.2 μm), 15 L of which was changed every two/three days. To do this, a 20 μm mesh was fitted onto the end of a siphon hose thereby allowing the capture of any plastic fragments lost from the system. Once the water had been siphoned off, the mesh was removed from the hose and re-submerged into the allotted tank to ‘re-input’ any plastic fragments previously removed during the water change process. The tanks were aerated continuously. Individual treatment tanks contained three urchins, five mussels (when present) and five ragworms. Species were added to the tanks in such a way that the biomass between tanks was similar for each species.

After the 13 day exposure was completed, all organisms were removed, weighed and immediately snap frozen in clear individual PE sampling bags. These were kept in a -12 °C freezer for further analysis. The bulk of the water was then filtered over separate 20 µm mesh filters. These individual meshes were then folded, kept in glass petri dishes before they were resuspended in Milli-Q water for 6hrs allowing the plastics to separate from the mesh. Once separated, the water containing the plastic fragments was vacuum filtered onto a 20 µm nylon mesh and kept in a clean glass petri dish before further analysis. A sub-sample of sediment from each tank was taken for analysis by dividing each tank up into a numbered grid and using a random number generator to pick where the samples were taken from. Using falcon tubes, three 50 ml cores were obtained from each tank and were kept in the freezer at -12 °C until further analysis.

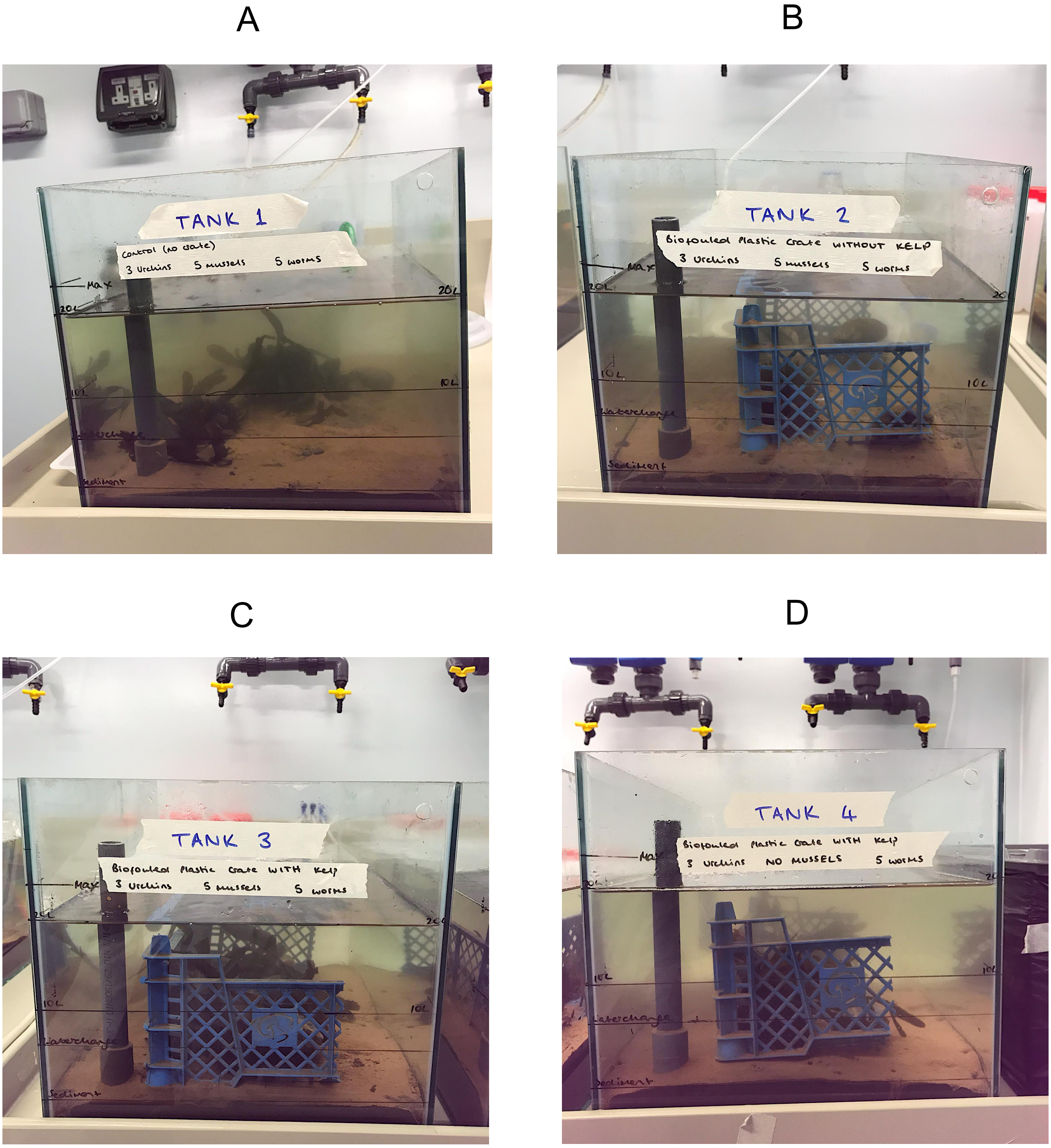


Figure 3.2: Pictures taken mid-experiment for **(A)** Treatment 1 (no plastic control), **(B)** Treatment 2 (mussels, no macroalgae), **(C)** Treatment 3 (mussels and macroalgae) and **(D)** Treatment 4 (no mussels). Photographed using an iPhone 8.

3.2.5 Tissue digestions and plastic isolation from sediment

While still frozen, urchin and mussel tissue were carefully separated from the test and shell respectively using a blade and scissors and transferred into a 50 ml falcon tube. The ragworms were transferred directly into separate 50 ml falcon tubes. Twenty-five millilitres of 10% filtered potassium hydroxide (KOH) solution was added to the falcon tubes each containing individual ragworms. Twenty-five millilitres of 30% KOH solution was added to the falcon tubes each containing individual urchins and mussels.

Ragworms were left to digest at 60 °C for 24 hrs, and both the mussels and urchins at 60 °C for 48 hrs, after which the digested organisms were moved to a laminar flow hood where all subsequent steps were conducted. The organisms were vacuum filtered through 10 μm Cyclopore polycarbonate membrane filters. Filter papers were then transferred into petri dishes and sealed with a parafilm until further analysed.

Defrosted sediment from each falcon tube was placed into individual 500 ml beakers and left to dry in an oven at 60 °C for 12 hrs. From each of these samples (three samples per tank with three repeats), 50 g of dry sediment was isolated for the identification of potential plastics, resulting in a total of 1800 g of analysed sediment. Isolating this volume of sediment per sample allows for the use of Sediment-Microplastic Isolation (SMI) units, a custom-built piece of apparatus replicating the design and methods developed by Coppock *et al.*, (2017). This method uses the principle of density floatation to separate plastic-like particles from differing types of sediment with a high recovery mean efficiency (95.8%). A double-filtered (50 μm) solution of zinc chloride ($ZnCl_2$) at an optimal density of 1.5 gcm^{-2} was chosen as the floatation media as it balances the requirements for fine sediment to settle, whilst still being dense enough to enable the floatation and subsequent recovery of denser polymers. This procedure was carried out in a fume hood where after the $ZnCl_2$ sediment solution was vacuum filtered through 10 μm Cyclopore polycarbonate membrane filters.

3.2.6 Analysis of filters

Water samples as well as filtered material from each urchin, mussel, ragworm and sediment sample was analysed visually using an Olympus SZX16 dissecting microscope. Particles blue in appearance were identified by scanning the filter papers at 3.2 and 1.6 magnification for 5 minutes each. These particles were counted and photographed using an Olympus XC10, all of which were fragment shape. Each of the procedural blanks from previous stages underwent the same processing however only black and colourless fibres were found. As only blue PE polymers were of interest in this experiment, the finding of blanks containing black and colourless fibres was of no significance. Images of particles were uploaded onto ImageJ 1.47v where length, area and diameters of the blue PE fragments were measured.

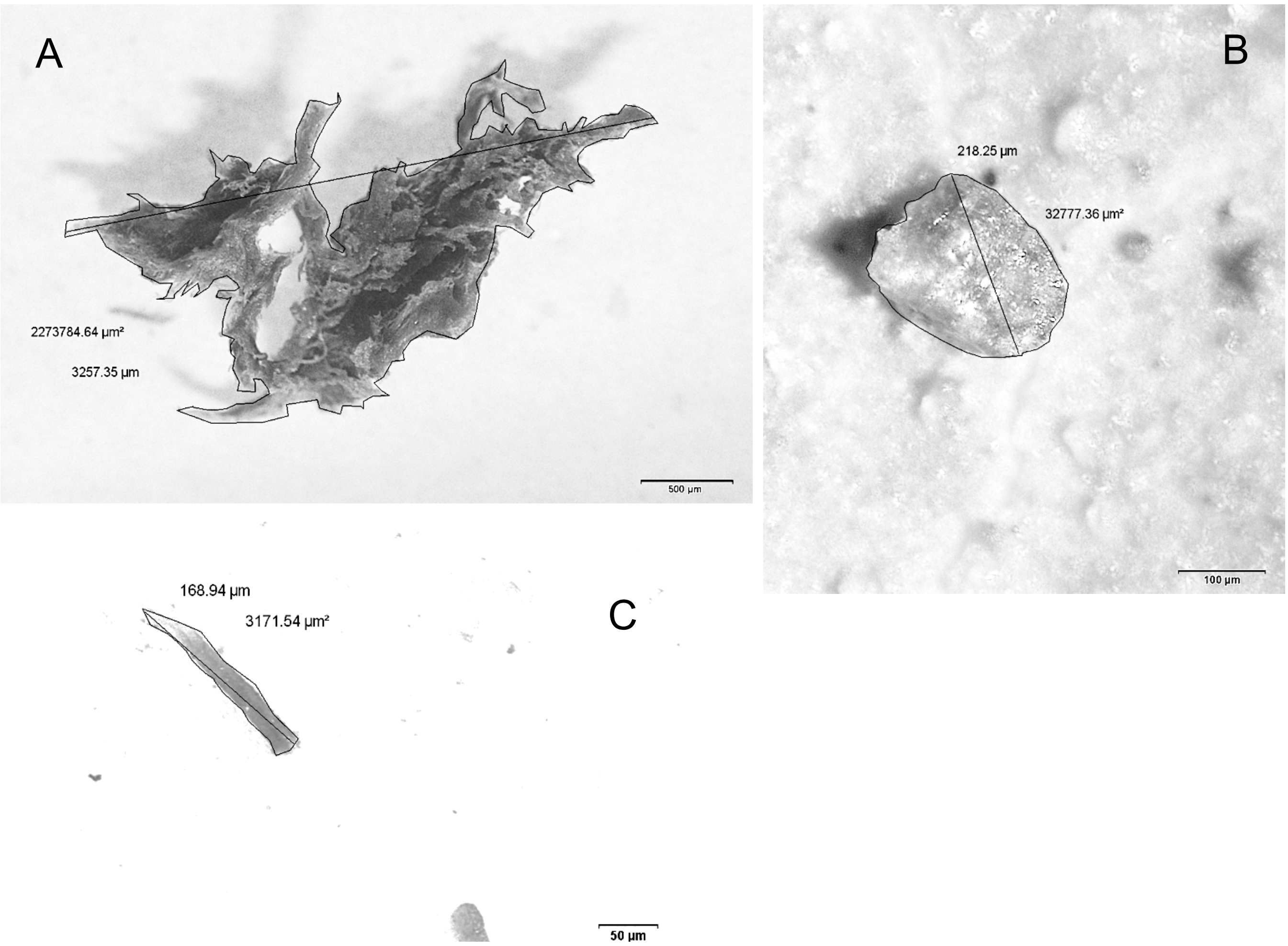


Figure 3.3: Example of images showing a selection of polyethylene fragments generated from the plastic crates by Urchins, *Paracentrotus lividus*. **(A)** a fragment found within an Urchin, **(B)** a fragment found within a Ragworm, *Alitta virens* and **(C)** a fragment found within a Mussel, *Mytilus edulis*. Photographed using an Olympus XC1 camera.

3.2.7 Contamination Control

Contamination was not of great concern in this experiment as the provenance of blue fragments within our exposure system was assumed, and then verified by FTIR, to be from the blue plastic crates. Only blue plastic items were counted and therefore airborne contamination was not a factor within our experiments.

3.2.8 Data analysis

All data presented is based on the particles observed following microscope analysis. SPSS Statistics 27 was used for statistical analyses with box plots plotted in RStudio v.1.3.1093. At each point treating tank as the level of replication, all data were tested for normality using Shapiro-Wilk test and for equal variances using Bartlett's test if normally distributed and a Levene's test if not normally distributed. Data is reported as mean ± standard error.

To test whether the numbers of plastic particles within the water, sediment, urchin, mussel and ragworm individuals varied between treatments, a One-way ANOVA was conducted followed by a Tukey's post-hoc test with number of particles found in each environmental matrix as the response variable and treatments as the explanatory variable.

To determine whether size of ingested particles varied between organisms, due to lack of normality within data, a Kruskal-Wallis test was conducted to test between length of particles within organisms. In these tests, the response variable was particle length and the explanatory variable used was organism type.

3.3 Results

3.3.1 Environmental partitioning of plastic particles

No microplastics were present in the water or sediment of any of the control (Treatment 1) tanks. Microplastic particles were observed in every tank to which a plastic crate was added, with a total of 408 small plastic particles sampled and analysed across Treatments 2, 3 and 4.

There was a significant difference in the number of polyethylene (PE) plastic fragments within the water between different treatments (One-way ANOVA; $F_{3,12} = 13.14$, $P < 0.001$). A post-hoc Tukey test confirmed that the presence of additional food for the urchins contributed to significantly higher number of PE fragments, with the number of PE fragments in Treatments 3 (mussels and macroalgae) and 4 (macroalgae but no mussels) being almost three times that recorded for Treatment 2 (mussels and no additional food) (Figure 3.4A).

A significant effect of treatment on the number of PE fragments present within the sediment of the tanks was also observed (One-way ANOVA; $F_{3,12} = 14.29$, $P < 0.001$). The highest number of PE fragments (6.4 ± 1.0 particles per 50 g) was present in the sediments of Treatment 3 where additional food for the urchins was provided (Figure 3.4B). This was significantly higher (revealed by a post-hoc Tukey test) than the number present in sediments of the other three treatments. There was no significant difference between the number of PE fragments within Treatment 2 (mussels, no macroalgae) and Treatment 4 (no mussels) which means that we cannot accept Hypothesis 3: that the presence of Blue mussels will increase the amount of PE fragments in the sediment, with food availability and the presence/absence of mussels likely interacting.

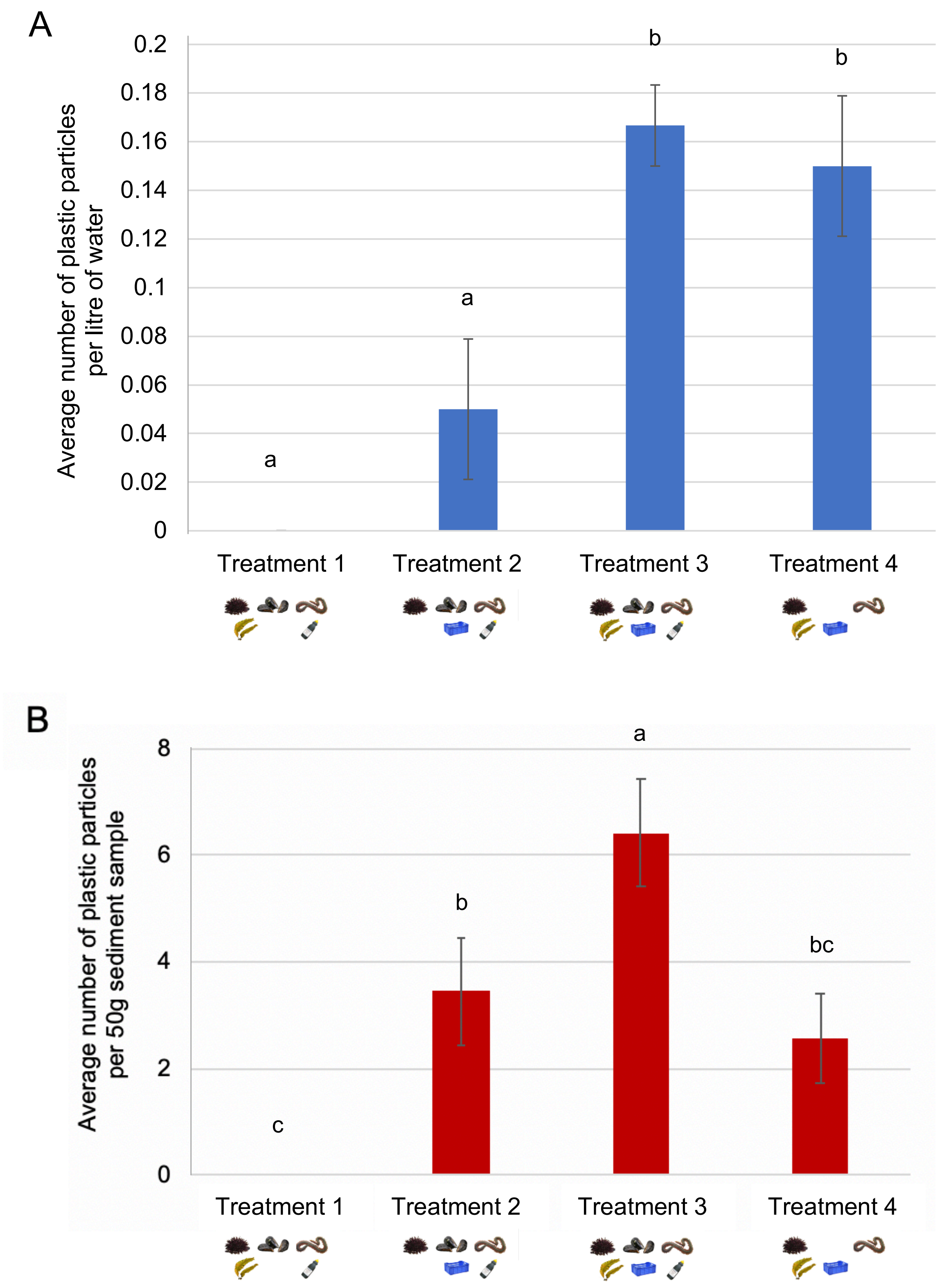


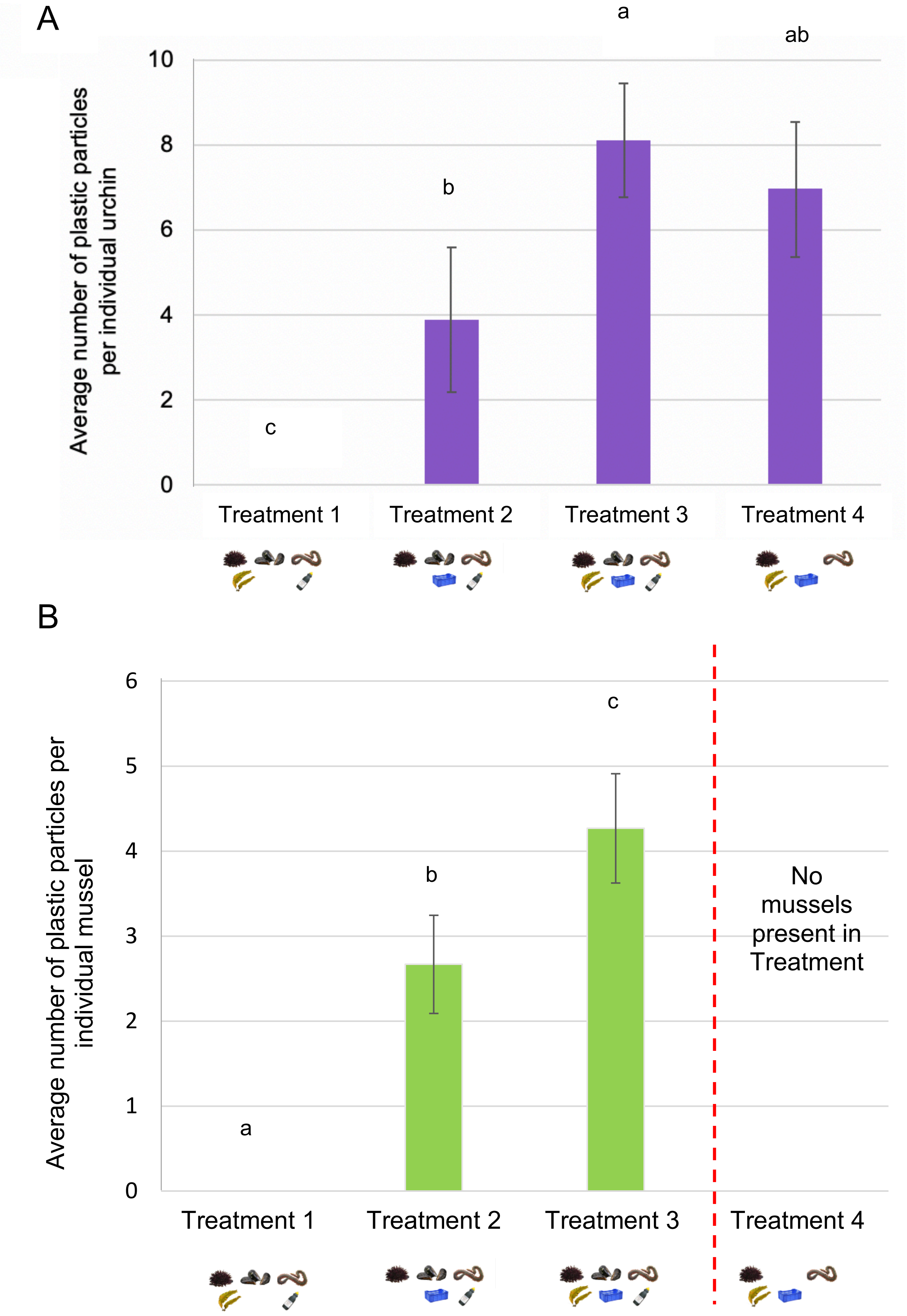
Figure 3.4: Abundance of plastic particles amongst the four treatments in **(A)** a litre of water (N = 12), **(B)** 50g of Sediment (N = 36) (± SE) after a 13 day mesocosm exposure experiment. Bars that do not share a letter are significantly different (Tukey's pairwise comparison).

A total of 104 PE fragments were found in the 36 urchins used in the experiment with fragments present in 100% of all organisms analysed. 98 PE fragments were found in the 45 mussels, present within 62.2% of all mussels analysed and 90 PE fragments were found within the 60 ragworms, present within 65% of all ragworms analysed (Figure 3.5). Hypothesis 1 theorising that the fragmentation of macroplastic crates into plastic fragments by the Purple sea urchin will lead to uptake of plastic fragments by the Blue mussel and the King ragworm within a mesocosm can therefore be accepted.

A significant treatment effect was observed in number of PE fragments found within individual urchins (One-way ANOVA; $F_{3,12} = 21.76$, $P < 0.001$) with a post-hoc Tukey test confirming Treatment 2 (mussels, no macroalgae, 3.8 ± 1.8 (SE) particles per individual) contained significantly fewer PE fragments than Treatment 3 (mussels and macroalgae, 8.1 ± 1.6 particles per individual) (Figure 3.5A). Here, there is a clear increase in uptake of PE fragments by urchins when food is present. Both Treatments 2 and 3 however were not significantly different from Treatment 4 (no mussels, 7.0 ± 1.4 particles per individual).

A significant treatment effect was observed in number of PE fragments found within individual mussels (One-way ANOVA; $F_{2,9} = 56.0$, $P < 0.001$) with a post-hoc Tukey confirming Treatment 2 (mussels, no macroalgae, 2.7 ± 0.5 (SE) particles per individual) contained significantly fewer PE fragments than Treatment 3 (mussels and macroalgae, 4.2 ± 0.7 particles per individual) (Figure 3.5B). This suggests urchin food availability contributes to the increase in the amount of PE fragments bioavailable to subsequent organisms.

The treatment scenarios also significantly affected the number of PE fragments found within the ragworms (One-way ANOVA; $F_{3,12} = 39.69$, $P < 0.001$, Figure 3.5C). Four times more PE fragments (3.6 ± 0.5 particles per individual) were found within the ragworms in Treatment 3, where mussels and macroalgae were both present, than in Treatment 4 with macroalgae but no mussels present (0.9 ± 0.2 particles per individual; significantly different as confirmed by a post-hoc Tukey test). There was also significantly fewer plastic particles within ragworms in Treatment 2 (mussels, no macroalgae, 2.2 ± 0.7 particles per individual) compared to Treatment 3 (mussels and macroalgae, 3.6 ± 0.5 particles per individual), further suggesting urchin food availability contributes to the increase in plastic particle availability to subsequent organisms. These results support Hypothesis 4: that the ragworm will have more plastic fragments available to it and thereby ingested in the presence of Blue mussels.


A
a
ab
10
8
6
4
2
0
b
c
Average number of plastic particles per individual urchin
Treatment 1
Treatment 2
Treatment 3
Treatment 4
B
6
5
4
3
2
1
0
c
b
a
Average number of plastic particles per individual mussel
No mussels present in Treatment
Treatment 1
Treatment 2
Treatment 3
Treatment 4

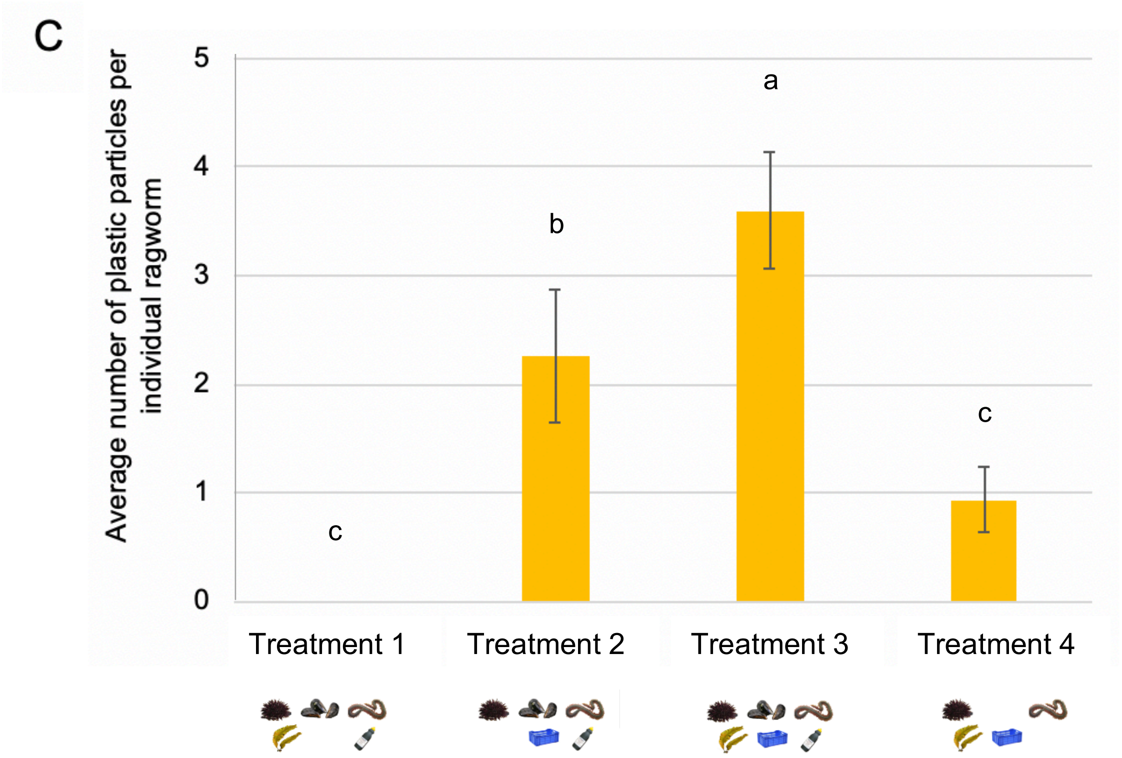


Figure 3.5: Abundance of plastic particles amongst the four treatments in **(A)** Mean plastic count per individual Urchin, *Paracentrotus lividus* (N = 36), **(B)** Mean plastic count per individual Mussel *Mytilus edulis* (N = 45) and **(C)** Mean plastic count per individual Ragworm, *Alitta virens* (N = 60) (± SE) after a 13 day mesocosm exposure experiment. Bars that do not share a letter are significantly different (Tukey's pairwise comparison).

3.3.2 Size of ingested plastic particles

There was a significant difference in the sizes of particles within the three different benthic species (H(2) = 176.41, P < 0.001) with urchins containing significantly larger fragments (range 90.2 μm to 3452.9 μm, mean of 1354.4 μm ± 65.4 μm) compared to the mussels (range 10.2 μm to 1921.0 μm, mean 230.2 μm ± 30.7 μm) and ragworms (range 38.7 μm to 806.6 μm, mean 197.0 μm ± 15.5 μm) (Figure 3.6). The standard deviation of particle lengths within the urchins (S.D. 663.9) was approximately twice that in the mussels (S.D. 301.1) and five-times that in the ragworms (S.D. 145.0).

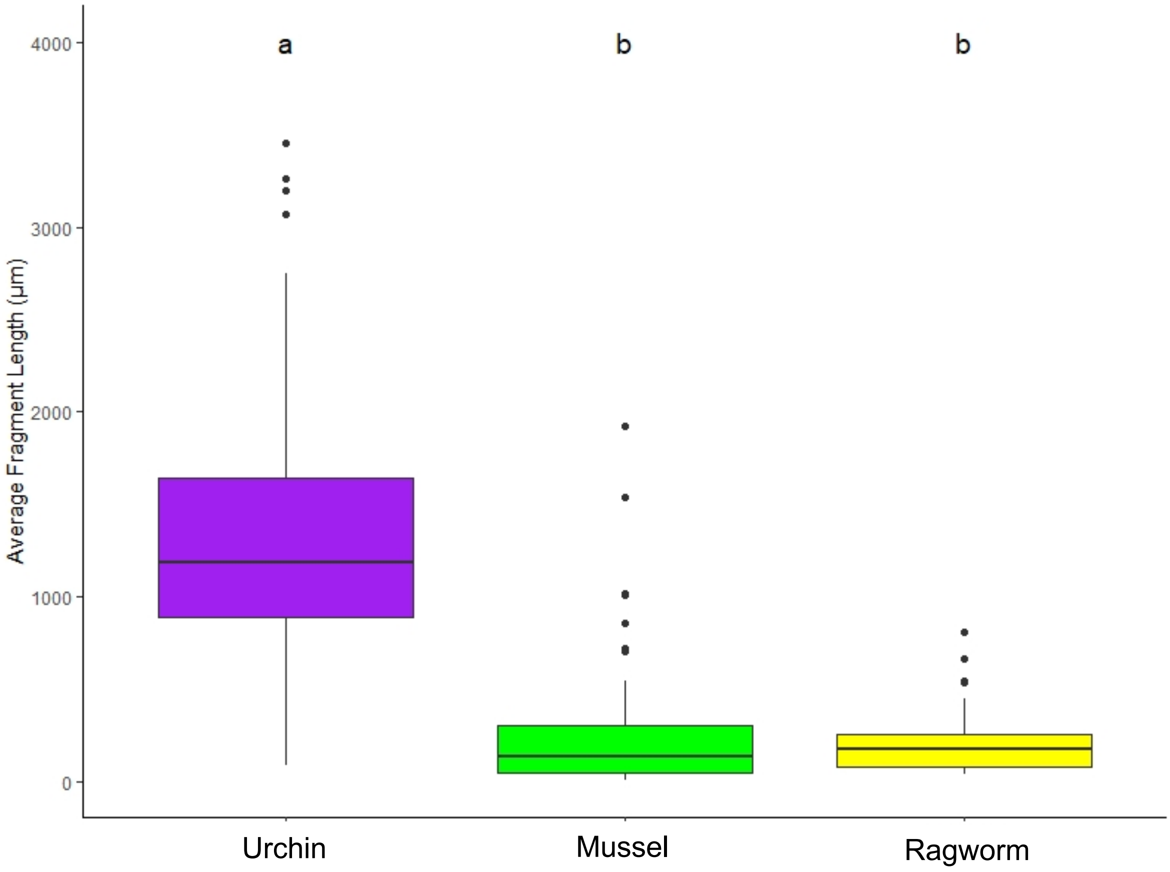


Figure 3.6: Mean lengths of plastic fragments within benthic organisms (μm) (± SE); the Urchin *Paracentrotus lividus* (N = 36), Mussel *Mytilus edulis* (N = 45) and Ragworm *Alitta virens* (N = 60). Bars that do not share a letter are significantly different (Tukey's pairwise comparison). On the graph, the horizontal line is the median, the coloured box represents the interquartile range associated with the median and the whiskers represent the upper and lower extremes.

3.4 Discussion

Using an experimental mesocosm comprising three benthic species with different functional roles, this study demonstrates the potential for benthic biota to influence the size, movement and fate of plastics through a benthic marine ecosystem. Plastic crates, previously biofouled under natural conditions to mimic time spent as litter on the seabed, were the only source of plastic introduced to each tank, as confirmed using the control tanks of Treatment 1. The Purple sea urchin was found to be effective at generating small plastic fragments (size range 10.2 μm to 5816.6 μm) from the plastic crates under all conditions tested. The presence of blue polyethylene (PE) plastic fragments within the benthic filter-feeding mussels and sediment dwelling polychaetes within the mesocosms where plastic crates were added, demonstrate that these smaller fragments generated by the urchin's feeding activity are then bioavailable for uptake by benthic biota. Clear evidence of food availability influencing the number of smaller plastics generated was indicated by more PE fragments within the urchins and the water when food was present, which in turn led to more PE fragments being taken up by the mussels. It also provides proof of principle that an otherwise buoyant plastic, generally thought to reside in the surface layers, can in fact become bioavailable to benthic-dwelling organisms. A combination of urchin food availability and the presence of mussels acted to increase the bioavailability and uptake of PE fragments into sediment-dwelling ragworms.

The idea that marine biota might play a role in the movement and fate of microplastics has been raised by a number of previous studies, but the experimental evidence to support these hypotheses to date has been limited. The idea was first conceptualised by Clark *et al.*, (2016) focussing on the ingestion and excretion of microplastics by copepods, incorporating microplastics into faecal pellets and altering the microplastic density as a result. The mechanism by which floating microplastics can be vertically transported away from surface waters to the sediments below was then proved experimentally in a study assessing the transport of plastics via sinking copepod faecal pellets (Cole *et al.,* 2016). Focussing on other marine species, Galloway *et al.,* (2017) hypothesised that the expelled waste water and pseudofaeces of mussels could draw down microplastics from the water column to the benthic boundary layer, leading to its incorporation into sediments by burrowing species. This study provides experimental evidence in support of the notion that interactions with benthic species act to facilitate the fragmentation and then subsequent movement of microplastic fragments.

In this study a total of 408 small PE fragments were generated through the grazing activity of urchins, with an average of 12.23 fragments generated per urchin. Bioerosion of macroplastic by the grazing activity of urchins has been previously demonstrated (Porter *et al.,* 2019), and has also been shown in a range of other species including crustaceans (Davidson, 2012; Mateos-Cárdenas *et al.,* 2020), polychaetes (Jang *et al.*, 2018), and even microbes (Zettler *et al., 2*013). The bioerosion rates recorded in these studies far exceed physical or ‘natural’ fragmentation rates from weathering and abrasions, increasing the rate of release of microplastic fragments into the marine environment.

One study investigated the generation of microplastics fragments from the boring of isopods, *Sphaeroma peruvianum* on polystyrene floats (Davidson, 2012). A maximum of 4630 microplastics varying in size and shape were created from a single burrow 17.4 mm long over a 2 month period, with a mean maximum length of 462.6 ± 29.2. Although isopods here have been shown to generate more microplastics than urchins predictably would over the same time scale, a key limitation of many studies of this type is the lack of natural food source as an alternative to the plastic for the study organism. One study that did take this into account is the recent work by Porter *et al.,* (2019) which found the grazing behaviour of sea urchins on the plastic within their tanks was strongly influenced by the presence or absence of a natural food source, and by the fouling of the plastic crates. They found urchins produced fewer microplastics from virgin (i.e., not-yet fouled) plastic crates when a natural food source was present, possibly suggesting that some discrimination between the unfouled plastic crate and natural food surfaces occurs. In the present study, additional presence of food resulted in an increase in PE fragment generation from fouled plastic crates, resulting in a ~ two-fold increase in the fragment counts found within the urchins and a ~ three-fold increase in the water. This significant increase was also reflected in the sediment, ragworm and mussel PE fragment counts when food was present. Comparing these results to the Porter *et al.,* (2019) study suggests some variability in this response. However, in all scenarios in this present study, as in the Porter *et al.,* (2019) study, plastic fragments were generated by urchins grazing on the plastic crate.

Virgin PE is a buoyant polymer, yet can be found in the guts of deep sea animals (Jamieson *et al.,* 2019), so clearly its density can be altered by its interactions with the marine environment. Porter *et al.,* (2019) found 90.5 % of microplastics generated by urchin grazing were incorporated into faecal matter, with the remaining 9.5 % floating to the surface when urchins were exposed to the biofouled trays with kelp. In the present study small PE fragments (some of which were covered in faecal matter) are still sinking without the influence of filter-feeding organisms, here shown by the presence of PE fragments found within the ragworms in a Treatment without mussels present. This confirms that urchins are not just fragmenting the plastic, they are altering its pathway through the ecosystem by altering the plastics propensity to sink.

An interesting part of this study was determining the influence mussel presence had on the proportion of PE fragments found within the sediment and ragworms within the mesocosm tanks. With mussels and food present, there were four times as many PE fragments present within the ragworms than where there was food but no mussels, strongly suggesting a role of mussels in the drawdown of plastics from the water column to the sediment. However, the picture is less clear for sediment plastic concentrations where food availability also alters the amount of plastics present. It is therefore likely that both food availability and mussel feeding behaviour influence plastic generation and the fate of those plastic particles. Further work confirming that the bentho-pelagic coupling role of mussels via their filter feeding activity applies not only to algae and nutrients (Gergs *et al.,* 2009; Griffiths *et al.,* 2017) but also to the movement and fate and plastic fragments within the water column is warranted. This might act as a pathway for buoyant plastic fragments floating at the surface water of the treatment tanks or within the water column to be redistributed and made bioavailable to sediment-dwelling biota, in this case the ragworms. This phenomenon has recently been proposed by Piarulli and Airoldi, (2020) who found the Mediterranean mussel, *Mytilus galloprovincialis* to be effective in removing microplastics from the water column by incorporating them into biodeposits. The effect was particularly evident in the smaller microplastics (41 µm) whose deposition was enhanced 15% by the action of mussels, also acting to more than double the amount of microplastic uptake by the benthic-dwelling polychaete, *Hediste diversicolor.* My study provides further evidence in support for this concept and is the first to use biologically fragmented and biofouled plastics rather than commercially available virgin plastics to highlight that urchin food availability and the presence of mussels act to increase the bioavailability and uptake of plastic particles into sediment-dwelling ragworms within a multi-species benthic mesocosm system.

Many of the reported effects studies (those looking at the biological effects of ingesting microplastic) are weighted towards invertebrate species for which microplastic represents the size range of natural food items and as a result, there is some evidence of the impact of microplastic ingestion. Microplastic exposure, ingestion and interactions have been shown to compromise the survival, growth and reproduction of organisms (Jemec *et al.,* 2016; Jeong *et al.,* 2016; Naidoo & Glassom, 2019; Rist *et al.,* 2016; Ziajahromi *et al.,* 2017) with sublethal mechanistic effects in benthic organisms including endocrine perturbation and hepatic stress, oxidative stress, reduced enzyme activity and cellular necrosis (Rochman *et al.*, 2013; Wright *et al.*, 2013; Lei *et al.*, 2018). Most of these studies however use commercially available 'virgin' microplastics which are typically spherically shaped and do not accurately represent those that we tend to encounter in the natural environment (Burns & Boxall, 2018). More commonly we find partially weathered, irregularly shaped microplastics whose impact on organisms may differ to the impacts observed using pristine microplastics in the lab.

Chronic exposure studies are also necessary to gain a better understanding of microplastic impacts. One of the few studies combining both irregularly-shaped microplastic particles with a chronic exposure timescale exposed three different *Cladoceran* species (*Daphnia magna, Daphnia pulex* and *Ceriodaphnia dubia*) to both pristine, spherically-shaped and irregularly-shaped cryomilled microplastic particles for up to 21 days (Jaikumar *et al.,* 2019). The reproductive output of all species declined in a dose-dependent manner and interestingly pristine microplastics were shown to have greater toxic potential relative to the artificially degraded microplastics. This highlights the importance of using environmentally relevant microplastic test materials in such studies. The body of evidence pointing towards the detrimental impact microplastic ingestion has on invertebrates has rightly become a cause for concern. Given these results highlight the ever-increasing abundance of bioavailable microplastics on the benthos, more research is required to investigate the impact of environmentally relevant biologically fragmented plastic concentrations on the health of benthic invertebrates.

Natural populations of organisms from all over the world have been reported to contain microplastics within their guts, often with higher numbers than reported in this study. The globally vast distribution of bioeroders such as crustaceans, amphipods, barnacles and certain fish species for example, may add considerably to the large amounts of microplastics bioavailable to a much larger range of species than just those that might eat the macroplastic item whole. Additionally, the capacity for one blue mussel to process large volumes of water is extensive with one study indicating a 21.5 mm sized blue mussel will filter an average of 15 mL min^{-1} under optimal algae conditions (Riisgård *et al.,* 2011). If one were to extrapolate this result to the volume of water filtered by a mussel bed 50 strong over a 24 hour time period, an equivalent of 1080 litres of water would be processed. The potential for their feeding activities to facilitate the drawdown of plastics could be great.

The partitioning of microplastics in the marine environment is of great interest to scientists ascertaining the risk to organisms. Seeing that benthic sediments are increasingly considered a major sink for plastic debris (Van Cauwenberghe *et al.*, 2013; Woodall *et al.*, 2014; van Sebille *et al.*, 2015; Courtene-Jones *et al.*, 2017; Munari *et al.*, 2017; Peng *et al.*, 2017), knowledge of the partitioning and redistribution of smaller plastic fragments on the benthos is crucial. This work provides insight into the ability of the purple sea urchin, *Paracentrotus lividus* to fragment macroplastic items acting to re-partition small plastic particles on the benthos. It also demonstrates that these otherwise buoyant fragmented plastics are bioavailable for uptake by other benthic-dwelling organisms and that the co-founding influence of both macroalgae and mussel, *Mytilus edulis* presence could be critical factors directly influencing the uptake of plastic fragments in the benthic-dwelling bioturbator, *Alitta* virens. This study demonstrates the complexities of processes surrounding bioerosion and partitioning of microplastics in the marine environment and highlights how the movement of plastic debris through marine ecosystems is complex, not only driven by physical processes such as currents and turbulent vertical mixing, but also by the organismal interactions influencing their bioavailability and its fate. Understanding these mechanisms is essential to increase our knowledge of the risks plastic pollution poses to marine ecosystems.

Chapter 4: General Discussion

This study adds evidence highlighting the accumulation of microplastic on the benthos (Courtene-Jones *et al.,* 2017; Munari *et al.,* 2017; Peng *et al.,* 2017; Van Cauwenberghe *et al.,* 2013) as well as microplastic ingestion by benthic-dwelling species (Taylor *et al.*, 2016). In the fieldwork study, average synthetic particle counts of 92 particles kg^{-1} were found in sediments from three sites in South Devon, UK (the Plym Estuary in Plymouth, Exe Estuary in Exton and Kingsbridge Estuary at Bowcombe Creek, Salcombe), while average counts within the benthic-dwelling polychaete, *Hediste diversicolor* was 0.74 particles per individual. Of all the worms analysed, 48% contained synthetic particles with buoyant polymers found both within the sediment samples and the worms, supporting previous work highlighting how polymers of varying densities are found in the guts of benthic organisms (Woodall *et al.*, 2014; Taylor *et al.*, 2016). It appeared only the size range of smaller synthetic particles in the sediment was bioavailable to the worms.

The mesocosm study also clarifies how interactions amongst certain organisms move plastic litter around benthic ecosystems, as originally hypothesised by Galloway *et al.,* (2017). This study looked at pathways by which interactions with three marine invertebrates with different functional roles might fragment and transport plastics to the benthos. Here, the action of sea urchins grazing on macroplastic polyethylene crates generated plastic fragments bioavailable to the mussels and ragworms present in the tanks. This aligns with the previous work by Porter *et al.,* (2019) and further highlights how bioeroders can contribute to the production of microplastics in the benthos. Plastic fragments were found within 62 % of mussels and 65 % of ragworms within the mesocosm set up, with clear evidence of food availability influencing the number of smaller plastics generated and both food availability and mussel feeding behaviour influencing plastic generation and the fate of those plastic particles. This adds light to previous evidence demonstrating the bentho-pelagic coupling role the Blue mussel plays in the drawdown of plastics to the sediment (Piarulli & Airoldi, 2020), and the subsequent increase in microplastic uptake in the benthic-dwelling polychaete.

Understanding the fragmentation, transport and fate of plastic debris within the ocean is a key aspect of understanding which species, habitats and ecosystems might be most impacted by its presence (Galloway *et al.,* 2017), yet this remains poorly understood. Much of the evidence to date on the pathways and mechanisms by which plastic might be transported to and within the benthos has been generated using modelling approaches. For example, Lebreton *et al.,* (2012) used the Langragian approach, otherwise known as 'numerical particle tracking', to model microplastic transport, distribution and accumulation below the surface. Here, virtual microplastic particles moving freely in the water column are simulated by ocean circulation models. The modelling results show the formation of five accumulation zones in the subtropical latitudes of the major ocean basins, while also determining the relative contributions of different source regions to the total plastic debris in a particular accumulation zone. However, only a few studies have come up with models accounting for buoyancy, friction and the settling of microplastics through biofouling and fragmentation (Ballent *et al.,* 2012; 2013; Isobe *et al.,* 2014) highlighting how the accuracy of such models is based solely on the quality of the hydrodynamic input data is that underpins them.

It's important to note however that methods other than computer modelling are used to investigate the accumulation and distribution of microplastics. A recent large-scale oceanographic study used *in situ* stand-alone pumps deployed simultaneously at three discrete depths at various stations with the Atlantic Ocean (Pabortsava & Lampitt, 2020). Here they showed that concealed in the mid-layer of the ocean, are high loads of small-sized plastic debris with the combined mass of the three most-littered plastics (polyethylene, polypropylene and polystyrene) of 32 – 651 µm size-class suspended in the top 200m of the Atlantic Ocean is 11.6 – 21.1 million tonnes. This highlights a large portion of microplastics residing in the mid-layer of our oceans that scientists have not formerly measured, tending to focus more commonly on surface-waters and benthic regions.

While particle tracking models are becoming increasingly sophisticated in the parameters that are accounted for, as of yet no models have included the impact organismal interactions have on the movement and partitioning of microplastics. From the data collected for this thesis, it is clear that the interactions between benthic organisms and plastic litter also have the potential to play a significant role in the fragmentation, re-partitioning and fate of plastic items and particles. We are not at the stage however to successfully include biological interactions in the already established physical mechanisms used in current models. While novel studies highlight for example how the incorporation of polystyrene particles into the faecal pellets of copepods alters the sinking rates of microplastics (Cole *et al.,* 2016), we are yet to understand the intricacies surrounding just how much plastic sinks faster when incorporated into faecal matter. Similarly just as the bentho-pelagic coupling role of mussels acting to facilitate the drawdown of microplastics to the sediment has been observed (Piarulli & Airoldi, 2020), we are yet to comprehend the extent to which mussels may re-partition these microplastics. Due to being non-selective filter feeders, a recent study found salps to also act as important vectors of marine debris transport from the surface waters to the benthos (Brandon *et al.,* 2020). This is another example of a biological interaction that might influence the movement of plastic. Realistically, with the role of other species in the transport of plastics yet to be understood and with the complexities associated with such mechanisms, substantially more knowledge is required before we can include biological interactions with plastic in these models. On reaching that stage, it would give us a greater understanding as to whether microplastics get locked away in the sediment or instead get re-suspended into the water column, altering their bioavailability to different species.

Understanding the partitioning and distribution of microplastics and the role that marine biota play is not just important to understand where plastic is going but is also vital to then identifying any impacts it may have on ecosystem functioning of benthic habitats. Multiple functionally different species residing at varying depths of the marine benthos have been shown to influence the complex factors that drive ecosystem function (Mermillod-Blondin & Rosenberg, 2006; Solan *et al.*, 2008; Schenone & Thrush, 2020). Bioturbation (the process by which many species affect the substratum in which they live (Kristensen *et al.,* 2012) for example, has been shown to directly affect ecosystem functioning shown in one study by generating biogeochemical conditions conducive to maximum efficiency of remineralisation (Aller & Cochran, 2019). Bioturbation is only one of numerous mechanisms by which ecosystem function is maintained in the benthos. As microplastics are known to contaminate benthic regions, knowledge of the impact these particles have on the functioning and structure of sedimentary habitats is crucial. The role bioturbation plays in the movement of microplastic particles was studied using the northern Baltic Sea clam, *Limecola balthica*, the polychaete *Marenzelleria* spp. and the amphipod *Monoporeia affinis* (Näkki, *et al.,* 2019). Using a 10-week mesocosm experiment the authors investigated whether sediment infauna might promote microplastic return to the sediment. Thin layers of frozen sediment containing environmentally realistic concentrations (<1,300 microplastics per kg of dry sediment) of acrylonitrile butadiene styrene microplastic fragments in two class sizes (> 500 µm and 100 – 300 µm) were added to depths of 2 cm and 5 cm in experimental cylinders filled with sediment. Although the results suggest bioturbation does not markedly transport buried microplastics upwards in sediments, the burial of microplastics in this case did reduce their availability to macrofauna (Näkki *et al.,* 2019). While there was a lack of evidence to suggest bioturbation did not directly transport microplastics upwards in this study, the ability of other bioturbating species to do this must be analysed, especially considering the multiple functional roles different sediment-dwelling organisms play in benthic ecosystems.

Studies directly showing ecosystem level impacts of macroplastics remain limited, but one study has investigated the ecological impacts on the biodiversity and ecosystem functioning of bivalve-dominated habitats (Green *et al.,* 2017). Here intact sediment cores containing European flat oysters (*Ostrea edulis*) and blue mussels (*Mytilus edulis*) were exposed to two different densities (2.5 or 25 μL^{-1}) of biodegradable or conventional microplastics in seawater in outdoor mesocosms. While after 50 days, ecosystem functioning within the *M. edulis* mesocosm was not affected by microplastic exposure, the opposite occurred amongst *O. edulis.* Porewater ammonium and biomass of benthic cyanobacteria decreased with exposure to microplastics and the infaunal invertebrate assemblages differed, with significantly less polychaetes and more oligochaetes in treatments exposed to microplastics (Green *et al.,* 2017). It appears that the effect of microplastics on ecosystem functioning in this study was dependent on the dominant bivalve present. Knowing the importance of such organisms in ecosystem functioning, these findings highlight the potential of microplastics to impact the functioning and structure of sedimentary habitats, the effect of which is currently unknown.

Despite the rapid increase over recent years in research and publications on the abundance and impacts of marine plastic pollution, our ability to identify real world impacts of microplastic ingestion remains lacking. A key issue that currently hinders any risk assessments on microplastic exposure is the difficulty in accurately assessing exposure. Digestion analysis experiments only produce a ‘snapshot’ of what is present within the gut of any organism at the time of sampling. Feeding strategies in many of the animals investigated have meant that ingestion of microplastics is followed relatively quickly by egestion/excretion (Cole *et al.,* 2016; Van Cauwenberghe *et al.,* 2015a), with little evidence of accumulation (Sfriso *et al.,* 2020). This means the time spent by microplastics within the organisms can be relatively short, hence calculating total exposure over any time cannot be gained from this experimental approach. Until a method is created analysing environmental plastic exposures in organisms over long timescales, scientists are limited by their knowledge.

It has come to light that results of dose-response experiments must always be interpreted in light of environmental concentrations, yet the experimental concentrations of some experiments are orders of magnitude higher than those reported from field sites (Burns & Boxall, 2018; Lenz *et al.,* 2016). One study investigating the impact of exposure to polystyrene microplastics on oyster reproduction used microplastic concentrations based on sediment data from a pollution hot-spot area close to a ship-breaking yard in India (Sussarellu *et al.,* 2016). Although the study used concentrations closest to those found in nature, these results are unlikely to be representative of general concentrations beyond the local area. When other studies assessing the effects of plastic on organisms use exposure concentrations higher than environmentally relevant levels (von Moos *et al.,* 2012; Besseling *et al.*, 2013, 2014), it makes us question whether microplastic represent a real environmental risk (Lenz *et al.,* 2016). Notably, just because scientists have not yet measured effects does not mean there is a lack of effect, simply that we do not know (Leslie & Depledge, 2020). With the knowledge we have gained so far from laboratory experiments stressing the threat of microplastic pollution, there is little evidence to suggest that the microplastic concentrations we are finding currently in the environment are chronically harming organisms. However, with plastic flow into the ocean expected to triple by 2040 (The Pew Charitable Trusts, 2020), it is possible that the environmental concentrations may begin to replicate that of the laboratory exposure experiments. In other scientific disciplines, future projected scenarios are used as a basis to predict the impact of change in future. Representative Concentration Pathways (RCPs) for example are scenarios used by climate scientists that describe alternative trajectories of carbon dioxide emissions and the atmospheric concentration from 2000 to 2100, encompassing a range of possible climate policy outcomes for the 21st century. Similarly, ocean acidification scientists use RCP scenarios to project the likely response of ocean acidification to a range of emission scenarios. As microplastics are increasingly contaminating our environment, it would seem reasonable to also adopt a projected scenario system once the more comprehensive modelling has been addressed, including that of clearly detailed pathways and adsorbed chemicals. Scientists could then use this to predict how a range of microplastic concentrations impact organisms.

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