Microplastics in the Marine Environment: From Top to Bottom

Submitted by **Rachel Louise Coppock** to the University of Exeter
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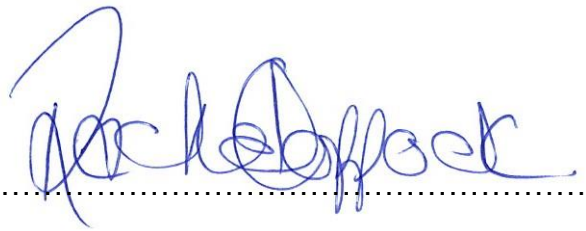
In collaboration with
Plymouth Marine Laboratory

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: 

“It is a curious situation that the sea, from which life first arose, should now be threatened by the activities of one form of that life. But the sea, though changed in a sinister way, will continue to exist; the threat is rather to life itself”
Rachel Carson
The Sea Around Us, revised edition 1961

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“The sea, once it casts its spell, holds one in its net of wonder forever”

Jacques Cousteau

Abstract

The first reports of small plastic debris floating at the ocean surface were recorded in the 1970s, but it is only in the last decade that scientific and media attention has soared. Microplastics (plastic 1 µm – 5 mm) have since been acknowledged as a global marine contaminant, raising concerns about the interactions between anthropogenic debris and natural biological processes. In this thesis, I explore the hypothesis that microplastics can be transported via biotic-driven mechanisms through the water column and into coastal sediments, leading to adverse impacts on the health and functioning of marine fauna and ecosystems. In Chapter 2, I demonstrate that a key pelagic species, the copepod *Calanus helgolandicus,* alter their prey selection dependent upon the size or shape of the plastic in their ambient surroundings, with the capacity to reduce feeding. I also establish that *C. helgolandicus* faecal pellets sink slower when contaminated with low density polyethylene (PE), whereas sinking rates increase when contaminated with high density polyethylene terephthalate (PET), highlighting potential impacts to marine nutrient flux. In Chapter 3, I develop a method utilising the differential density of sediment and plastic to isolate and recover microplastics from sediments; I apply this method in Chapter 4, and latterly discuss harmonisation of microplastic estimates between studies and its use across the wider international field (Chapter 5). In Chapter 4, I employ a multi-faceted study to explore the role that benthic fauna play in the uptake of microplastics by the seabed. My environmental data demonstrate that microplastics are being permanently buried in coastal sediments, and that this process is ubiquitous across sampled sites and seasons. I further identify that benthic faunal functional groups that move sediment vertically (“conveyors”) and randomly (“biodiffusers”) influence sediment plastic loading differently, affecting ultimate burial and deep sediment loading. Furthermore, experimental data indicate that a key benthic species, the brittlestar *Amphiura filiformis*, buries nylon fibres along its burrow structure and that burial activity deep in the burrow is impaired when plastic is consumed. Collectively, my research contributes to our understanding of the mechanisms governing microplastic transport through the water column and into the sediment matrix, highlights risks posed to marine fauna and ecosystems and provides evidence that coastal sediments are final sinks for microplastics.

Acknowledgements

I can't quite believe that I've got to the point of writing the acknowledgements for my PhD! What a journey it's been. I am lucky to be able to say that, on the whole, I have absolutely loved doing my PhD and feel completed humbled and privileged to have had this amazing opportunity. But I can't take all the credit, I would not have got through it without the wonderful support network I have. Trying to balance doing a PhD with being a wife and mother has been pretty challenging at times, but I am incredibly lucky to have had such amazing support from friends, family and supervisors, reminding me that whilst I may feel like a complete imposter in this academic world and somehow become so good at blagging it for so long that I've completely fooled all the very smart and intelligent people around me, that those feelings will pass and that I have actually done some pretty cool stuff worth shouting about.

Firstly, I must say a heartfelt and massive thank you to my fabulous supervisors, Pennie, Matt, Ana and Tamara – you have always given me advice when I've asked for it, encouragement when it's been needed and confidence in my own thoughts and ideas to help propel me forwards. You have always encouraged me to grab every opportunity, ensuring I made the most of my PhD. I will be forever in your debt for all of your friendship, support and guidance.

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And to my family, wow, you never doubted me for a moment. Grant, you are my rock and my soul mate. I would never have had the confidence to do a degree or less still, a PhD, without your unequivocal belief in me, your love, support and

encouragement. You have always encouraged me to take that risk and step into the unknown so I can grow. Words cannot express how much your love and support has kept me going and I thank you from the bottom of my heart. To my awesome daughters, Mollie and Grace, thank you for your love, understanding and patience. Working all those late nights, Saturdays, conferences, training courses, Norwegian research, Arctic cruises…… you never complained or made me feel like I had to choose, you already knew how hard it was for me not to be with you. I am so incredibly proud of the smart, funny, caring and wonderful human beings you have become. To my sister Stephie, thank you for being on the end of the phone when I've needed and for proof reading my 'really boring sciencey writing'! And finally, a massive thank you to my wonderful parents, who moved from the other side of the country to help with childcare so I could pursue my marine biology dream, you really are the best. Mum, thank you for always being bowled over by all my achievements over these past years, your unwavering admiration and love has helped buoy me along the way. And to my Dad, you would have made a fabulous scientist, all your note taking and research, maybe it's always been in my genes. I wish you could have seen me finish this PhD, I think you would be pretty proud. I love you.

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The crew of Plymouth Marine Laboratory's RV Quest are thanked for help during sampling, which was supported within activities of the Western Channel Observatory, as part of the UK Natural Environment Research Council's national capability. Prof. Richard Thompson and Andrew Tonkin (Plymouth University) are thanked for the use of FT-IR and help with analysis, Steven Cooper (University of Exeter) for his help producing the SMI units, Grant Coppock for helping with prototypes, Dr. Andy Watts (University of Exeter) for preparation of manufactured polyethylene microplastics and Sarah Nelms for her help with map preparation and general support. We also thank three anonymous reviewers for their comments to improve the manuscript. Project funded by the Natural Environment Research Council GW4+ PhD studentship (NE/L002434/1) awarded to RLC. AMQ and PL acknowledge funding from the UK Dept. of Environment, Food and Rural Affairs and the Natural Environment Research Council through the Marine Ecosystems Research Programme (NE/L003279/1). PL and TSG acknowledge funding from the Natural Environment Research Council discovery grant (NE/L007010).

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Contents

Author's declaration

My thesis is presented as three research papers (two published and one being prepared for submission). All work has been the product of my planning and implementation, and I am the lead author on all the papers presented here; the contributions from my supervisors and co-authors are described at the beginning of each chapter. Papers have been reformatted to provide a unified editorial and referencing style throughout, with figures embedded within the text. References are compiled into a single bibliography at the end of the thesis.

List of definitions and abbreviations

AFDW	Ash free dry weight
BIP_c	Benthic community bioirrigation potential
BP_c	Benthic community bioturbation potential
DC	Strict downward conveying sediment-dwelling fauna
DMS	Dimethyl sulphide
FT-IR	Fourier Transform Infrared spectroscopy
FSW	Filtered sea water
GFF	Glass fibre filter
Macroplastic	Plastic pieces > 5 mm
Microplastic	Plastic pieces 1 µm - 5 mm
MP	Microplastic
MPSS	Munich Plastic Sediment Separator
MSFD	Marine Strategy Framework Directive
NaCl	Sodium chloride
NaI	Sodium iodide
PE	Polyethylene
PET	Polyethylene terephthalate
PLA	Polylactic acid (“bio-plastic”)
PML	Plymouth Marine Laboratory
POM	Particulate organic matter
PS	Polystyrene
PVC	Polyvinyl chloride
S	Salinity
SMI	Sediment-microplastic Isolation unit
SST	Sea surface temperature
UC	Strict upward conveying sediment-dwelling fauna
UC/DC	Sediment-dwelling fauna that move particulates both upwards and downwards
WW	Wet weight
$ZnCl_2$	Zinc chloride

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Chapter 1:

General Introduction

Microplastics – what are they and what is the problem?

Microplastics are a global, pervasive pollutant. Microplastic debris has been reported globally from every marine habitat including shorelines (Browne et al., 2011), surface waters (Eriksen et al., 2014), Arctic Sea ice (Obbard et al., 2014; Peeken et al., 2018), marine protected areas (Barnes et al., 2018; Cooper et al., 2012) and deep sea sediments (Bergmann et al., 2017; Woodall et al., 2014). Despite reports of tiny plastic pieces floating at the ocean surface dating back to the 1970s (Carpenter and Smith, 1972; Colton et al., 1974; Gregory, 1977), it is only in the last decade that scientific and media attention has soared, shedding light on the scale of this anthropogenic pollutant which has been highlighted as a major contaminant of global concern (eg; 2008/56/EC Marine Strategy Framework Directive, Descriptor 10, United Nations Sustainable Development Goal 14 target 14.1.1). Since the 1950s, when the new 'wonder material' began mass production, global plastic manufacture has increased exponentially from 1.5 million tonnes per annum (PlasticsEurope, 2015) to almost 350 million tonnes per annum in 2017 (PlasticsEurope, 2018). In 2010 alone, an estimated 4–12 million tonnes of plastic waste entered into the oceans, and this figure is predicted to rise by an order of magnitude by 2025 (Jambeck et al., 2015). Microplastic abundances in surface waters are highly variable; an average of 0.27 microplastics m^{-3} were observed in the western English channel (Cole et al., 2014) compared to a mean concentration of 2000 microplastics m^{-3} in the northeast Pacific (Desforges et al., 2014). These differences are due in part to the heterogeneity of different water bodies and proximity to land based pollution sources (Clark et al., 2016; Erni-Cassola et al., 2019), but also to differing sampling methodologies, with the net size dictating the smallest particle size in waterborne techniques, and also potentially due to differences in nomenclature. The term "microplastic" was coined in 2004, where it was hypothesised that plastic particles <5 mm could explain the missing fraction of plastic from measured waterborne concentrations, based on modelled predictions (Thompson et al., 2004). Since then, estimates reporting a range of differing size and shape classifications have contributed to a lack of standardised

terminology and consensus to describe micro and macroplastic debris measured in the natural environment. Hartmann et al., (2019) proposed a standardised set of criteria to unify parameters such as chemical composition, size categories, shape and structure. Microplastic size is proposed at 1 to <1000 µm in any dimension, mesoplastic 1 to < 10 mm and macroplastics >1 cm. However, the upper limit of 5 mm for microplastics (though in which dimension is still ambiguous) appears to be most commonly adopted by the scientific community and wider public alike. Similarly for shape characterisation, conflicting nomenclature between studies hinders meta-analyses that seek to draw comparisons and build a picture of the global, marine plastic loading. Going forward, adopting a standardised framework, such as the one proposed by Hartmann et al., (2019) would aid cross study comparability and mitigation steps.

Table 1.1 Examples of plastics found in the marine environment with associated common uses and densities (adapted from Andrady, 2011)

Polymer type	**Common uses**	**Density (g cm^{-3})**
Polyethylene	Plastic bags, storage containers	0.91—0.95
Polypropylene	Rope, botte lids, car interiors	0.90—0.92
Polystyrene (expanded)	Hot drink cups, floats, insulation	0.01—1.05
Polystyrene	Utensils, containers	1.04—1.09
Polyvinyl chloride	Film, pipe, containers	1.16—1.40
Polyamide/nylon	Fishing gear, textiles	1.13—1.15
Polyethylene terephthalate	Bottles, strapping	1.34—1.39
Cellulose Acetate	Cigarette filters, sanitary products	1.22—1.24

Synthetic polymers are derived from fossil fuels and constitute a wide variety of plastics and uses (Table 1.1). Polyethylene (PE) and polypropylene (PP) are the most commonly manufactured polymers globally and constitute almost half of all plastics produced in Europe (PlasticsEurope, 2018). Polyethylene terephthalate (PET) is the main constituent of single use drinks bottles whilst polyvinyl chloride (PVC) is commonly used by the construction industry. Due its versatility and durability, plastic is commonplace in our everyday lives with a wide and varied range of uses such as food packaging, medical equipment and technological applications. Ironically, it is this durability and the inability to bio-

degrade that is raising environmental concerns. Indeed, aside from incinerated plastics, it is estimated that every piece of plastic that has ever been produced still exists in one form or another today; either whole or fragmented (Thompson, 2005). Microplastics can be purposefully manufactured (primary microplastics), as with virgin pre-production pellets, known as “nurdles” or “mermaid’s tears”, that wash up on beaches around the globe, and microbeads in consumer products such as toothpaste and facial scrubs (Napper et al., 2015). Recent UK legislation banning the inclusion of microbeads in ‘rinse-off’ cosmetic products (The Environmental Protection (Microbeads) (England) Regulations 2017, No. 1312) was a progressive step toward reducing microplastic pollution in the ocean, however microbeads are rarely reported from the marine environment (Burns and Boxall, 2018). More commonly, marine microplastic debris results from the fragmentation of larger plastics over time (secondary microplastics), breaking into ever smaller pieces through mechanisms such as weathering (Andrady, 2017), photo-degradation (Barnes et al., 2009) and abrasion (Boucher and Friot, 2017; Horton et al., 2017a). The most frequently reported type of microplastics in the marine environment however, are small fibres (Burns and Boxall, 2018), which stem from the shedding of synthetic materials such as clothing (Napper and Thompson, 2016) and fishing equipment (Welden and Cowie, 2017). Potential routes of microplastics into the marine environment include waste water outlets (Browne et al., 2011), airborne dispersal (Dris et al., 2016), runoffs from snow and ice (Bergmann et al., 2019; Obbard et al., 2014) and via streams and rivers (Hurley et al., 2018). Indeed, global models estimating plastic inputs into the world’s oceans due to mismanaged waste, estimate between 1.2—2.4 million tonnes of plastic flowing into the oceans from global riverine systems, with the top 20 most polluting rivers located mostly in Asia and peak inputs linked to rainy seasons (Lebreton et al., 2017).

It is not generally possible to pinpoint the source of microplastics, yet the majority of macroplastic on beaches is from land based sources (Nelms et al., 2017), with most of this originating from single use items due to the mismanagement of waste (Jambeck et al., 2015). Plastics can also act as a source of chemical contamination, potentially containing plasticizers and additives incorporated during manufacture. They may be vectors for chemicals sorbed onto the surface from the surrounding seawater, concentrating harmful

pollutants (Teuten et al., 2009) and potentially resulting in detrimental effects to marine life (Cole et al., 2011). Due to their small size, microplastics can be ingested by a vast array of taxa, ranging from marine megafauna (Duncan et al., 2019; Nelms et al., 2019) to invertebrates such as pelagic zooplankton and benthic polychaete worms near the base of the food chain (Cole et al., 2013; Wright et al., 2013). Evidence also exists for microplastics to be transferred through the marine food web; for example, from mussels to shore crabs (Watts et al., 2014), from mesozooplankton to macrozooplankton (Cole et al., 2016; Setälä et al., 2014), and from wild caught fish fed to captive grey seals (Nelms et al., 2018). Microplastics have also been found in seafood destined for human consumption (Rochman et al., 2015), highlighting the potential for transfer to humans.

Through experimental studies, it is clear that at the individual level, microplastic ingestion can adversely affect feeding, reproductive output, energy reserves and development in lower trophic organisms such as copepods (Cole et al., 2019, 2015) and lugworms (Green et al., 2016; Wright et al., 2013), and reduced predatory performance has also been reported in fish (de Sá et al., 2015). Little is known about the effects microplastic pollution has at the ecosystem level, however this was alluded to in an experimental study using oysters, where altered benthic invertebrate assemblages were detected after exposure to polyethylene and polylactic acid (PLA, a “degradable” plastic; Green et al., 2017) microplastics. As a consequence of microplastic pollution, there is a high potential for altered behaviour of keystone species to significantly impact ecological functioning (Galloway et al., 2017). However, the extent of any negative effects resulting from experimental exposures appears highly dependent upon the microplastic type and concentration used, which is extremely variable between studies. Indeed, there has been a mismatch between the type (ie; shape, size, polymer) and abundance of plastic used in laboratory experiments and those reported in the natural environment (Botterell et al., 2019; Lenz et al., 2016), with researchers now aiming to strike a balance between observed measurements from the environment, and the ability to uncover potential effects of microplastic pollution on biota and ecosystems.

Microplastic transport in marine coastal systems

Once plastic enters the ocean, it persists and accumulates in water bodies (Cole et al., 2011), transported laterally via wind and currents in surface waters (Cózar et al., 2014; Eriksen et al., 2014) and vertically through the ocean interior and to the seabed below (Figure 1.1). The majority of plastics are positively buoyant in seawater (Hidalgo-Ruz et al., 2012), yet a substantial proportion of low density polymer plastics, such as polyethylene and polypropylene (0.9 g cm^{-3}) have been found in ocean sediments (Bergmann et al., 2017). A number of biologically mediated routes by which microplastics may

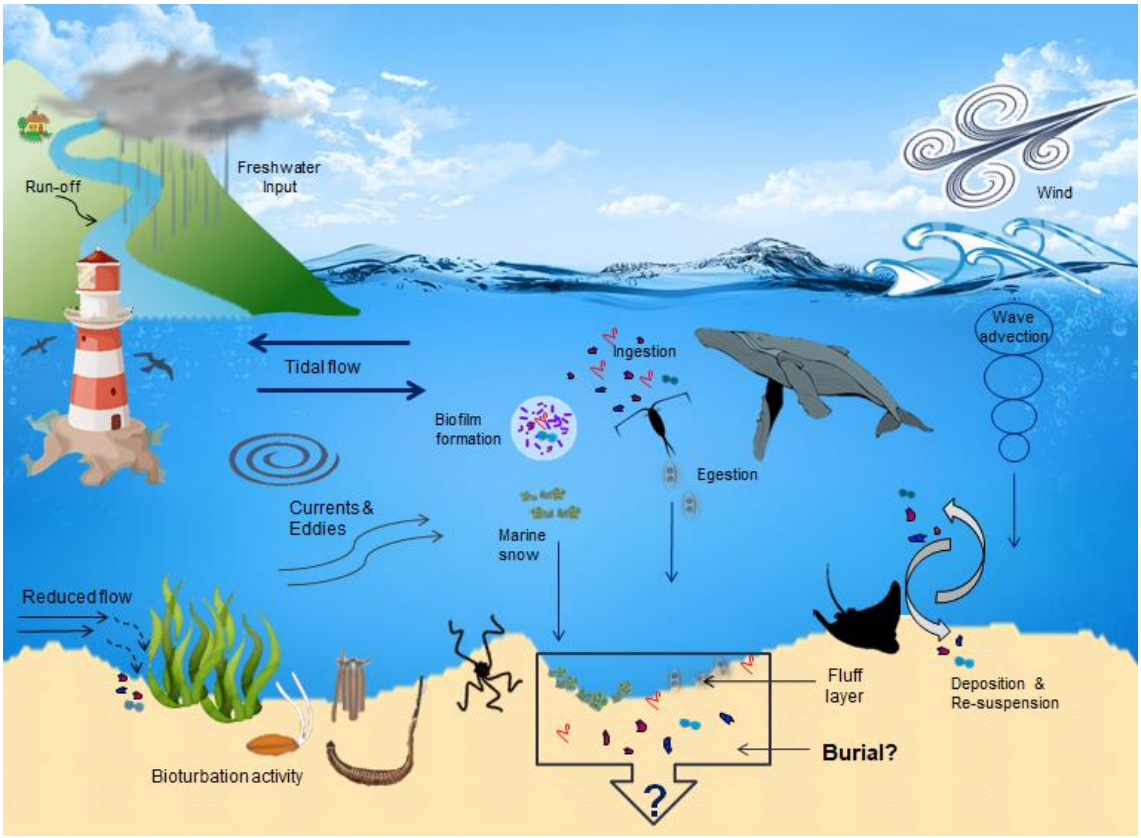

Figure 1.1. Graphical representation indicating some of the factors dictating abiotic and biotic mediated microplastic movement through the water column and into the seabed.

be vertically transported through the water column have been identified and reflect wider benthic-pelagic coupling routes. Biofouling of plastics by micro and macro organisms occurs within hours of entering the marine environment (Donlan, 2002) and can have profound effects on particle buoyancy (Kooi et al., 2017; Lobelle and Cunliffe, 2011; Ye and Andrady, 1991), ballasting the plastics and facilitating sinking. Biofouled microplastics are more palatable to marine

life, as demonstrated in copepods (Vroom et al., 2017), promoting their ingestion by animals in the water column. Ingested microplastics can subsequently be repackaged and egested in faecal pellets, potentially altering the rate at which these vectors sink through the water column (Cole et al., 2016). Marine snows; organic-rich aggregations of phytoplankton, faecal pellets and other particulate matter, have also been shown to be vectors for the transport of microplastics (Long et al., 2015), enhancing bioavailability to benthic macrofauna (Porter et al., 2018). These organic transport routes play an instrumental role in the biological carbon pump (Figure 1.2), exporting carbon and nutrients from surface waters to the deep ocean and sea floor (De La Rocha and Passow, 2007). Zooplankton are an important link between primary producing phytoplankton and higher trophic levels in marine food webs (Kiorboe, 1997; Turner, 2004), grazing on the phytoplankton and together, forming organic matter that sinks through the ocean interior (ie; phytoplankton detritus, zooplankton moults, carcasses and faeces). This organic material is then either decomposed by bacteria, consumed by other organisms or deposited onto the seabed (Turner, 2002). Copepods constitute a high proportion of oceanic zooplankton biomass, with calanoid copepods making up to 90% of total mesozooplankton biomass during bloom conditions in productive, temperate regions such as the North Sea (Bonnet et al., 2005). Copepod faecal matter constitutes a substantial contribution to marine carbon flux (Turner, 2004). Changes to this flux as a result of microplastic contamination, may result in a shift in carbon export from surface waters, potentially impacting on the ability of the ocean floor to accumulate organic carbon fixed in surface waters.

The fate of microplastics in marine sediments

Distribution and abundances of microplastics in benthic sediments are much less reported than in surface waters (Underwood et al., 2017), however there is clear potential for microplastic to accumulate in marine sediments (Erni-Cassola et al., 2019; Ling et al., 2017). Indeed, elevated abundances of microplastic in deep sea sediment compared to waterborne plastics (per unit volume), prompted the hypothesis that deep sea sediments are a sink for microplastics

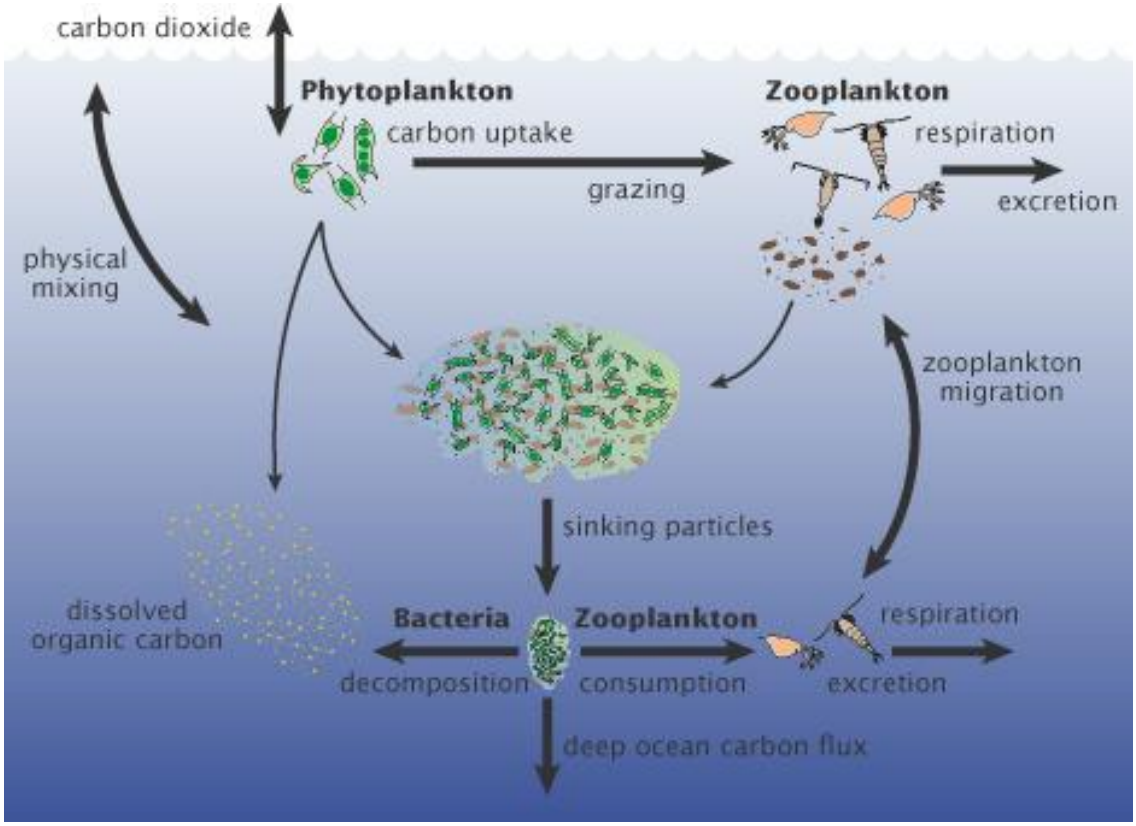

Figure 1.2. Simplified illustration of the biological carbon pump. Phytoplankton photosynthesise at the ocean surface, fixing atmospheric carbon which then sinks or is grazed by zooplankton. Illustration adapted by NASA Earth Observatory from US Joint Global Ocean Flux Study (JGOFS) 2001

(Woodall et al., 2014), providing an explanation for the mismatch of predicted and actual plastic occurrences at the sea surface (Eriksen et al., 2014; Thompson et al., 2004). As with waterborne estimates, known microplastic abundances in sub-tidal sediments are highly variable, and again, methods to quantify plastics vary widely. Many existing methods to extract microplastics from sediments are time consuming, complex or costly (Claessens et al., 2013; Hidalgo-Ruz et al., 2012; Imhof et al., 2012; Nuelle et al., 2014), but if we are to gain a clearer understanding of the risks posed to marine life and ecosystems, there is an urgent need for accurate estimates of microplastic loadings in sediments. We currently do not know the residency time of microplastics within the sediment matrix. Whether a particle becomes deposited on the seabed or re-suspended back into the water column is largely dictated by the local physics at a particular site, such as bottom currents and seabed topography (Figure

1.1), however biogenic interaction may also substantially influence particle uptake and deposition in sediments.

Bioturbation (sedimentary particle mixing and pore water flux exchanges (i.e. bioirrigation) mediated by burrowing fauna (Kristensen et al., 2012)) mediates fundamental benthic-pelagic exchange pathways, including the organic matter exchanges with the water column, and nutrient remineralisation (Queirós et al., 2019, 2015; Zhang et al., 2015). Bioturbating animals significantly alter the structure and pore-water content of soft sediment through foraging and feeding, dispersal, and mating behaviours, burrow flushing and aerobic respiration. These activities enhance sedimentary habitat complexity and mixed layer depth, promoting oxidising conditions within ocean sediments that are essential drivers of global ocean biogeochemical ecosystem function (Boyle et al., 2014; Kristensen and Kostka, 2004; Teal et al., 2008). This impact on benthic-pelagic exchange pathways makes it extremely likely that bioturbators affect sedimentary plastic burial in natural environments. In the Baltic Sea, while biology plays a role in microplastic deposition (Näkki et al., 2019, 2017), physical hydrodynamics are likely to be the dominant factor, as macrofauna in the Baltic tend to be small, shallow-burrowing deposit feeders. In contrast, UK coastal sediments are highly diverse, species-rich environments, lending the potential for high occurrences of animal led microplastic uptake and burial in sediments.

Microplastic in the marine environment; from top to bottom

Despite the vast growing body of evidence pertaining to microplastics in the marine environment, many questions remain. We are still far from understanding the mechanisms governing microplastic transport to, and into, the seabed, the fate once there and the implications of such widespread pollution on individuals, populations and ecosystems. Coastal shelf seas are highly productive, contributing 15 – 21% of the total oceanic primary production (Jahnke, 2010). Due to their close proximity to land based pollution sources, interactions between marine life and microplastic are likely to be high, rendering these interactions paramount in understanding microplastic fate in coastal systems (Clark et al., 2016). In this thesis, **“Microplastics in the marine**

environment; from top to bottom", I explore some of the mechanisms governing transport of microplastic through the water column, entrance into the seabed and the ultimate fate of microplastics in marine coastal ecosystems. I also investigate the impacts of environmentally relevant microplastics to key pelagic and benthic fauna.

In **Chapter 2, "Microplastics alter feeding selectivity and faecal density in the copepod, *Calanus helgolandicus*"**, I present a two-component study investigating interactions between different microplastics and the copepod, *Calanus helgolandicus,* a key component of temperate, pelagic marine systems. I firstly investigate whether prey selection by *C. helgolandicus* will be altered depending upon the relationship between prey shape and/or size and that of microplastics available in their surrounding medium, and secondly I explore whether the resulting copepod faecal pellets, contaminated with plastics of varying density, alter their sinking rates through the water column. In **Chapter 3, "A small-scale, portable method for extracting microplastics from marine sediments"**, I present a novel method for extracting microplastics from sediments and demonstrate its effectiveness on a range of sediment types. This method is then used in **Chapter 4, "Benthic fauna contribute to permanent microplastic burial in coastal sediments"**, where, for the first time in a sub-tidal environmental setting, I investigate microplastic burial in a coastal system and explore the role that benthic fauna play in microplastic sequestration in marine sediments. Here I use a key benthic species, the brittlestar *Amphiura filiformis*, in a targeted study to gain a mechanistic understanding of microplastic burial, and investigate the effects of the microplastic on brittlestar behaviour and oxygen consumption. In **Chapter 5**, I discuss my key findings and contributions to the field of microplastic research in the marine environment.

In addition to the five chapters outlined above, I have also contributed to a research paper, **"Effects of Nylon Microplastic on Feeding, Lipid Accumulation, and Moulting in a Coldwater Copepod"**, for which I am second author, and a book chapter, **"Microplastics in marine food webs" in Microplastic Contamination in Aquatic Environments: An Emerging Matter of Environmental Urgency**, for which I am co-author. These are presented in their published formats in the Appendices.

Chapter 2:

Microplastics alter feeding selectivity and faecal density in the copepod, *Calanus helgolandicus*

This chapter is a reformatted version of my publication:
Coppock, R. L., Galloway, T. S., Cole, M., Fileman, E. S., Queirós, A. M., and Lindeque, P. K. (2019). Microplastics alter feeding selectivity and faecal density in the copepod, *Calanus helgolandicus*. *Science of the Total Environment*, *687*. https://doi.org/10.1016/j.scitotenv.2019.06.009.

RLC, MC and PKL designed the experiments. RLC carried out the experiments, data collection, conducted statistical analysis and wrote the manuscript. All authors contributed to editing and improving the final manuscript.

Microplastics (1 µm – 5 mm) are a ubiquitous marine contaminant of global concern, ingested by a wide range of marine taxa. Copepods are a key component of marine food webs, providing a source of food for higher trophic levels, and playing an important role in marine nutrient cycling. Microplastic ingestion has been documented in copepods, but knowledge gaps remain over how this affects feeding preference and faecal density. Here, we use exposure studies incorporating algal prey and microplastics of varying sizes and shapes at a concentration of 100 microplastics mL^{-1} to show: (1) prey selection by the copepod *Calanus helgolandicus* was affected by the size and shape of microplastics and algae they were exposed to; Exposure to nylon fibres resulted in a 6% decrease in ingestion of similar shaped chain-forming algae, whilst exposure to nylon fragments led to an 8% decrease in ingestion of a unicellular algae that were similar in shape and size. (2) Ingestion of microplastics with different densities altered the sinking rates of faecal pellets. Faeces containing low-density polyethylene sank significantly more slowly than controls, whilst sinking rates increased when faeces contained high-density polyethylene terephthalate. These results suggest that *C. helgolandicus* avoid ingesting algae that are similar in size and/or shape to the microplastic particles they are exposed to, potentially in a bid to avoid consuming the plastic.

Introduction

Microplastics (plastic pieces, 1 µm - 5 mm) are pervasive marine pollutants, which have been highlighted as a contaminant of global environmental concern (UN Sustainable Development Goal 14 target 14.1.1, GESAMP 2016). Microplastic particles and fibres have been documented ubiquitously throughout the marine realm, including surface waters (Cózar et al., 2014), polar regions (Bergmann et al., 2017; Cincinelli et al., 2017) and deep sea sediments (Woodall et al., 2014). These synthetic particles can be purposefully manufactured, such as cosmetic exfoliates or virgin pre-production pellets, or result from the fragmentation of larger items such as fibres from textiles (Napper and Thompson, 2016), wear of tyres (Boucher and Friot, 2017) and the breakdown of single-use plastics that have degraded over time (Andrady, 2011). Microplastic ingestion has been documented in a wide range of marine organisms including corals, (Hall et al., 2015), fish (Lusher et al., 2013) , marine mammals (Nelms et al., 2019), turtles (Duncan et al., 2019), seabirds (Lourenço et al., 2017;) and commercially important shellfish (Murray and Cowie, 2011; Rochman et al., 2015). Exposure to microplastics can result in adverse health effects, including reduced feeding and fecundity in copepods (Cole et al., 2015), reproductive disruption in oysters (Sussarellu et al., 2016), intestinal damage (Lei et al., 2017) and behavioural changes in fish (de Sá et al., 2015).

Zooplankton are an important link between primary producing phytoplankton and higher trophic levels in marine food webs (Kiorboe, 1997; Turner, 2004). Copepods constitute a high proportion of the total zooplankton carbon biomass and *Calanus* species, which are amongst the largest copepods, may account for more than 90% of mesozooplankton biomass in regions such as the North and Celtic seas (Bonnet et al., 2005). Experimental studies have demonstrated that zooplankton have the capacity to ingest microplastics (Cole et al., 2013) and field studies have showed that zooplankton, including copepods, euphausiids, jellyfish and fish larvae, consume microplastics in the wild (Desforges et al., 2015; Steer et al., 2017; Sun et al., 2017). Food selectivity has been widely evidenced in copepods, with the capacity to discriminate between algal prey and microplastics (Donaghay and Small, 1979; Huntley et al., 1983). The drivers of this selectivity might include the chemosensory

properties of the particles, for example when covered in biofilms (Vroom et al., 2017), the size, which alters capture efficiency, and shape, that may affect handling and capacity for ingestion. This may result in negative effects including, reduced food intake and energy available for growth and reproductive success (Cole et al., 2015).

Copepod faecal material substantially contributes to the flux of carbon and nutrients to deeper waters and to the seabed. Through ingestion of phytoplankton and subsequent repackaging into dense faecal pellets, calanoid copepods play an instrumental role in the biological carbon pump. Their faecal pellets transfer atmospheric carbon dioxide in the form of photosynthetically produced organic matter, or fixed carbon, to the deep ocean, thereby providing food for benthic dwelling organisms and facilitating microbial degradation and remineralisation by microzooplankton (Turner, 2002). A change to the sinking rate of this faecal material has potential ecological consequences affecting a wide range of factors including carbon and nitrogen export out of the euphotic zone, shifting the balance of particulate organic matter (POM) remineralisation and reducing food to the benthos. In a prior study, the sinking rates of copepod faecal pellets contaminated with polystyrene (PS) microspheres were significantly reduced (Cole et al., 2016). If translated to natural systems in highly polluted waters, slower faecal sinking rates may alter POM export, cause faecal pellets to remain in the upper reaches of the ocean for longer and hence increase the likelihood of consumption by microzooplankton (coprophagy), fragmentation (coprohexy) or degradation by protozoan and microbial communities.

Many previous studies have used PS spheres as representative microplastics, and it has been highlighted that a wider range of plastics, with greater ecological relevance, should be included in exposure studies to better understand the risks microplastics pose to marine life (Botterell et al., 2019; Lenz et al., 2016) . Numerous environmental studies report fibres as the predominant particle type (Cole et al., 2011; Lusher et al., 2016) and 50% of microplastics isolated from copepods in the North Pacific (Desforges et al., 2015) were fibrous. It is currently unclear whether the bioavailability or sinking rates of copepod faecal matter will change with different types of plastic that vary in size, shape and polymeric composition. In this study, we test the

hypotheses: (1) that prey selection by the copepod *C. helgolandicus* will be altered depending upon the relationship between prey shape and/or size and that of microplastics available in their surrounding medium; and, (2) that the resulting contamination of copepod faecal pellets with plastics will alter their sinking rates, with buoyancy primarily affected by the density of the polymer. We test these using a mixed-prey exposure containing chain-forming and unicellular algae with copepods over a 24 hour period to gain a mechanistic insight into copepod feeding strategies and resultant changes to faecal buoyancy.

We predict that the temperate copepod *Calanus helgolandicus* will ingest all types of plastic within their prey size range but that shape and size will influence selection of their algal prey. We also predict buoyant plastic (e.g. polyethylene (PE)) will dramatically reduce sinking rates of contaminated faecal matter, while denser plastics (e.g. polyvinyl chloride (PVC), polyethylene terephthalate (PET)) will substantially increase sinking rates.

Methods

Experimental treatments comprised field collected *Calanus helgolandicus* copepods and algal solutions containing assemblages of cultured microalgae, spiked with different types of microplastic at a density of approximately 100 plastics mL^{-1}. Whilst our exposure concentrations are higher than those reported in the natural environment, the majority of data has been collected using a much larger net size of 333 µm (see Shim et al, (2018) and references therein). Microplastic abundance increases with decreasing size (Lenz et al., 2016), therefore we would expect much higher concentrations at the microplastic sizes used here. Experiments set out to; 1) investigate the effect of microplastic on algal selection and 2) measure the sinking rate of microplastic contaminated faecal pellets.

Sample Collection

Zooplankton were sampled in January and May 2017 from the Plymouth Marine Laboratory's RV Plymouth Quest from the Western Channel Observatory (station L4; 50°15′N, 4°13′W; https://www.westernchannelobservatory.org.uk/),

a site approximately 12 km south-west of Plymouth Sound, UK, which combines coastal influence from the Tamar Estuary and continental shelf conditions (Smyth et al., 2015). Zooplankton were collected via horizontal surface tows using 735 µm mesh plankton nets. Samples were transported in 2 L of seawater, enclosed within a cool box to a temperature controlled laboratory (matched to ambient sea surface temperature at the collection site; SST Jan 10 °C, May 11 °C) at Plymouth Marine Laboratory (Plymouth, UK) within 3 hours of collection. On arrival, adult female *Calanus helgolandicus* copepods were carefully, manually selected using a low power microscope (Wild M5-49361; x20-x50 magnification) and stork billed forceps. They were immediately transferred to a 10 L glass beaker, aerated and maintained in 0.2 µm filtered seawater (FSW; Salinity 34.5-35‰; 24h darkness; SST) collected from L4, for 72 hours during preconditioning to experimental diet treatments (see *Algal cultures* below).

Algal cultures

Three algal prey species, the unicellular chlorophyte *Dunaliella tertiolecta* (11 µm), the chain-forming diatom *Thalassiosira rotula* (24 µm) and the dinoflagellate *Prorocentrum micans* (35 µm; Figure 2.1.), are representative components of *C. helgolandicus* natural prey (Widdicombe et al., 2010) and were selected for their size and shape to assess prey selection by the copepods. All prey species were cultured at Plymouth Marine Laboratory after purchase from Swansea University (*P. micans*) and Culture Collection of Algae and Protozoa (*D. tertiolecta* CCAP 19/6B*, T. rotula* CCAP 1085/20) using Guillard's F/2 media for *D. tertiolecta* and *P. micans*, with additional meta-silicates (1 mL L^{-1} of seawater) for *T. rotula* (15°C; 16:8 light regime; S 34.5-35‰).

Microplastic preparation

Dried powder suspension

Fluorescent PE microspheres (0.09g; Cospheric) were added to 15 mL falcon tubes and 10 mL of 0.01% Tween 20 surfactant solution (Thermo Fisher Scientific) was added to aid particle solubilisation. Solutions were thoroughly mixed through vigorous shaking, vortexing and sonicating for 15 minutes in an ultrasonic bath (Guyson KC3).

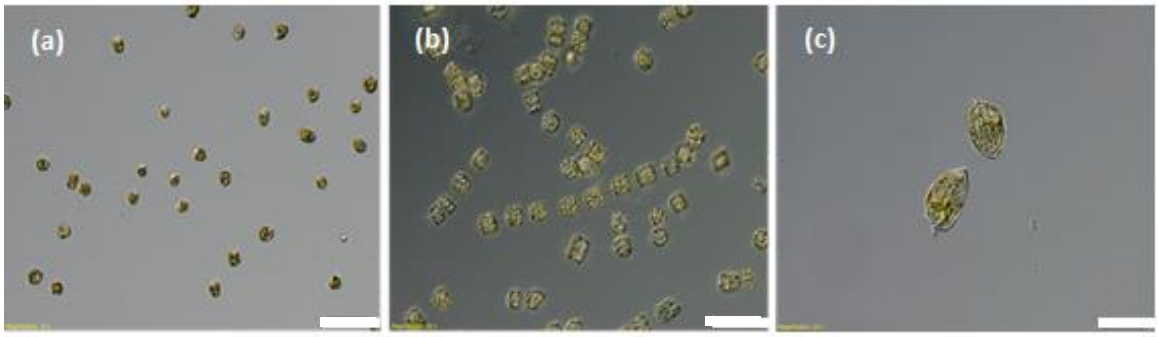

Figure 2.2. Cultured single cell algae used in experiments; (a) unicellular chlorophyte, *Dunaliella tertiolecta* (11 µm), (b) chain forming diatom *Thalassiosira rotula* (24 µm) and (c) dinoflagellate, *Prorocentrum micans* (35 µm). Magnification x20, white scale bars measure 50 µm.

Nylon and PET fibres

Nylon 6,6 microfibres were produced using an established 'cryotome' protocol (Cole, 2016). To summarise, nylon 6,6 and PET polyfilaments (Goodfellow) were aligned and embedded in a glycol freezing solution (Neg 50™, Richard-Allan Scientific) and frozen (10 min, -80°C, New Brunswick U570 ultra low temperature freezer); frozen fibres were sectioned into pre-determined lengths (Table 2.1.) using a cryogenic microtome (Leica CM1950). Sections were thawed and 'rod' shaped microfibres retrieved via filtration and washed with ultrapure water. For imaging purposes, Nile Red was used to fluorescently dye the nylon microfibres using a solvent-extraction protocol (Cole, 2016). Recovered fibres were suspended in MilliQ water and quantified using Sedgwick Rafter counting cells and stereo microscope (x20 magnification; Wild, M5-49361), where their shape and size were also quantified.

Nylon fragments

Nylon fragments (20 µm) were prepared by size fractionating nylon 6 powder (Goodfellow; AM306010) using 20 µm and 25 µm nylon meshes. Size and shape were visually inspected and quantified using a graticule and stereo microscope (x20 magnification; Wild, M5-49361). The fragments were then fluorescently dyed using Nile Red as per section above.

Microplastic uptake

Uptake assays were conducted to guide selection of the most appropriate size of each of three common microplastic types that differ in density (Table 2.1.) for

use in both the copepod feeding selectivity and sinking rate experiments; low density PE, medium density nylon and high density PET. A single adult female *C. helgolandicus* was transferred to a 50 mL lidded glass bottle (n=4), containing 100 microplastics mL^{-1} and filled with FSW (S 34.5‰; SST; total volume: 74 mL). Controls contained either FSW alone or FSW with equivalent volume of 0.01% Tween 20 surfactant solution as used to disperse PE microspheres, and a single *C. helgolandicus.* Lids were securely fastened and bottles installed onto a rotating plankton wheel. After 24 h, the experiment ended and individuals were filtered through a 50 µm mesh, taking care to retain the copepod and any faecal pellets, and preserved in 4% formalin for 48 h before washing thoroughly and storing in 95% ethanol. Microplastic presence and abundance was qualitatively assessed in preserved copepods and faecal pellets under UV light, using an Olympus IMT-2 inverted microscope to guide appropriate size selection for the ingestion studies.

Ingestion study

To determine the impact of different shaped plastics on algal ingestion rates, we conducted a 24 h feeding study. In brief: 500 mL Duran bottles were filled with 615 mL of FSW, (S 35‰), containing 120 µg C L^{-1} of a mixed, autotrophic algal assemblage (*Prorocentrum micans;* 5 cells mL^{-1} ≈ 25 µg C L^{-1}*, Dunaliella tertiolecta;* 166 cells mL^{-1} ≈ 35 µg C L^{-1} and *Thalassiosira rotula;* 38 cells mL^{-1} ≈ 60 µg C L^{-1}), representing natural carbon availability during spring bloom conditions (Harris et al., 2000; Widdicombe et al., 2010). Abundances were calculated using a Sedgewick Rafter counting chamber and carbon biomass was estimated using a conversion factor of 5 nL biovolume ≈ 1µg C (Jones et al., 2002). Guillard's F/2 nutrient media was added to algal stocks to ensure algae were nutrient replete prior to study, negating the effects of additional nutrient input from copepod excretions. Treatments were prepared as follows: 1) control without predation; 2) control with predation; 3) nylon fibres (10 x 40 µm; 100 fibres mL^{-1}) and 4) nylon fragments (20 µm; 100 fragments mL^{-1}). Environmental concentrations of microplastics in this size range are not well reported, however there is considerable evidence that concentrations increase with decreasing size (Lenz et al., 2016). Our decision to use 100 microplastics mL^{-1} balanced the desire to achieve near environmental concentrations with the ability to determine any potential effects arising from the microplastic

exposures. We therefore used an algae to microplastic ratio of 2:1 to allow a mechanistic insight into prey selection. Five adult female *C. helgolandicus* were added to each bottle (n = 5), with the exception of the 'control without predation' treatment, used to ascertain the natural growth of algae over the experimental period. Bottles were rotated on a plankton wheel for 24 h (<5 r.p.m.; 24h darkness; SST). After 24 h, 200 mL from each bottle was fixed (Lugols 1% final concentration) for algal cell and microplastic quantification using an Olympus IMT2 inverted microscope (x150 magnification: *T. rotula*, *P. micans*, fibres; x300 magnification: *D. tertiolecta*, fragments) and Utermöhl counting technique (Utermöhl, 1958). Samples were homogenised through inversion before settling 100mL subsample for treatments 2, 3 and 4 or 50mL for treatment 1 and leaving to settle for > 24 h (50 mL) or > 48 h (100mL). Clearance (mL copepod^{-1} day^{-1}) and ingestion (µg C copepod^{-1} day^{-1}) rates for algal prey and microplastics were calculated using formulae of Frost, 1972.

Egestion; Faecal pellet sinking study

To collect faecal pellets for this study, five adult female *C. helgolandicus* were incubated in 500 mL bottles (n = 4) containing FSW, (S 35‰) plus 105 µg C L^{-1} of the mixed, autotrophic algal assemblage (*P. micans;* 9 cells mL^{-1} ≈ 30 µg C L^{-1}*, Dunaliella tertiolecta;* 108 cells mL^{-1} ≈ 20 µg C L^{-1} and *Thalassiosira rotula;* 43 cells mL^{-1} ≈ 55 µg C L^{-1}). In addition to the algal mix, treatments were prepared as follows: 1) control with nothing else added; 2) control plus 0.01% Tween 20 at volume corresponding to PE prep; 3) high density PET fibres (17x60 µm; 100 fibres mL^{-1}); 4) low density PE spheres (10-20 µm; 100 spheres mL^{-1}) and 5) medium density nylon fibres (10x40 µm; 100 fibres mL^{-1}). As per *section 2.5*, experimental bottles were rotated on a plankton wheel for 24 h (<5 r.p.m.; 24h darkness; SST). After 24 h, faecal pellets were collected using a 50 µm mesh sieve and washed into a Petri dish using FSW then stored in the refrigerator at 4°C for the sinking study, which was completed within 3 days of pellet collection.

Adapting the method of Cole et al., (2016), a clean 2 L measuring cylinder was filled with filtered seawater (34.5 ‰ S), covered to prevent dust particles entering and placed on a stable workbench at a constant temperature (15°C). The cylinder was marked at intervals of 40 mm, the first mark occurred 80 mm below the surface to allow for deceleration of the pellets. Using a stereo

microscope (Wild M5-49361, x50 magnification) and eyepiece graticule, faecal pellet length, width and number of encapsulated plastics were recorded. Faecal pellets were then carefully drawn up using a liquid-pipette and gently released once the liquid-pipette tip was submerged just below the water surface; the time taken for the faecal pellet to travel at a constant speed between the two markers was recorded. For analysis, the volume of microplastic in each pellet was determined using the average size of each plastic type used, calculating the volume of the shape (eg; cylinder for nylon fibres and sphere for nylon fragments and PE spheres) and multiplied by the number observed.

Statistical analyses

All data were analysed using R statistical software V 1.0.136 (R. Core Team, 2016).

Ingestion rates

All data were tested (Shapiro-Wilk) and visually inspected for distribution and homogeneity of variances and were found to violate *apriori* requisites for linear, parametric tests. Kruskal-Wallis non-parametric tests were therefore performed to assess how each response variable (clearance rate of each algal species) was influenced by the explanatory variable (treatment: control, nylon fibres or fragments) and Dunn's post-hoc pairwise test applied.

Egestion; Faecal pellet sinking rates

Generalised linear modelling (GLM) was conducted to investigate how the explanatory variables (volume of microplastic contained in faecal pellets, faecal pellet volume and polymer type) influenced the response variable (sinking rate). First, a linear regression was conducted to assess the relationship between microplastic volume and faecal pellet volume; collinearity was found to occur therefore microplastic volume was removed from the model, as this variable only applies to plastic treatments and not controls. To achieve model parsimony, a full model was built which included main effects (faecal pellet volume and polymer type) as fixed terms, treatment replicate (n=4) as a random term and main effect interactions. The significance of the random term was tested with GLS and lme functions (nlme package) using REML estimation. The model without a random term returned the lowest AIC value and models which included the random term generated non-significant model coefficients,

therefore was excluded from further models. All fixed terms in the model were then tested for significance using GLM. Terms were dropped sequentially and models tested for significance, determined by ANOVA "*F*" test and AIC comparison. Models including interaction terms suggested these resulted in a greater model AIC value and generated non-significant model coefficients, which were also excluded from the final model. Gaussian distribution with 'Identity' link function was used and the model was validated by visually inspecting error distributions and homogeneity of variances relative to linear model assumptions (See Table SI 2.1).

Results

Ingestion

Microplastic uptake

Adult female *Calanus helgolandicus* readily ingested microplastic fibres, beads and fragments (Table 2.1; Figure 2.2). The copepods showed a preference for particles in the size range of 10-20 µm for PE, whilst PET was ingested in greater quantities in the 17x60 µm size range. Nylon was readily ingested in both granule and fibre form, the most commonly ingested fibre size being 10x40 µm.

Ingestion of algal prey

There was an overall impact to clearance rates of algal prey when exposed to microplastics (H = 45.81, *df* = 2, *p* = 0.05; Figure 2.4). When exposed to nylon fibres, there was an overall reduction in the amount of food ingested by *C. helgolandicus* (*H* = 5.81, *df* = 2, *p* = 0.05) and a shift in algal preference compared to the control treatment. We observed a reduction in the clearance rates of both *Prorocentrum micans* (*H* = 3.17, *df* = 2, *p* = 0.04) and a highly significant reduction in clearance rates of *Thalassiosira rotula* (*H* = 8.97, *df* = 2, *p* = 0.001), which are similar in size and shape (respectively) to the 10x40 um fibres (Figure 2.5). There was no difference in the clearance of *Dunaliella tertiolecta* (*H* = 5.49, *df* = 2, *p* = 0.14) compared to controls. When exposed to nylon fragments, total clearance rates were significantly reduced compared to control treatments (*H* = 5.81, *df* = 2, *p* = 0.01). When assessing clearance rates

of individual algal prey, we observed no difference in the clearance rates of *P. micans* (H = 3.17, df = 2, p = 0.11) or *T. rotula* (H = 8.97, df = 2, p = 0.16) when compared with control treatments, however there was a significant reduction in the clearance rate of *D. tertiolecta* (H = 5.49, df = 2, p = 0.01) which is similar in size and shape to the fragments (Figure 2.5.). When considering the proportions of each algal prey type ingested, the mean proportion of *P. micans* ingested did not vary with treatment (Figure 2.6), however exposure to fibres resulted in a 5.7% decrease in ingestion of the similar shaped *T. rotula* and a 5.9% increase in ingestion of *D. tertiolecta*. Conversely, exposure to fragments led to a 7.4% increase in consumption of *T. rotula* but a 7.8% decrease in the similar shaped *D. tertiolecta*.

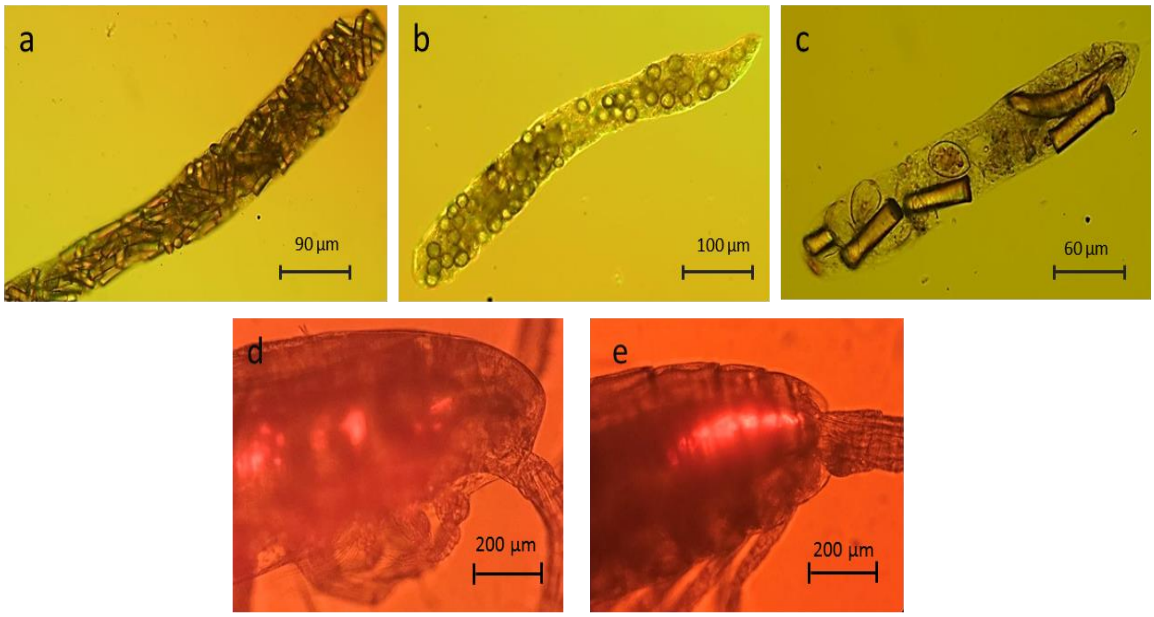

Figure 2.3. Images of contaminated *C. helgolandicus* faecal pellets (a – c) after exposure to solutions containing mixed algal assemblage and a) nylon fibres, b) PE spheres and c) PET fibres and *C. helgolandicus* with fluorescently labelled nylon fibres (d) in digestive tract and (e) being formed into a faecal pellet in the hind gut. All exposures at concentrations of 100 microplastics mL^{-1} with an algae to plastic ratio of 2:1.

Table 2.1. Polymer, shape, density, size and mass concentration of microplastics used to assess uptake in the copepod, *C. helgolandicus* to guide particle selection for ingestion and sinking rate experiments.

Polymer	**Shape**	**Density (g cm^{-3})**	**Size (µm)**	**Mass concentration at 100 MP mL^{-1} (g mL^{-1})**
Polyethylene	Sphere	0.91-0.96	10-20	$2.8\ x10^{-8}$ – $2.2\ x10^{-7}$
Polyethylene	Sphere	0.91-0.96	20-27	$2.2\ x10^{-7}$ – $5.5\ x10^{-7}$
Polyethylene	Sphere	0.91-0.96	27-32	$5.5\ x10^{-7}$ – $9.2\ x10^{-7}$
Nylon 6,6	Fragment	1.15	20	$4.8\ x10^{-7}$
Nylon 6,6	Fibre	1.15	10 x 40	$3.6\ x10^{-7}$
Nylon 6,6	Fibre	1.15	23 x 100	$4.8\ x10^{-6}$
Polyethylene terephthalate	Fibre	1.38	17 x 60	$4.0\ x10^{-6}$
Polyethylene terephthalate	Fibre	1.38	23 x 70	$1.9\ x10^{-6}$

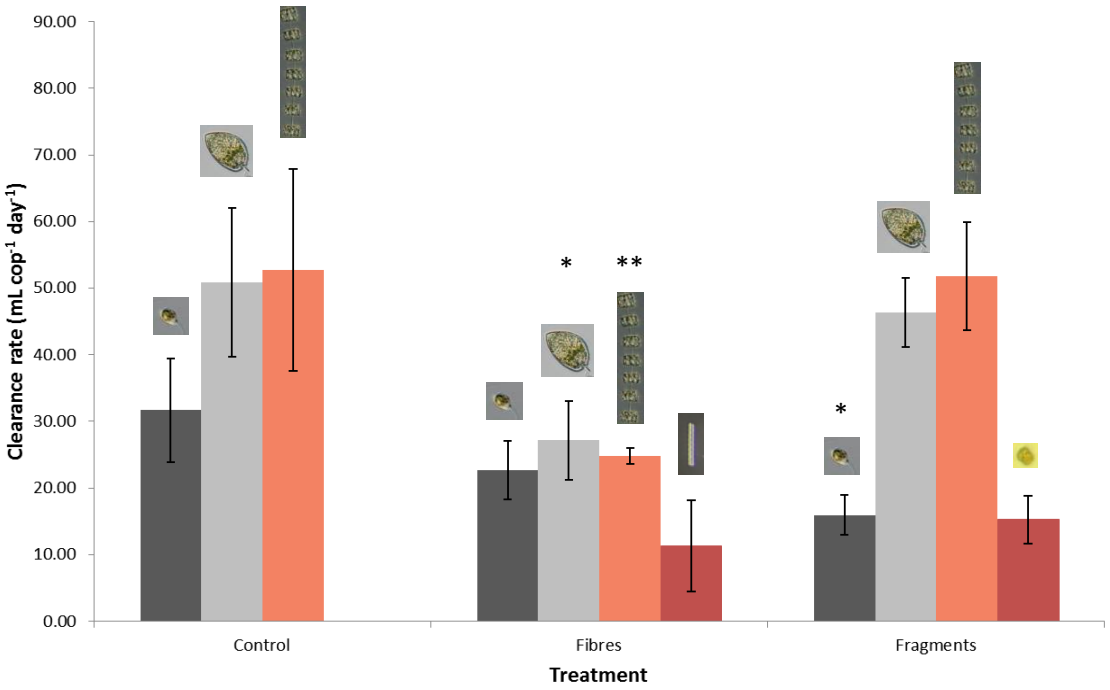

Figure 2.4. Mean (±SE) clearance rate (volume of water swept clear of particles) of each algal species (dark grey bars, *D. tertiolecta*; light grey bars, *P. micans*; orange bars, *T. rotula*) and plastic (red bars) cleared per copepod, per day for each treatment. * denotes statistical significance at <0.05, ** at <0.001, Kruskal Wallis (n = 5).

Egestion

Microplastic presence in *C. helgolandicus* faecal pellets altered their sinking rate, but this was dependent on the type of plastic ingested. Treatment (GLM $F_{4,92}$ = 34.74, p = <0.001; Table SI 2.1) and faecal pellet volume (GLM $F_{1,91}$ = 29.30, p = <0.001) were both significant predictors of faecal pellet sinking rates. Faecal pellets contaminated with low density PE sank significantly slower than the controls ($F_{4,92}$ = 34.74, p = <0.001; Figure 2.7), in contrast to the high density PET contaminated pellets which sank significantly faster than controls ($F_{4,92}$ = 34.74, p = <0.01). Neither nylon ($F_{4,92}$ = 34.74, p = 0.25) or the Tween 20 control ($F_{4,92}$ = 34.74, p = 0.48) had any significant influence on sinking rates.

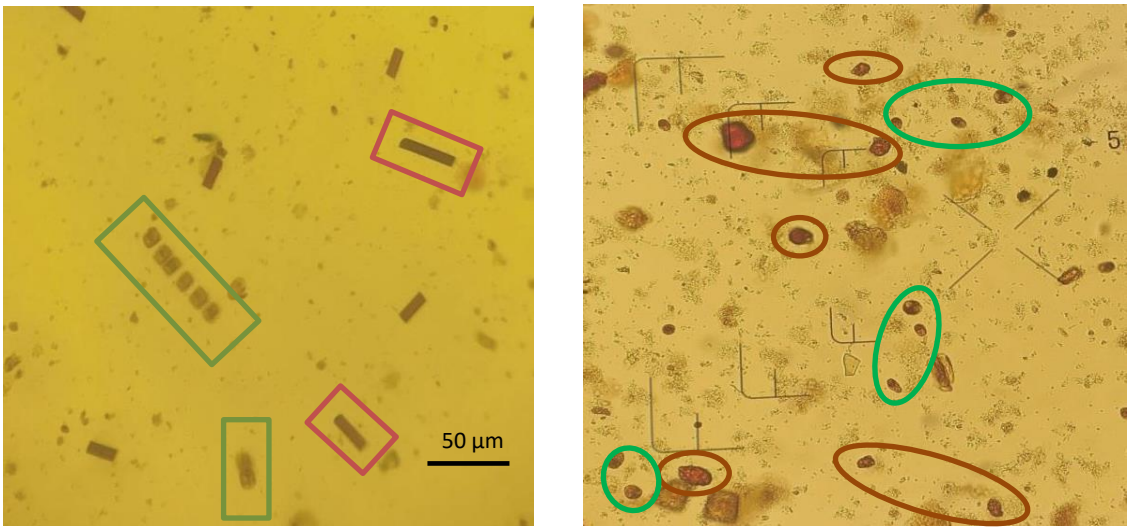

Figure 2.5. Images showing similarity between a) nylon fibres (red rectangles) and chain-forming algal prey, *T. rotula* (green rectangles) and b) nylon fragments (circled red) and algal prey species, *D. tertiolecta* (circled green).

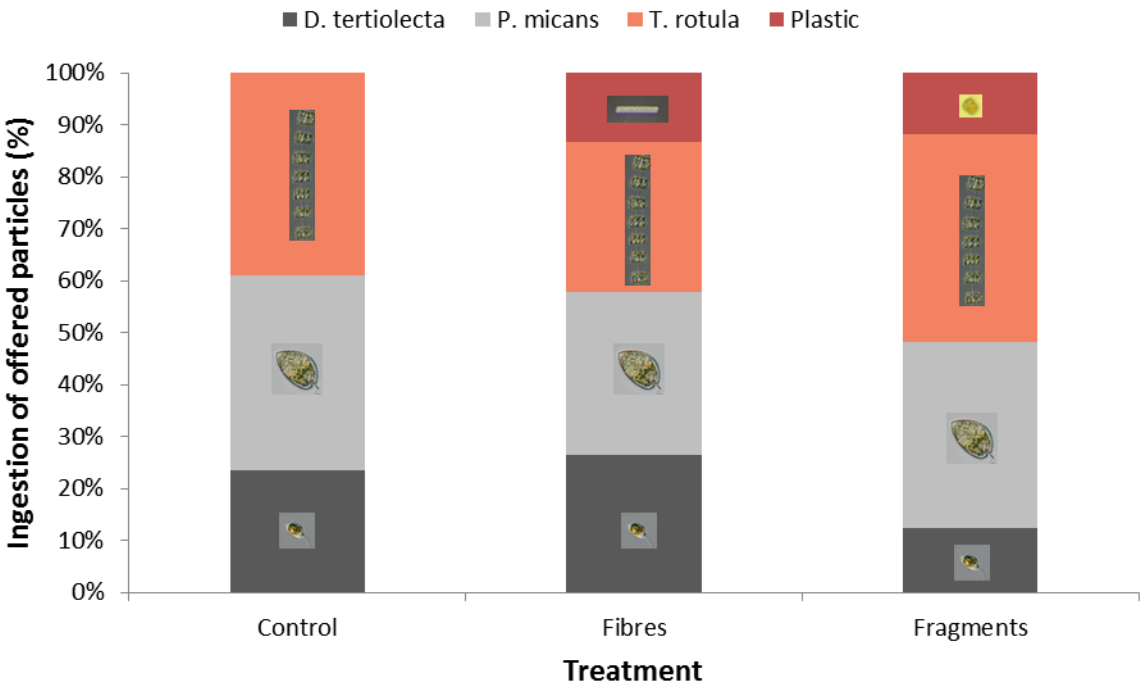

Figure 2.6. Proportion of each offered particle type ingested for each treatment (n = 5). Dark grey blocks = *D. tertiolecta*, light grey = *P. micans*, orange = *T. rotula* and red= plastic.

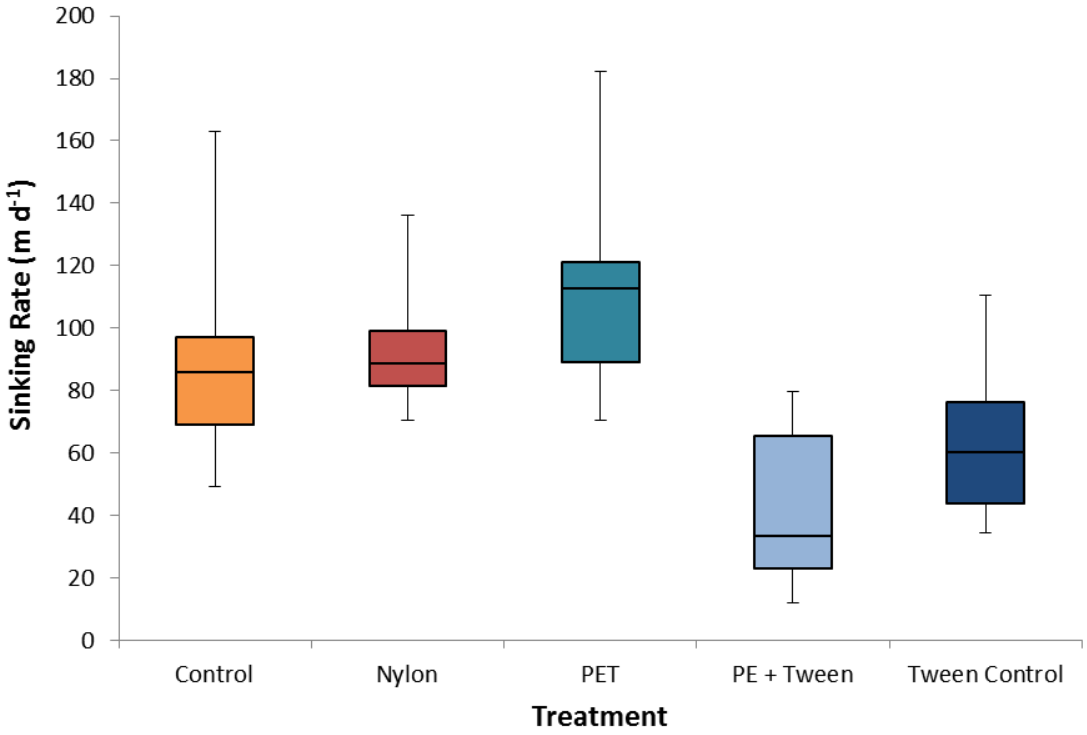

Figure 2.7. Box and whisker plots showing the median, interquartile and full range of sinking rates (m d^{-1}) of control and microplastic contaminated faecal pellets. * denotes statistical significance at <0.01, ** at <0.001, GLM (n = 4).

Faecal pellet volume was positively influenced by microplastic volume when contaminated with all plastic treatments (Figure 2.7); PE ($F_{1,16}$ = 9.32, p = 0.006, r^2 = 0.29), PET ($F_{1,18}$ = 9.32, p = 0.007, r^2 = 0.34) and nylon ($F_{1,16}$ = 6.72, p = 0.02, r^2 = 0.30) and is therefore a factor in faecal pellet sinking rates. There was no correlation between the volume of microplastics and sinking rates for PE ($F_{1,23}$ = 3.14, p = 0.09, adj R^2 = 0.08, Figure 2.7.) or PET contaminated pellets ($F_{1,18}$ = 2.34, p = 0.143, adj R^2 = 0.07) but there was a correlation when contaminated with nylon ($F_{1,23}$ = 26.6, p = <0.001, adj R^2 = 0.32). There was no difference in the size of faecal pellets between control and nylon ($F_{4,5.58}$ = 19.95, p = 0.66), PE ($F_{1,5.58}$ = 19.952, p = 0.98) or PET ($F_{4,5.58}$ = 19.95, p = 0.19) treatments but Tween 20 control faecal pellets were smaller than all other treatments ($F_{4,5.58}$ = 19.95, p = <0.001).

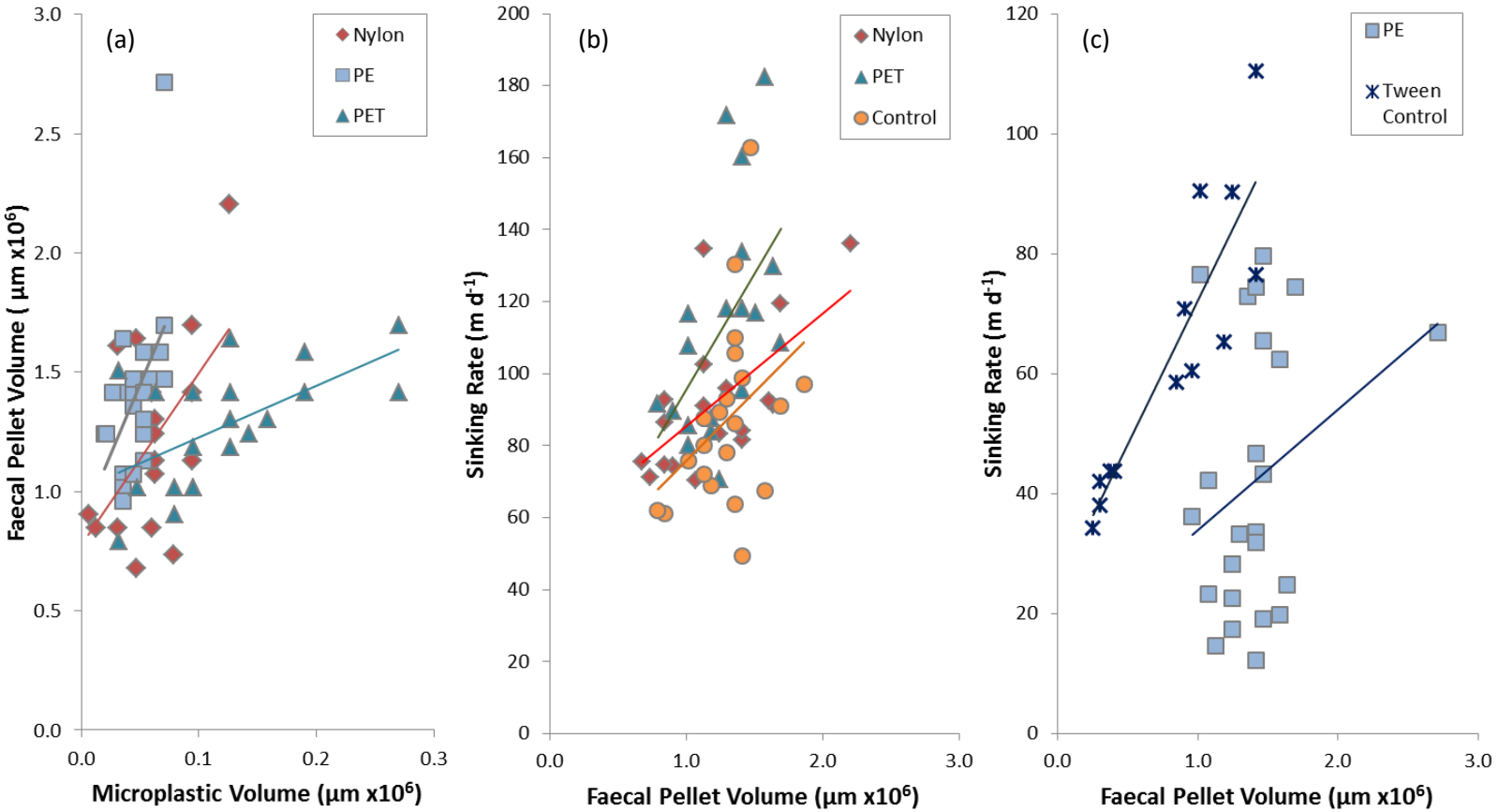

Figure 2.8. Relationship between (a) volume of microplastic per faecal pellet, (µm x10^6; nylon, red diamonds; PE, blue squares; PET, green triangles) and faecal pellet volume (µm x10^6) and sinking rates (m d^{-1}) and faecal pellet volume (µm x10^6) for (b) nylon, red diamonds; PET, green triangles; control, yellow circles; and (c) PE, blue squares; Tween 20 control, blue stars. Slopes represent linear relationship (see Results section for r^2 values), lm (n = 4).

Discussion

Ingestion

Our results reveal that exposure to microplastics at concentrations of ~100 plastics mL^{-1} not only caused an overall reduction in *Calanus helgolandicus* feeding, but also influenced prey selection. Nylon fibres impeded ingestion of algae of a similar size or shape and caused a shift in the preference of consumed prey. The copepods *C. helgolandicus* reduced their intake of the similarly shaped chain forming diatom *Thalassiosira rotula* and the similar sized dinoflagellate *Prorocentrum micans*, but ingestion of the small flagellate *Dunaliella tertiolecta* remained unchanged. Exposure to nylon fragments did not alter the total consumption of algal prey, however there was a significant reduction in the ingestion of *D. tertiolecta*, which is similar in size and shape to the fragments. These results suggest that *C. helgolandicus* avoided ingesting algae that were similar in size and/or shape to the microplastic particles they were exposed to, potentially in a bid to avoid consuming the plastic.

Calanus sp. copepods primarily feed by generating a feeding current using appendages around their mouth (Cannon, 1928). Copepods have demonstrated complex selective capabilities when it comes to particle ingestion. A previous study observed a 40% reduction in the total carbon biomass ingested by *C. helgolandicus* when exposed to microplastic and this was due to a subtle shift in algal cell size preference away from the PS microplastics that were present (Cole et al., 2015). Some studies suggest selectivity is a function of size (Harvey and Sc, 1937; Meyer et al., 2002), others have reported selection based on nutritional value; i.e. phytoplankton cells versus PS beads (Fernández, 1979) or that live food is preferable to detritus determined by chemo and mechanoreceptors in the zooplankton (Paffenhöfer and Sant, 1985). How and why zooplankton select one particle over another has been widely debated, with unselective feeding also reported (Djeghri et al., 2018; Leiknes et al., 2014); often highly variable feeding rates are seen and interpretation of copepod feeding strategies is notoriously difficult. Differences in these rates may be explained by a wide variety of factors, including light conditions, temperature, food quality, size and abundance and pre-exposure to the experimental diet (Huntley, 1988). The copepod *Acartia clausi* has demonstrated complex grazing behaviour which includes the ability to optimise

capturing food particles whilst avoiding non-food particles and to reject food post-capture (Donaghay and Small, 1979). Similarly, when offered mixtures of phytoplankton cells and PS beads, *Calanus pacificus* were able to discriminate between particles of different types, although they were not wholly efficient at rejecting the non-food PS beads (Huntley et al., 1983). It is possible that as the copepods are unable to digest the plastics, they display a learned behavioural response by attempting to avoid food of a similar size or shape which may explain the results seen in our study. It has not been possible to differentiate from our results, or predict, whether it is size or shape that is more important in the particle selection seen here, however size was determined more influential than shape in experimental studies investigating microplastic ingestion and entanglement in mysid shrimp, *Praunus* sp. and three-spined sticklebacks, *Gasterosteus aculeatus* (Lehtiniemi et al., 2018), prompting further investigations to quantify.

Mechanoreception, used in the handling of individual particles, is a recognised mechanism for prey detection in many calanoid copepods. Legier-Visser et al., (1986) suggested that copepods could detect and work out the size and location of a particle based on the pressure disturbance created in the feeding current. This mechanism would give credence to our suggestion here that *C. helgolandicus* may be rejecting food particles that mimic the size and shape of the microplastic. It has been suggested however, that mechanoreception can only be triggered when chemoreceptors are activated (Paffenhöfer and Jiang, 2016), based on historical studies using PS spheres as non-food particles when conducting mechanistic feeding trials. Adult female *Eucalanus pileatus* rejected PS spheres once three or more had been passed to the mouth, only ingesting the plastic once phytoplankton cells were also offered and detected in the feeding current (Paffenhöfer and Sant, 1985). More recently however, microplastic nylon fibres infused with dimethyl sulfide (DMS), an infochemical produced by many phytoplankton species, were ingested by *C. helgolandicus* up to three times more readily than non-infused nylon fibres (Procter et al., 2019), but the copepods did still ingest the non-DMS infused fibres despite no phytoplankton being offered. Behavioural studies are recommended to investigate this matter further.

Recorded microplastic abundance in marine surface waters is highly variable, both spatially and temporally, ranging from zero in some studies to > 100,000 microplastic particles m^{-3} in a Swedish industrial harbour (Noren, 2007) (also see Shim et al., 2018 and references therein). Due to methodological constraints, environmental concentrations of microplastics in the size range of those used in this study are not well known, however there is evidence to suggest microplastic concentrations increase with decreasing size (Lenz et al., 2016). The vast majority of waterborne microplastic concentration data has been obtained using a 333 µm net, therefore current reported environmental concentrations typically refer to microplastic particles larger than those used in this study. Whilst enhanced concentrations were used in our study compared to those reported for larger microplastics in the environment (x 10^3 to 10^6), fragmentation of plastic (Andrady, 2011) will likely increase the number of plastics in the small size fractions; a scenario where the concentrations used in our study may potentially represent future microplastic hotspots or accumulation zones. Due to high biological productivity and the close proximity to land-based pollution sources, coastal areas are predicted convergence hotspots of zooplankton and microplastic accumulation (Clark et al., 2016). In coastal waters off California, USA, the ratio of microplastics to zooplankton was reported as 1:3 (Lattin et al., 2004) and near Plymouth, UK, microplastics outnumbered fish larvae by 27:1 (Steer et al., 2017). By altering their prey selection, copepods may shift the balance of phytoplankton community composition and such shifts have been known to lead to the development of harmful algal blooms (Hallegraeff, 2010). However, given current concentrations and the wide range of shapes and sizes of microplastics sampled from the marine environment, such a shift would seem unlikely. A bigger concern may be for the health of the copepod themselves, where chronic exposure to plastic leads to nutrient deficiency, reduced feeding and impeded reproductive output (Cole et al., 2015). The increased handling times involved in the copepod selecting the food items (Tiselius et al., 2013) may also lead to carbon deficits which in turn would have consequences for the health of the individual.

Egestion

Our results confirm that microplastic contamination of copepod faecal pellets alter their sinking rates, and those rates are primarily affected by the density of

the polymer. These results compliment a previous experiment that demonstrated *C. helgolandicus* faecal pellets contaminated with low density PS, sank more slowly than uncontaminated pellets (Cole et al., 2016). Faecal matter produced by zooplankton play a significant role in the ocean's biological carbon pump, the transport of photosynthetically-produced organic matter, or fixed carbon, away from surface waters to deeper water and sediments, and the remineralisation through grazing by zooplankton and microbial degradation (Turner, 2002). Plastic-contaminated faecal pellets may alter this flux of carbon to the seabed, extending or decreasing transport times depending on the type and potentially, quantity, of plastic ingested. Our results support the idea that zooplankton faecal pellets contaminated with low density plastics such as PE may remain in surface waters for longer than uncontaminated pellets. Slowly sinking faeces are less likely to reach the sea floor (Turner, 2015), which increases the potential for repackaging of microplastics through coprophagy, the ingestion of faecal pellets (Cole et al., 2016; Iversen and Poulsen, 2007), or degradation by the microbial community (De La Rocha and Passow, 2007). Slower sinking rates may also increase the propensity for fragmentation by other zooplankters, breaking the pellets into smaller pieces and thus reducing sinking even further. Whilst not quantified in our study, Cole et al. (2016) observed increased fragmentation of faecal pellets when contaminated with PS beads, potentially increasing retention in the photic zone further and releasing free microplastics back into the water column. Reduced sinking rates may also allow for the degradation of the organic matter contained in the pellet to be taken up by microorganisms in the surface waters, shifting the balance of nutrient recycling from the water column to the surface, and affecting the flow of carbon to the seabed; thus alternatively fuelling faster mineralisation near the warmer water surface, than in the deeper ocean. This biogeochemical cascade may have potentially significant implications for the ocean carbon cycle and the ability of the seafloor to accumulate organic carbon fixed in photic waters, requiring future research. Furthermore, faecal pellets containing low density polymers may remain within the upper surface waters and undergo predominantly lateral advection rather than vertical flux, potentially altering also the geographical location of carbon stores due to extended buoyancy.

In contrast to low density polymers, faeces contaminated with high density polymers such as PET may increase the rate at which the carbon-rich pellets are conveyed away from surface waters. Total carbon flux varies both spatially and temporally, alongside phytoplankton, zooplankton and microbial abundance and species composition (Wilson et al., 2013), potentially also influencing microplastic dispersal. For example, krill faecal pellets were highly abundant in sediment traps deployed along the Western Antarctic Peninsula during January 2009, but were completely absent at the same location the following month (McDonnell and Buesseler, 2010). Similarly, faecal pellets contributed up to 48% of the total particulate carbon flux during a 15 year time-series study in the northeast Pacific deep sea (Wilson et al., 2013). Diel vertical migration, the synchronous daily migration of many zooplankton species and a wide range of other taxa, may also present a potential route for microplastic transport from surface to deeper waters (De La Rocha and Passow, 2013). Whilst our study did not extend to fish faecal pellets, microplastics have been identified in the gastrointestinal tracts of adult (Lusher et al., 2013) and juvenile (Steer et al., 2017) fish and it is plausible to suggest that pellet density of small fish may also be influenced by ingested microplastics and contribute to altering carbon transport. Microplastic contamination of faecal pellets may therefore directly influence the lateral and vertical distribution of microplastics at locations where high densities of zooplankton or shoaling fish co-occur with microplastic hotspots and result in a significant shift in carbon export from surface waters.

No distinct relationship was observed between faecal pellet volume and the sinking rates in either PET or PE treatments. Whilst this was unexpected and contrary to many studies (Turner, 2002), it is in agreement with previous observations made between *Calanus* faecal pellet sinking rates and volumes when offered different diets (Bienfang, 2010). One explanation for our results may be due to potential variation in the size of each of the plastics ingested. The size of the plastics used were variable, however microplastic size in each pellet was not calculated and only mean size was used to calculate plastic volume.

Here, we have highlighted that animals respond very differently to microplastics of differing size, shape and polymer, and would advocate that it is important to move away from using solely PS beads as a representative plastic if we are to

gain a fuller understanding of the threat microplastics pose to marine life. Our results suggest that microplastic fibres will have a more pronounced effect on copepod feeding than fragments, leading to subsequent health implications. Fibres are by far the largest reported fraction of microplastic in the marine environment and therefore pose a significant threat to copepod health and ecosystem functioning. With increasing amounts of plastic entering the oceans each year; an estimated input of up to 24 million tonnes annually by 2025 (Jambeck et al., 2015), whilst it is unlikely that current estimated microplastic levels in the ocean will significantly alter the biological pump balance, it is important to investigate and consider future scenarios based on plastics continuing to enter the oceans at predicted rates. We have demonstrated that pelagic biota can play an instrumental role in altering the properties and redistribution of plastic in the marine environment and it is now prudent to uncover the role benthic biota may impart on plastic burial in marine sediments.

Chapter 2: Supplementary Information

Table SI 2.1. Model outputs and interpretation after model parsimony achieved for General Linear Model (GLM; Response variable; Sinking Rate m d^{-1}, Explanatory variables; Treatment, Faecal Pellet Volume) and linear regression (lm; Microplastic Volume, Faecal Pellet Volume) arising from Faecal Pellet Sinking experiment.

Model	**Treatment factors**	**Output**	**Interpretation**
Fmodel <- glm (sr ~ treatment + fpvol, family = gaussian (link = "identity"), data = Sinking)	Full model output	GLM $F_{4,92}$ = 34.74, *p* = <0.001	Model predictors (faecal pellet volume and treatment) had a significant effect on sinking rate
	Control : nylon	GLM $F_{4,92}$ = 34.74, *p* = 0.249	Nylon did not have a significant effect on sinking rate
	Control : PET	GLM $F_{4,92}$ = 34.74, *p* = <0.001	PET had a significant effect on sinking rate
	Control : FPvol	GLM $F_{1,89}$ = 29.30, *p* = <0.001	Faecal pellet volume had a significant effect on sinking rate
	Control : Tween20 Control	GLM $F_{4,92}$ = 34.74, *p* = 0.476	No difference between controls
	Tween20 Control : PE + Tween	GLM $F_{4,92}$ = 34.74, *p* = <0.001	PE had a significant effect on sinking rate
Lm (fpvol ~ mpvol, data = Sinking)	Nylon treatment	$F_{1,16}$ = 6.72, p = 0.019, r_2 = 0.296	Correlation between Microplastic vol and Faecal Pellet vol
	PET treatment	$F_{1,18}$ = 9.32, p = 0.007, r_2 = 0.341	Correlation between Microplastic vol and Faecal Pellet vol
	PE treatment	$F_{1,23}$ = 9.32, p = 0.006, r_2 = 0.288	Correlation between Microplastic vol and Faecal Pellet vol

Chapter 3:

A small-scale, portable method for extracting microplastics from marine sediments

This chapter is a reformatted version of my publication:
Coppock, R. L., Cole, M., Lindeque, P. K., Queirós, A. M., and Galloway, T. S. (2017) 'A small-scale, portable method for extracting microplastics from marine sediments', *Environmental Pollution*. Elsevier Ltd, 230, pp. 829–837.

RLC developed the method, carried out sample collection, conducted data collection, statistical analysis and wrote the manuscript. All authors contributed to editing and improving the final manuscript.

Microplastics (plastic particles, 0.1 µm–5 mm in size) are widespread marine pollutants, accumulating in benthic sediments and shorelines the world over. To gain a clearer understanding of microplastic availability to marine life, and the risks they pose to the health of benthic communities, ecological processes and food security, it is important to obtain accurate measures of microplastic abundance in marine sediments. To date, methods for extracting microplastics from marine sediments have been disadvantaged by complexity, expense, low extraction efficiencies and incompatibility with very fine sediments. Here we present a new, portable method to separate microplastics from sediments of differing types, using the principle of density floatation. The Sediment-Microplastic Isolation (SMI) unit is a custom-built apparatus which consistently extracted microplastics from sediments in a single step, with a mean efficiency of 95.8% (± SE 1.6%; min 70%, max 100%). Zinc chloride, at a density of 1.5 g cm^{-3}, was deemed an effective and relatively inexpensive floatation medium, allowing fine sediment to settle whilst simultaneously enabling floatation of dense polymers. The method was validated by artificially spiking sediment with low and high density microplastics, and its environmental relevance was further tested by extracting plastics present in natural sediment samples from sites ranging in sediment type; fine silt/clay (mean size 10.25 ±SD 3.02 µm) to coarse sand (mean size 149.3 ±SD 49.9 µm). The method presented here is cheap, reproducible and is easily portable, lending itself for use in the laboratory and in the field, eg. onboard research vessels. By employing this method, accurate estimates of microplastic type, distribution and abundance in natural sediments can be achieved, with the potential to further our understanding of the availability of microplastics to benthic organisms.

Introduction

Microplastics (plastic 0.1 µm–5 mm in size) are ubiquitous throughout the marine environment and are widely regarded as a contaminant of global concern (2008/56/EC Marine Strategy Framework Directive, Descriptor 10, United Nations Sustainable Development Goal 14 target 14.1.1). Over the past 75 years, plastic production has increased dramatically from 1.5 million tonnes to 322 million tonnes per year globally (Plastics Europe, 2015); an estimated 4–12 million tonnes of plastic is predicted to have entered the marine environment from land-based sources in 2010 alone (Jambeck et al., 2015). Microplastic debris is widespread, impinging upon the poles (Obbard et al. 2014), deep sea (Woodall et al. 2014), open ocean (Barnes et al. 2009) and shorelines worldwide (Browne et al., 2011; Nelms et al., 2017). Microplastics are formed in a variety of ways, including: (1) direct manufacture, whereby microscopic or small plastics are purpose made (e.g. cosmetic exfoliates, virgin pre-production pellets); (2) fragmentation of larger pieces of plastic that have degraded after prolonged exposure to the elements (Andrady, 2011); (3) microfibres shed from ropes (Welden and Cowie, 2017) and textiles (Browne et al. 2011; Napper and Thompson, 2016); and 4) tyre and road paint particles transported via run-offs from roads (Boucher and Friot, 2017; Horton et al., 2017a).

Owing to their small size, microplastics are bioavailable to a wide range of organisms. Ingestion has been documented in animals throughout the marine food web, including zooplankton (Desforges et al. 2014), fish (Bellas et al. 2016; Lusher et al. 2013), marine mammals (Lusher et al. 2015; Bravo-Rebolledo et al. 2013), turtles and seabirds (Tourinho et al. 2010). This ingestion of microplastics can negatively affect food intake, reducing the amount of energy available for growth and reproductive success (Cole et al. 2015; Sussarellu et al. 2016; Wright et al. 2013). Plastics can act as a source of chemical contamination, containing plasticizers and additives incorporated into the plastic during manufacture. They may also be vectors for chemicals sorbed onto their surface from the marine environment (Teuten et al., 2009). Plastic debris has been shown to concentrate harmful pollutants up to one million times higher than that of the surrounding seawater (Mato et al., 2001) and consumption of

this polluted plastic may result in detrimental effects to marine life (Koelmans et al., 2016).

Benthic sediments have been identified as a potentially important sink for microplastics (Clark et al. 2016; Woodall et al. 2014; Zalasiewicz et al. 2016). Highly impacted coastal sediments can contain up to 3% microplastics by weight (Carson et al. 2011), and Woodall et al. (2014) conservatively estimates that 4 billion fibres km^{-2} are littering Indian Ocean seamount sediments. Environmental studies (Lusher, 2015 and references therein) have reported the presence of a wide range of microplastic polymer types in sediments, including typically buoyant polymers. Biofouling (Lobelle and Cunliffe, 2011), mineral adsorption (Corcoran et al., 2015) and incorporation of microplastics into faecal pellets (Cole et al. 2016) and marine aggregates (Long et al. 2015) can decrease the buoyancy of plastics, facilitating their movement to the seafloor. Within the sediment, microplastics may therefore become bioavailable to a wide range of benthic fauna, including commercially important species, such as Norway lobster (Murray and Cowie 2011) and shellfish (Rochman et al. 2015) that contribute to biochemical and nutrient cycling processes (Queirós et al. 2015; Zhang et al. 2015). Following exposure to polyvinylchloride (PVC) microplastics, ecologically important intertidal polychaete lugworms, *Arenicola marina,* suffered a 50% reduction in energy reserves (Wright et al., 2013), increased metabolic rates and reduced bioturbation (Green et al., 2016) with impacts on its role in ecosystem process mediation (Volkenborn et al., 2007).

To gain a clearer understanding of microplastic availability to marine life, and thus of risks posed to the health of benthic communities and associated ecological processes, it is important to obtain accurate measures of microplastic abundance in sediments. Indeed, a recent review highlighted the difficulties in developing a global picture of benthic microplastic prevalence due to the lack of reliable microplastic abundance measurements (Underwood et al. 2017). This is largely due to the costs, impracticalities or inefficiencies associated with existing methods. We therefore need to promote harmonised, practical and representative sampling, sample preparation and microplastic detection (Horton et al. 2017; Van Cauwenberghe et al. 2015). The principle of density floatation is commonly employed to separate less dense plastic polymers from denser

sediment particles, and a range of high-density salt solutions have been used to extract microplastics from marine sediments (Hanvey et al., 2016; Horton et al., 2017a; Thompson et al., 2004). However, such methods have been disadvantaged with a number of drawbacks, including complexity (Claessens et al. 2013), expense (Imhof et al. 2012), low extraction efficiencies (Hidalgo-Ruz et al. 2012; Imhof et al. 2012), incompatibility with very fine sediments (Claessens et al. 2013; Fries et al. 2013), particle degradation from flotation media (Lusher et al. 2016), and expense of consumables, e.g. Metatungstate solution in the NOAA approved protocol (Masura et al. 2015). The decanting of floating plastic whilst simultaneously avoiding disruption of the settled sediment poses a challenge, typically yielding low extraction efficiencies and hence requiring repeat extractions (Hidalgo-Ruz et al. 2012; Imhof et al. 2012). Other methods require several steps to retrieve microplastics (Claessens et al. 2013; Fries et al. 2013; Nuelle et al. 2014; Stolte et al. 2015) and may include equipment that suit extraction from coarse sediments such as an elutriation step (Claessens et al. 2013) or use of a separation funnel (Fries et al. 2013), but clog when using very fine sediments (pers. comms. Dr. Andy Watts, University of Exeter and pers. obs.). The Munich Plastic Sediment Separator [MPSS - (Imhof et al. 2012)] isolates microplastics above a shut-off valve and achieves recovery rates of 95.5% (microplastics < 1 mm). However, the MPSS was designed for use with large quantities of sediment (6 kg) and is fabricated from stainless steel standing at approximately 1.75 m tall, thereby expensive to produce and limiting its portability and feasibility when processing numerous replicates of small samples.

Here, we describe the construction and application of a small-scale, portable microplastic extraction unit that mirrors the design of the MPSS, and compare the viability and financial cost of three high-density salt solutions: sodium chloride (NaCl), sodium iodide (NaI) and zinc chloride ($ZnCl_2$), tested for use with the unit. We test the efficiency of the unit by artificially spiking sediment with known quantities of microplastics (polyethylene, polyvinyl chloride and nylon) and validate its use with environmental samples of varying type. We present an optimised method that is applicable for use with a range of sediment types, suits most budgets and which can be used both in the field and the laboratory to isolate microplastics from benthic samples.

Table 3.1. Examples of existing floatation methods commonly used to extract microplastics from sediments

Floatation extraction technique	Floatation media	Repetitions	No. steps	Sediment type	Efficiency	Size	References
Decanting	NaCl; NaI	2 - 5	1	Fine (estuary) Coarse (beach)	35 % (pers. obs.)	< 1 mm	(Hidalgo-ruz et al. 2012; personal observations)
Modified decanting	NaCl; NaI; $ZnCl_2$	1 - 2	1 - 2	Fine (river; mangrove)	99 %; 40 – 72 %	1 - 5 mm < 1 mm	(Imhof et al. 2012; Mohamed et al. 2014)
Elutriation, aeration & centrifuge	H_20; NaCl; NaI;	1 x NaCl elutriation 3 x NaI centrifuge	2	Coarse (sand)	97 - 98 %	< 1 mm	(Claessens et al. 2013; Wessel et al. 2016)
Aeration & ball valve (MPSS)	$ZnCl_2$	1	1	Fine (river)	100 % 96 %	1 - 5 mm < 1 mm	(Imhof et al. 2012)
Froth floatation	Pine oil, froth conditioner, dishwasher tablet	1	1	Fine (river)	55 %	1 - 5 mm	(Imhof et al. 2012)
Air-induced overflow, oxidation & decanting	NaCl; NaI	5	3	Coarse (beach)	68 - 99 %	1 mm	(Nuelle et al. 2014)
Separation funnel	NaCl	2	2	Coarse (beach)	80 – 100 %	1 mm	(Fries et al. 2013)
Pipetting & decanting	$CaCl_2$	1	2	Coarse (beach)	55 %	< 1 mm	(Stolte et al. 2015)
Overflow	NaI; NaCl	3	1	Fine (sound)	48 %	< 1 mm	(Personal observations)

Methods

Flotation media

Solutions of sodium chloride (NaCl), sodium iodide (NaI) and zinc chloride ($ZnCl_2$) were prepared by dissolving the salts in ultrapure water to achieve densities in the range 1.2-1.8 g cm^{-3} (Table 3.3.); solutions were filtered (10 µm Whatman nucleopore membrane) to remove any contaminants prior to use. The financial cost (GBP L^{-1}) of media was calculated by averaging the cost of salts from three scientific suppliers (i.e. Fisher Scientific, Sigma Aldrich and APC Pure; December 2016) and adjusted for the preparation of solutions (amount added to 1 L ultra-pure water) at the appropriate density (Table SI 3.3.; Supplementary Information).

Sediment-Microplastic Isolation (SMI) unit

In evaluating existing microplastic extraction protocols (Table 3.1), we identified the need for a method that allows rapid, simple and efficient extraction of microplastics from a range of sediment types. We set out to design a compact extraction unit that can be easily decanted in a single step and quickly cleaned to avoid cross-contamination. Following optimisation, we constructed the Sediment-Microplastic Isolation (SMI) unit (Figure 3.1). The unit was constructed using 63 mm PVC piping and ball valve, secured to a PVC plate with PVC welding rod for stability (see Table SI 3.2 for material information and costs) with dimensions of 130 (w) x 130 (d) x 380 mm (h), and a weight (excluding floatation media) of 1.5 kg. The unit was designed so that all internal sides were smooth with no protruding surfaces, allowing free movement of the particles, thus avoiding any microplastics becoming trapped within the unit.

Cleaning, purging and priming the SMI

All SMI components were thoroughly rinsed with ultrapure water prior to assembly; particular attention was given to cleaning the ball valve owing to its relative complexity. Following assembly, 700 mL of filtered $ZnCl_2$ solution (1.5 g cm^{-3}) was poured into the SMI unit, ensuring the ball valve was completely submerged. The ball valve was primed by opening and closing several times, making sure the internal cavity was filled so as to avoid agitation upon valve closure during sample processing. The solution was topped back up to

approximately 90 mm above the open valve (approximately 50 mL) and the unit left for 5 minutes to allow any externally-derived contaminants to float to the surface. After 5 minutes, the valve was set in the open position and the $ZnCl_2$ solution filtered through a 25 µm nylon mesh into a clean flask for continued use, rotating the unit to ensure all internal sides were clear of contamination. This step was undertaken prior to each extraction and took no more than 10 minutes.

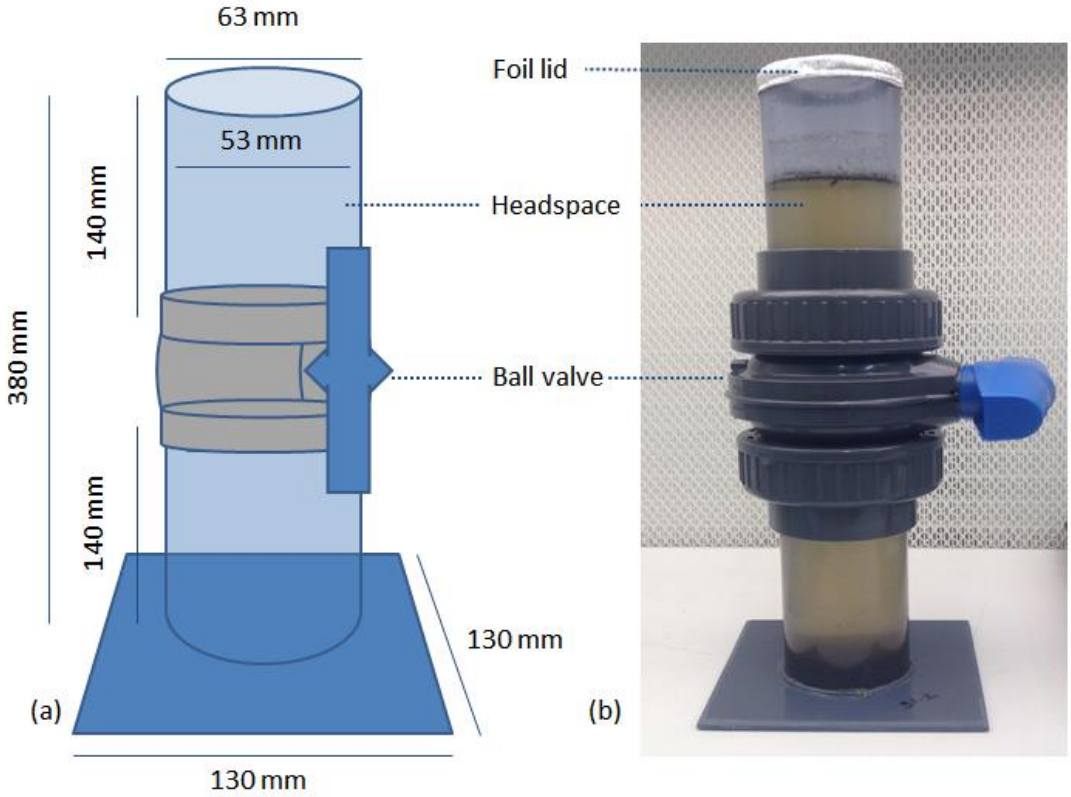

Figure 3.1. Schematic (a) and photograph (b) of Sediment-Microplastic Isolation (SMI) unit. Photograph depicts SMI unit with ball valve in closed position, denser sediment settled at the bottom of $ZnCl_2$ solution (1.5 g cm^{-3}) and less dense particles floating on top.

Microplastic extraction from sediment

During extraction from sediment samples, all cleaned equipment was placed inside a laminar flow hood and covered with clean aluminium foil to minimise contamination. On each occasion, a dry (30-50 g) sample, clean magnetic stir bar and 700 mL of $ZnCl_2$ were added to the purged SMI unit. A magnetic stirring

plate was used to mix the sediment for 5 minutes, and then the sediment allowed to settle for 5 minutes, followed by 3 short stirring pulses to allow the escape of trapped air bubbles. The unit was left to settle until the supernatant was clear of sediment. Next, the valve was carefully closed and the supernatant in the headspace vacuum filtered (Millipore) through a 30 µm nylon mesh (or split over multiple meshes if high quantities of organic material present), retaining the zinc chloride for further use. The headspace was rinsed thoroughly with ultrapure water to recover any remaining particles. Meshes were transferred to a clean Petri dish and sealed with Parafilm, pending examination under a microscope. After each extraction, the SMI was cleaned with ultra-pure water and purged again before processing the next sample. Procedural blanks ($ZnCl_2$ excluding sediment) were carried out prior to first use and after every three samples as a contamination control measure.

Extraction of microplastic from artificially spiked sediments – SMI validation

To evaluate the extraction efficiencies of the SMI unit, we used natural and untreated fine sediment (Table 3.2) spiked with known concentrations of microplastics. Sediment samples were sourced from the entrance to the Plym estuary, Plymouth Sound, UK (N 50°21.717'; W 4°08.055') using a benthic multicorer (four Perspex cores measuring 50 cm long x 10 cm diameter). Samples were dried at 50 °C for approximately 72 hours then stored in a clean polyethylene bag (Sigma Aldrich Z162965). Artificially incorporated microplastics (Table SI 3.) included: (1) weathered polyethylene filaments (200-1000 µm), and (2) weathered nylon filaments (200-1000 µm long) both sourced from Cockleridge beach (Devon, UK; N50°28.136'; W03°87.150') in 2014 and hand-cut to the given sizes using dissecting scissors; (3) virgin polyvinylchloride (100-800 µm, Goodfellow); and (4) manufactured low density polyethylene (400-1000 um), prepared by milling clean milk bottle lids with a cryogenic-grinder (SPEX Freezer-Mill® 6870) and then cutting to appropriate size using dissection scissors. Spiked plastics were distinctive, both in colour and shape and obviously cut at both ends, ensuring that only spiked plastics were counted in the trials. The plastics were also inspected for signs of degradation. Microplastics (50 combined particles per replicate) were mixed with 30 g sediment in a clean, ceramic bowl, any solidified sediment was gently broken up

using the weight of a pestle. Plastic-spiked sediment samples (*n*=5) were then added to the SMI unit with 700 ml $ZnCl_2$ (1.5 g cm^{-3}).

Table 3.2. Description of sediments used for environmental testing of SMI unit.

Site	Lat. Long.	Descriptor	Type	Grain size (x̄ µm)	*n*	Sampling date
Plym Estuary	N 50°21'716" W 4°08'073"	Fine	Clay/silt	10.25 (± SD 3.02)	3	23.06.16
Plymouth Sound Breakwater	N 50°20'174" W 4°08'605"	Medium	Silt	20.78 (± SD 3.05)	3	23.06.16
Portwrinkle Beach	N 50°21'390" W 4°18'22.9"	Coarse	Coarse sand	149 (± SD 49.86)	3	13.12.16

Extraction of microplastic from natural sediment samples – environmental validation

The applicability of the SMI unit in isolating microplastics from natural sediment samples was also verified by testing the procedure on locally sourced sediments of varying grain size. Natural sediment was sourced from three sites in the western English Channel (Table 3.2). Fine and medium sediments (*n=3;* Table 3.2) were sampled using a benthic multicorer deployed from the RV Plymouth Quest at sites local to Plymouth; the top 2 cm of each core was used for microplastic extraction. Tide time was not controlled for due to logistic constraints. Coarse sand (*n*=3) was sampled using a clean stainless steel measuring cup from the intertidal zone at the cliff base at Portwrinkle beach, Cornwall during low tide. All samples were immediately transferred to a clean foil tray and sealed. Sediment was dried at 50 °C for approximately 72 hours then microplastics extracted using the SMI unit as previously described (up to 50 g dry sediment per extraction). Once complete, nylon meshes were visually examined under a microscope (Leica, x25 magnification) for particles with a synthetic appearance; i.e. lacking cell structure, unnatural appearance in shape,

colour or texture (Lusher et al. 2016). Isolated microplastics were photographed and characterised by quantifying the shape (fragment, fibre or nurdle), colour and size of each particle. Particles were chemically quantified by Fourier Transform Infrared spectroscopy (Agilent Cary 630 and Bruker Vertex 70 with Hyperion 1000 microscope). Data were normalised by the dry weight (g) of sediment added to the SMI for extraction.

Contamination control

Contamination controls and procedural blanks were implemented during field sampling and sample processing, per the protocols of Lusher et al. 2016. All equipment was rinsed first with tap water, then twice with ultra-pure water before covering with clean foil. A dampened glass fibre filter (GF/C) paper was left open to the air both on board RV Quest and in the laboratory at each stage of processing and screened for plastic contamination using a light microscope (Leica, x25 magnification). Procedural blanks were used throughout to control for equipment contamination and samples were processed inside a clean laminar flow cabinet. Bench tops and microscope were cleaned prior to picking microplastics from filtered samples, and care was taken to expose samples for minimal periods. At times when using the laminar flow cabinet was not appropriate, a clean polyethylene cape was created around the microscope (Figure SI 3.1; Supplementary Information). A cotton laboratory coat was worn at all times.

Results and Discussion

Floatation media

A range of densities of three different salt solutions were trialled to determine the optimal conditions to float microplastic particles from sediment samples, balancing the attainability of high-density solutions and financial cost (Table 3.3). Sodium chloride proved the cheapest option (£4.17 L^{-1}; referred to as '1 cost unit' for comparative purposes; (see Table 3.3), however the maximal achievable density is just 1.2 g cm^{-3}. Numerous field studies have reported microplastic concentrations following extraction using NaCl. These include high profile studies by Browne et al. (2011), who highlight that coastlines are

contaminated with microplastic particles on a global scale and positively correlated with densely populated areas, and Woodall et al. (2014), who identified that microfibres are prevalent in deep sea sediments in abundances of up to four orders of magnitude higher than that of contaminated surface waters. However, while saturated NaCl is adequate in extracting low density plastics from sediments, it precludes denser plastics such as PVC (1.3-1.45 g cm^{-3}) and polyethylene terephthalate (PET, 1.38 g cm^{-3}), commonly used in textiles and to produce plastic bottles, from being suspended. Therefore, whilst NaCl is a cheap, inert option to use in microplastic studies, its use could result in an underestimation of the abundance of plastics found, particularly high density plastics. Sodium iodide can be prepared to higher densities than sodium chloride, however achieving a density of 1.5 g cm^{-3} proved the most expensive option at £172.95 L^{-1} (20.5 cost units) and therefore was consequently eliminated from our trials. Where NaI has been used to extract microplastics, multi-step methods are necessitated to minimise the volume of NaI required (Claessens et al. 2013; Nuelle et al. 2014; Table 3.1). Zinc chloride as a floatation medium has the benefit of attaining densities >2 g cm^{-3} and is relatively inexpensive at £35.10 L^{-1} (8.5 cost units) to prepare a density of 1.5 g cm^{-3}, enabling its use at greater volumes at higher densities. As such, $ZnCl_2$ has been used to quantify microplastic abundance in a number of microplastic studies (Horton et al., 2017a; Imhof et al., 2012; Liebezeit et al., 2012). In this study, at very high densities (1.6 - 1.8 g cm^{-3}), the fine sediment used for SMI method validation remained in suspension, making it impractical to use for plastic extraction. Therefore, considering the relative achievable density of NaCl and the expense of NaI, from our results, $ZnCl_2$ was deemed the most appropriate salt solution for floatation of microplastics using the SMI unit, at an optimal density of 1.5 g cm^{-3} when extracting from fine sediment. This density, whilst it precludes aggregates or composites denser than 1.5 g cm^{-3}, balances the requirement for the sediment to settle, whilst still dense enough to enable floatation of denser plastics such as PVC and PET.

Table 3.3. Comparative amount of salts (g) added to 1 L ultra-pure water to achieve specific densities, and their associated costs.

Salt	Density (g cm^{-3})	Amount added to 1 L H_20 (g)	Cost (GBP L^{-1})	Relative Cost Unit
Sodium chloride (NaCl)	1.2	337	£ 4.17	1
Sodium Iodide (NaI)	1.3	494	£ 85.44	20.5
	1.5	1000	£ 172.95	41.5
Zinc chloride ($ZnCl_2$)	1.3	500	£ 18.06	4.3
	1.5	972	£ 35.10	8.4
	1.8	1800	£ 65.00	15.6

Sediment-Microplastic Isolation (SMI) unit

The Sediment-Microplastic Isolation (SMI) unit is a compact, portable device that extracts microplastics from different sediment types in a single step, with reproducible results. A prototype of the SMI was constructed from glass and steel, however we identified that $ZnCl_2$ reacted with the steel. The advantages of manufacturing the SMI using PVC include resistance to corrosion, plus ease of construction, reduced costs, durability and weight. A quotation was obtained to construct a version of the SMI from stainless steel, however at GBP £640 (excl. VAT) per unit, it was no longer a cheap option, therefore potentially hindering the harmonisation of microplastic extraction methodology across studies. Constructing the unit from plastic does have a potential downside; long term use has not been tested in this study, and over time there is potential the continued use of $ZnCl_2$ could result in the degradation (e.g. fracturing, cracking) of the SMI unit. With this in mind, if regular inspection and procedural blanks reveal contamination, the unit should be replaced, which is made feasible by the low cost of the unit. In following the prescribed purging method, the SMI unit extracted microplastics from different sediments whilst avoiding self-contamination (corroborated by procedural blanks); the unit can be dismantled for easy, thorough cleaning between samples. The SMI unit is straightforward in

design and use, relatively cheap to produce, with each unit costing around GBP £50 (excl. VAT; Table SI 3.2). This allows for multiple units to be manufactured and used simultaneously, increasing the scope for sample replication, and reducing the time required to process all samples. Its design mirrors that of the Munich Plastic Sediment Separator [MPSS - (Imhof et al. 2012)], whereby sediment is mixed at the base of a vessel, and density floatation is used to float plastics above a shut-off valve. The MPSS is designed to extract plastics from up to 6 kg of sediment using 12 L of dense salt media, with aeration to adjust the relative density. As such, the MPSS is constructed entirely of stainless steel, stands at approximately 1.75 m tall by 36 cm wide and includes a base equipped with an electric motor to stir the sediment. While the MPSS is well suited for isolating microplastics from large volumes of sediment, the expense and complexities of manufacturing, size, weight and volume of flotation media required, limit its functionality and feasibility when processing numerous replicates of small samples.

SMI validation

Results from artificially spiked sediments

Microplastics artificially incorporated into fine estuarine sediments were extracted using the SMI unit with $ZnCl_2$ at a density of 1.5 g cm^{-3}. Mean extraction efficiencies, based on fibrous and particulate microplastics of different densities retrieved in a single step, ranged from 92-98% (*n*=5, mean 95.8% ±SE 1.6; Table 3.2) and were comparable with those of the MPSS (Imhof et al. (2012)), for which a mean 95.5% recovery rate for <1 mm microplastics was identified. No degradation of spiked plastics was observed after immersion in $ZnCl_2$ for 24h. Losses in microplastic recovery were found to arise if the unit was not primed with the floatation media prior to adding the sample. Indeed, if the space inside the ball valve is not filled with fluid, the media will be agitated when the valve is opened as the liquid floods the cavity, potentially leading to loss of plastics otherwise retrieved within the headspace of the SMI. Other potential losses may occur if very small microplastics become trapped within the sediment as it sinks back down to the bottom of the unit. It is therefore important to ensure the unit is not overfilled with sediment, thus avoiding a sub-optimal

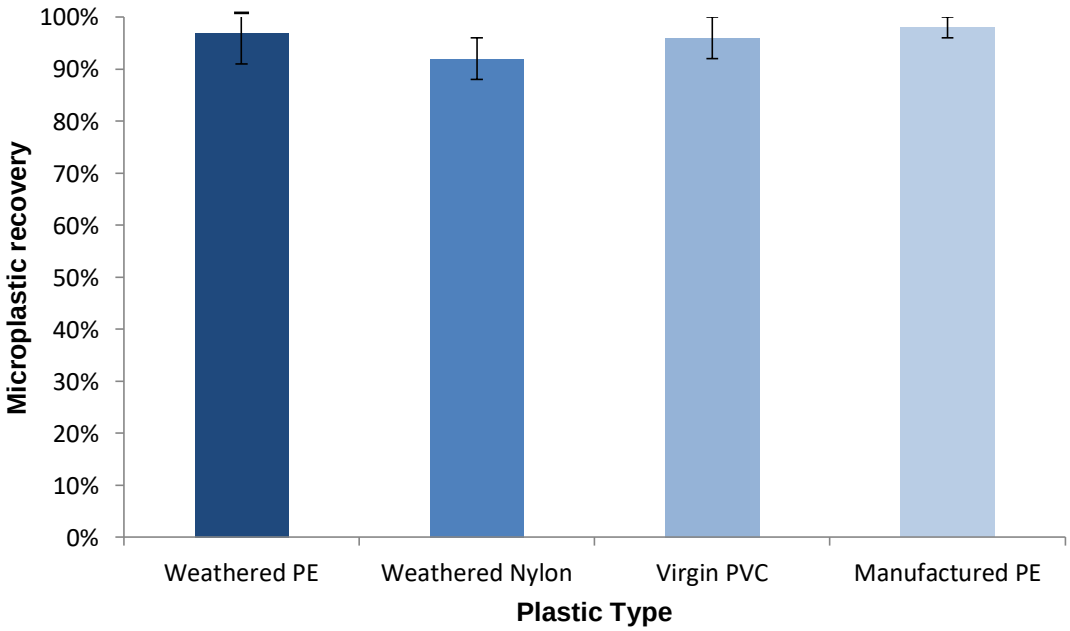

Figure 3.2. Mean (± SE 1.6; min 70%; max 100%) percentage recovery of microplastics (*n*=10 –20) from artificially spiked sediment (*n*=5).

ratio of sediment to floatation media, recommended here up to 50 ml sediment to 700 ml media. Similarly, it is also recommended that the sediment is briefly mixed again once the sediment begins to settle, to avoid microplastics becoming trapped within air bubbles in the sediment. Some key benefits of using the SMI unit in conjunction with $ZnCl_2$ (1.5 g cm^{-3}) over other microplastic extraction methods (see Table 3.1) are the combination of high extraction efficiency in a single step, simplicity, affordability and a compatibility with all sediment types.

The classic decanting method (Hidalgo-Ruz et al. 2012; Thompson et al. 2004), though simple in design, has a relatively lower recovery rate (35% pers. obs. 40% Imhof et al. 2012), due to plastics adhering to the inside of the vessel as the media is decanted. To combat this low extraction efficiency, the technique is often repeated 3–5 times, extending the processing period for each sample (Claessens et al. 2013; Fries et al. 2013; Nuelle et al. 2014). Studies employing this method may therefore underestimate the number of microplastics. Conversely, extending the sample processing time may increase the risk of external contamination. We propose, that in using the SMI unit, the user has the advantage of being able to rinse the entire headspace multiple times without re-

suspending the settled sediment, therefore reducing the need for repetitive processing and limiting opportunities for external contamination. The SMI has also proven compatible with finer sediments (e.g. estuarine silt). This contrasts with a number of existing methods (e.g. the elutriation and aeration chamber presented by Claessens et al. 2013), which works well with coarse sand but was found, when replicated for use in early trials, to clog irrevocably when using the fine, muddy estuarine sediments (pers. comms. Dr Andy Watts, University of Exeter).

Results from environmental samples

The SMI protocol extracted microplastic debris from all environmental samples, including coarse beach sediments and fine estuarine mud. Microplastic concentrations and type varied across samples and sites, ranging from 29.3 to 144.1 synthetic particles kg^{-1} dry sediment (mean ± SE: 67.4 ± 13.2) across the sites sampled (Figure 3.3). In the coarse sediments, 66.7 particles kg^{-1} (mean ± SE 17.6) were identified. Microplastics consisted of nurdles, fragments and fibres in a variety of colours (Figure 3.4), including blue, green, orange and mauve ranging in size from 100 µm to 10 mm in length and 30 µm to 4.3 cm wide, with mean dimensions of 3325 µm x 2117 µm. Polyethylene and ethylene copolymers were the dominant constituents of the microplastics found in the coarse sand (67%, Figure 3.4). These polymers are the most widely manufactured plastic type, commonly used in packaging. Polypropylene (8%), frequently used to make ropes, styrene (8%) and unidentified particles (17%) were also present. Fine sediment yielded 72.2 particles kg^{-1} (mean ± SE: 36.2), all were fibrous and ranging in length from 80 µm to 5000 µm, 20 µm to 40 µm wide and blue, red, black or transparent in colour (Figure 3.4). Semi-synthetic rayon, commonly used in textiles and sanitary products, was the predominant polymer type (67%, Figure 3.4), with polyester (13%), polyethylene terephthalate (PET; 7%), which is the main polymer used in plastic bottle production, polypropylene (7%) and unidentified particles of synthetic appearance (7 %) present. Medium sediment yielded 63.3 synthetic particles kg^{-1} (mean ± SE: 21.5) and were predominantly fibres, with one fragment present. The particles were red, grey, blue, transparent or green in colour (Figure 3.4) and ranged from 400 µm to 5000 µm in length and 30 µm to 200 µm wide. Polyester (25%), a common polymer in the manufacture of clothing,

and acrylic (25%), frequently used in optical applications and additives in paints, were the most abundant (Figure 3.4). Also present were ethylene propylene (12.5%), rubber widely used for its insulation properties, polypropylene (12.5%), rayon (12.5%), and unidentified particles (12.5%) (Figure 3.3).

Whilst this method has proven reliable in microplastic extraction from a range of sediment types, it is ultimately reliant on the user to manually sort and extract the plastics which is labour intensive and may introduce potential bias. Longer term, a shift to a more automated method of analysis is envisaged; however the infrastructure and technology are not currently available.

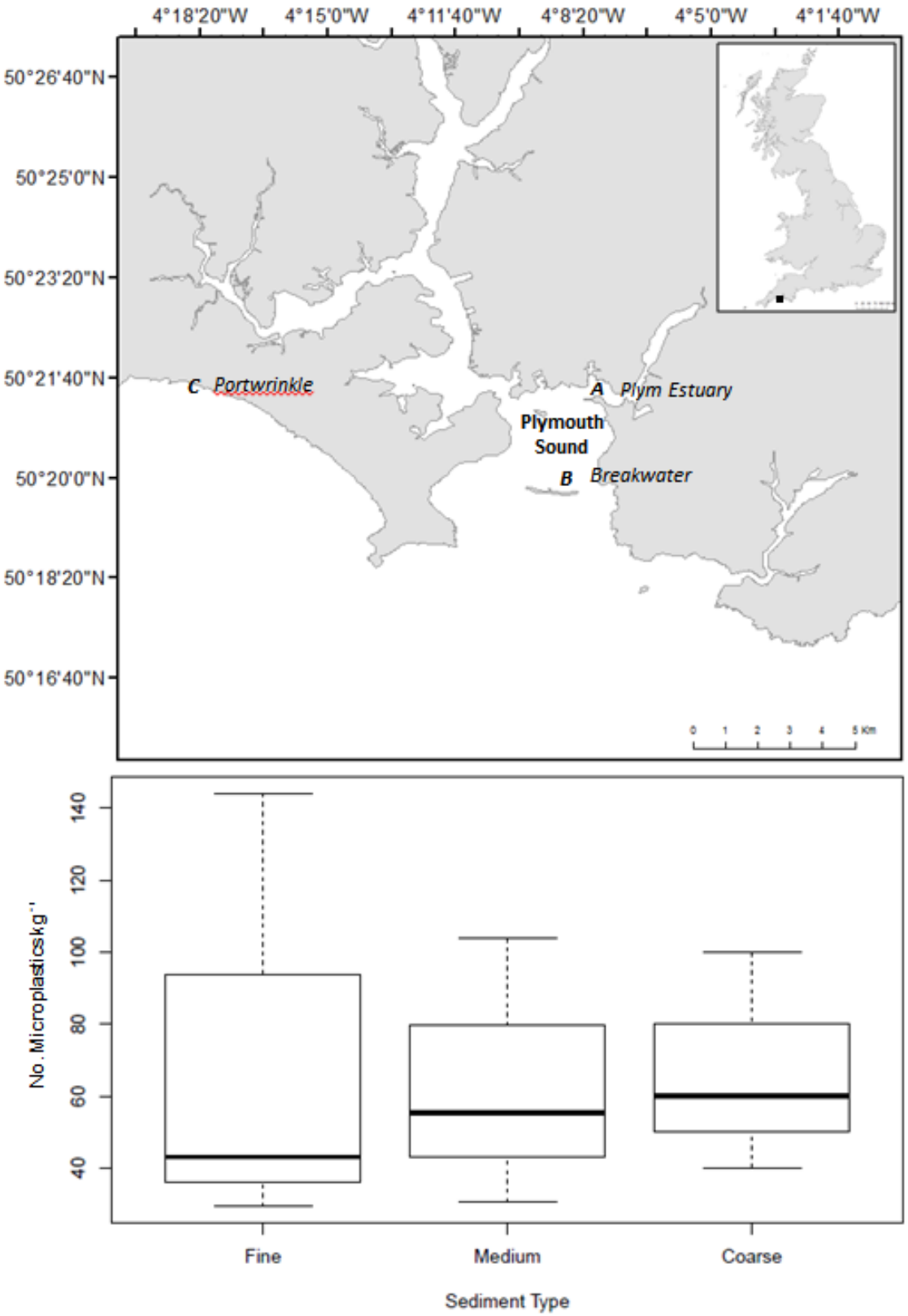

Figure 3.3. Sample collection sites for SMI validation, for fine (A), medium (B) and coarse (C) (unspiked) sediment (Table 2). Box and whisker plots show the median, interquartile and full range of microplastics extracted from each sediment type.

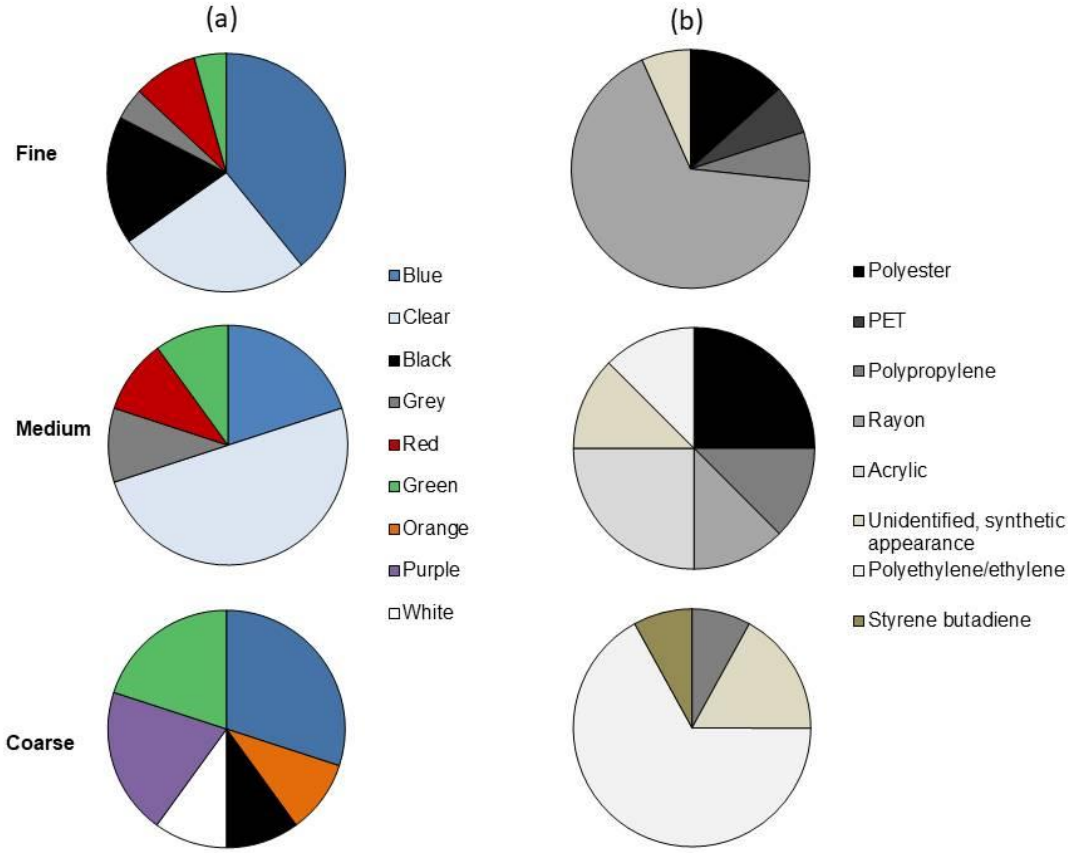

Figure 3.4. Composition of particles identified in fine (top row), medium (middle row) and coarse (bottom row) sediments. Breakdown of particles by colour (a) and polymer type (b).

Conclusions

A clear understanding of the microplastic availability within marine sediments requires accurate data on microplastic abundance in natural systems, of which there is a paucity at present. Despite calls for consistently applied sampling and extraction strategies, this is currently still lacking. Here we have presented a method to extract microplastics from sediments using a specially constructed Sediment-Microplastic Isolation (SMI) unit, in combination with zinc chloride solution (1.5 g cm^{-3}), able to extract microplastics from sediments with a mean recovery rate of 95.8% in a single step. The method is cost effective, encouraging universal use regardless of budget, thereby promoting harmonised sampling and working towards achieving comparable data sets across studies. The protocol is applicable to a range of sediment types, with microplastics

successfully isolated from estuarine silts and clay, and coarse beach sand. Zinc chloride was determined the most appropriate media for floatation of microplastics, achieving high densities with relatively low expense (GBP £35 L^{-1} at 1.5 g cm^{-3}). An optimal density of 1.5 g cm^{-3} was determined, balancing the requirement for media dense enough to allow floatation of different polymer types whilst allowing fine sediments to settle out of suspension to achieve the desired separation. The small dimensions and low weight lend the SMI for use in multiple settings, including laboratories and field based work such as on-board research vessels. Without accurate data on the field occurrence of microplastics in marine sediments we cannot regulate this widespread pollution of the marine environment and food web. A method to fill important data gaps regarding the availability of microplastics to benthic organisms is described here and made available.

Figure SI 3.1. Additional contamination control when not using laminar flow cabinet.

Table SI 3.2. Material cost for SMI unit (GBP£ excl VAT)

Equipment	Supplier	Product Code	Date of Costing	Cost	Cost per unit
63mm clear PVC pipe (2.5 m)	Pipestock.com	356685	22/08/2016	£ 64.81	£ 7.26
63 mm PVC ISO ball valve	Pipekit Ltd	GF161355007	22/08/2016	£ 45.12	£ 45.12
PVC sheet (250 x 250 x 3 mm)	DirectPlastics		10/12/2016	£ 1.38	£ 0.35
PVC beading	Barnes Plastic welding	WR.UPVC.3.GY.2.B	10/12/2016	£ 24.50	£ 0.25
				Total per unit	**£ 52.97**

Table SI 3.3. Details of costs, quantities and suppliers of chemicals for obtaining relative density mean costs. Where available, cost comparisons were for 1 kg of product, otherwise 2.5 kg products were used.

Supplier	Chemical	Quantity	Cost	Cost kg^{-1}
APC Pure	$ZnCl_2$	1 kg	£ 11.95	£ 11.95
Sigma	$ZnCl_2$	1 kg	£ 79.80	£ 79.80
Fisher	$ZnCl_2$	2.5 kg	£ 41.45	£ 16.58
			Mean	**£ 36.11**
APC Pure	NaCl	1 kg	£ 4.95	£ 4.95
Sigma	NaCl	1 kg	£ 20.00	£ 20.00
Fisher	NaCl	1 kg	£ 12.20	£ 12.20
			Mean	**£ 12.38**
APC Pure	NaI	1 kg	£ 179.00	£ 179.00
Sigma	NaI	2.5 kg	£ 561.00	£ 224.40
Fisher	NaI	2.5 kg	£ 288.60	£ 115.44
			Mean	**£ 172.95**

Table SI 3.4. Microplastics artificially incorporated into sediment to test efficiency of SMI unit

Microplastic	Shape	Size (µm)	Colour	Replicates	No. particles
Weathered PE	Filament	200-1000	Orange or Blue	10	20
Weathered Nylon	Filament	200-1000	Pale green	5	10
Virgin PVC	Granule	100-800	Pale pink	5	10
Manufactured PE	Fragment	400-1000	Bright green	5	10

Chapter 4:

Benthic fauna contribute to permanent microplastic burial in coastal sediments

This chapter is being prepared for publication. **Coppock, R. L**., Lindeque, P.K., Cole, M., Näkki, P., Birgani, H., Galloway, T.S. and Queirós, A.M.

RLC, PKL, MC, AMQ and TSG designed the studies. RLC carried out field sampling. RLC and PN processed field samples. RLC conducted experiments. RLC and HB processed experimental samples. RLC conducted statistical analysis, guided by AMQ. RLC wrote the manuscript and all authors contributed to editing and improving the final manuscript.

Microplastic (plastic 1µm to 5 mm) debris has been globally recognised as a pervasive pollutant of marine systems, and benthic sediments have been proposed as sinks. Despite the ubiquitous presence of microplastic in the marine environment, the mechanisms governing their entrance to and burial in the seabed are poorly understood. Benthic faunal activity such as bioturbation (the mixing and exchange of sediment particles and pore-water fluids) facilitates important bentho-pelagic coupling processes, including the recycling of nutrients and re-suspension of materials into the water column. Through a multi-faceted study, microplastic burial at three sites within a coastal system were investigated. Potential invertebrate contributions to that burial were estimated using a functional biodiversity classification of field data, further considering seasonal variations at one of those sites. Secondly, microcosm based experiments were used to quantify nylon fibre burial by a key benthic species in the study area, the brittlestar *Amphiura filiformis*. Environmental data confirmed that microplastic is buried within coastal sediments and there was no difference in microplastic burial pattern between sites or seasons. These results suggest that the process is ubiquitous in the study region. Sediment-dwelling fauna that move sediment vertically (“conveyors”) and randomly (“biodiffusers”) were found to significantly influence plastic loading. Furthermore, experimental data indicated that *A. filiformis* buries nylon fibres along its burrow structure, and plastic uptake significantly reduced burial activity deep in the burrows compared to the controls. Collectively, these results indicate that coastal sediments can act as permanent sinks for microplastics, with burrowing fauna contributing to that burial. Plastic uptake by burial fauna seems however to limit important fauna-mediated sedimentary processes.

Introduction

Microplastic debris (plastic particles and fibres 1 µm - 5 mm in size; Thompson et al., 2004) has been globally recognised as a pervasive pollutant of marine systems (2008/56/EC Marine Strategy Framework Directive, Descriptor 10, United Nations Sustainable Development Goal 14 target 14.1.1). An estimated 4–12 million tonnes of plastic litter enters the oceans annually and this figure is set to rise by an order of magnitude by 2025 (Jambeck et al., 2015). The majority of studies reporting marine plastic pollution stem from surface water measurements (Cózar et al., 2014), with a global study estimating >5 trillion pieces of plastic floating at the surface (Eriksen et al., 2014). This figure doesn't include particles <333 µm however, and there is now a growing body of evidence to suggest that microplastic concentrations increase with decreasing size (Lenz et al., 2016), substantially increasing surface water estimates. Microplastics have been shown to accumulate in benthic sediments (Woodall et al. 2014; Bergmann et al. 2017; Ling et al. 2017) although sedimentary loading of microplastic is much less well understood than in seawater (Underwood et al., 2017). Existing data suggest that there is currently a mismatch between expected and reported concentrations of microplastics in surface waters (Eriksen et al., 2014; Thompson et al., 2004), with much higher sedimentary values reported by four orders of magnitude (Bergmann et al., 2017; Erni-Cassola et al., 2019; Ling et al., 2017; Woodall et al., 2014). These values suggest that benthic sediments may serve as a final sink for microplastics.

Plastic and animal interactions are prominently reported for large pieces of plastic, however microplastic encounters are less well known. Due to their small size, microplastics can be ingested by a wide range of animals throughout the marine realm, including marine mammals (Nelms et al., 2019), turtles (Duncan et al., 2019), seabirds (Lourenço et al., 2017), fish (Lusher et al., 2013), corals (Hall et al., 2015), zooplankton (Cole et al., 2013,Coppock et al., 2019) and benthic invertebrates (Watts et al., 2015; Wright et al., 2013a). Microplastic ingestion can cause adverse health effects in benthic fauna, such as reduced feeding and energy budget in lugworms (Wright et al., 2013a) and crabs (Watts et al., 2015) and reproductive disruption in oysters (Sussarellu et al., 2016).

The routes by which microplastic could be transported to the benthos reflect wider benthic-pelagic coupling routes, including biologically mediated transport via biofouling (Kooi et al., 2017; Lobelle and Cunliffe, 2011), incorporation into organic matrices (Long et al., 2015) including marine snow (Porter et al., 2018) and faecal pellets (Cole et al., 2016; Coppock et al., 2019), and by physical hydrodynamics such as gravity, wind advection, currents and tides (Chubarenko et al., 2016). Physical, hydrodynamic forces are the dominant factors dictating particle exchanges in some areas; however fauna driven benthic-pelagic exchanges, including bioturbation (sedimentary particle mixing and pore water flux exchanges (i.e. bioirrigation) mediated by burrowing fauna (Kristensen et al., 2012)) are especially important determinants in the very productive and biologically active coastal regions (Queirós et al., 2019; Snelgrove et al., 2018). To date, no studies have investigated the role of burrowing fauna on microplastic burial in natural environments. This may, however, be especially important in coastal shelf seas, where macrofauna have large contributions to benthic-pelagic exchange, and proximity to coastal sources of microplastics is high (Clark et al., 2016). Bioturbation facilitates important benthic-pelagic coupling processes including the recycling of nutrients, burial and re-suspension of materials into the water column (Green et al., 2017; Queirós et al., 2019, 2015; Zhang et al., 2015). Bioturbating animals significantly alter the sediment structure of soft habitats through burrowing or feeding activities, enhancing the mixed layer depth (Teal et al., 2008). Associated exchanges of pore water via the sediment-water interface through the flushing of burrows during feeding and respiration (Kristensen and Kostka, 2004), often have a greater effect (by several orders of magnitude) on benthic-pelagic coupling than particle mixing (Berg et al., 2001, Queiros et al 2019). It is therefore likely that bioturbators affect sedimentary plastic burial in natural environments. Indeed, previous laboratory based experiments have highlighted benthic faunal contributions to plastic burial. Common Baltic infauna buried plastic fishing line (<1 mm lengths), with the highest abundance in the upper portion of sediment, decreasing with depth (Näkki et al., 2017). In a follow up experiment it was shown that plastic fragments were rarely brought back to the surface (Näkki et al., 2019).

Aims and hypotheses

This study is a first attempt to determine sub-tidal microplastic burial in marine systems in an environmental context and explore the role of marine benthic macrofauna on burial. Firstly, field observations are used to investigate the role that benthic faunal communities may play in the burial of plastic in coastal systems and how that might vary seasonally. Three studied sites within the Western Channel Observatory (Smyth et al., 2015) are dynamic, subtidal areas characterised by fine, muddy sediment beds and are predicted sedimentation accumulation zones (Uncles et al., In Press). A field program was devised to test the hypotheses that; (1) the 'fluff layer' adjacent to the sediment surface presents a viable transport route for microplastics to enter the benthos; (2) microplastics are being buried; (3) marine benthic fauna contribute to the burial of microplastics in coastal sediments; and, (4) the potential for plastic burial varies spatially and temporally. Secondly, a microcosm experiment was used to investigate the mechanisms underpinning plastic burial potential in a key benthic species, the brittlestar *Amphiura filiformis*, which is abundant in European coastal systems, and has been widely studied within the study area (Calder-Potts et al., 2018; Queirós et al., 2015; Widdicombe et al., 2004). Experimental work was used to test the hypotheses that; (5) microfibres are buried through bioturbation/bioirrigation activities; and microfibres in sediments alter (6) normal bioturbation activity and (7) oxygen uptake in a key benthic faunal species.

Methods

Environmental study

Sediment and fauna collection

Samples were collected from 3 sites in the Western English Channel (Plymouth, UK; Figure 1) on board Plymouth Marine Laboratory's (PML) RV Plymouth Quest. Site selection was guided by past studies into the hydrodynamics of Plymouth Sound (Uncles et al., 2015), and model simulations of particle transport and dispersal (Uncles et al., In Press; Chen et al., 2003). Sites were selected from a number of modelled possibilities; (1) The "Plym" site is located at the mouth of the Plym Estuary, and was selected as it receives direct inputs

from the River Plym which flows at a long-term mean rate of 1 m^3 s^{-1} (CEH, 2003) alongside the city of Plymouth where it receives industrial, maritime and wastewater inputs; (2) the “Breakwater (BW)” site is located inside the Plymouth Sound breakwater, an artificial barrier that reduces hydrodynamic flow and is therefore a likely deposition zone; (3) the “Rame” site is located 2.5 km off Rame Head and is one of the stations of the Western Channel Observatory sampling programme (https://westernchannelobservatory.org.uk). This site has been a dredge disposal site intermittently for over 100 years, initially used for munitions disposal but subsequently used for dredged material from the nearby ports, harbours and navigation channels (Bolam et al., 2011) and is thus likely rich in plastic debris. All sites were sampled during summer (June 2016), while the Plym site was sampled seasonally (January, April, June and September 2016). Sediment samples (*n*=3 per site, season and depth) were collected via a single deployment of a benthic multicorer, housing four cylindrical Perspex corers (length: 50 cm x diameter: 10 cm) that collect sediment and bottom waters preserving sedimentary structure, including the integrity of the sediment-water interface.

Fluff layer collection and processing

The ‘fluff layer’ (bottom water layer immediately above the sediment-water interface and rich in organic material (Queirós et al., 2019)), was sampled from each core, using 7.5 mL silicone tubing and a 100 mL syringe; gently syphoned off avoiding resuspension of the sediment water interface into a 500 mL Nalgene sample bottle, pre-rinsed with MilliQ water before and between samples. The water sample was filtered using a vacuum pump and filtering cup thoroughly pre-rinsed with MilliQ water, onto new 10 µm membrane filters (Whatman Nuclepore Track-Etch), and transferred immediately into a new, previously sealed, lidded Petri dish until microplastic extraction.

Sediment processing - Field

Each core was depth fractionated, sliced into three sections using a custom-made core extruder (section 1: top 2 cm; section 2: 4–6 cm; section 3: 6–10 cm depth) and a stainless steel plate (25 cm x 20 cm), which was rinsed clean with MilliQ water between slices and replicates. The top section (1) was immediately placed into a pre-rinsed, 1 L lidded pot (Kartell™) and preserved in a cool box

during transport to the laboratory for processing. For the remaining sections (2 and 3), 3 x 10 mL subsamples (30 mL per section) were taken for quantification of microplastic abundance using a pre-rinsed 20 mL syringe with the end sliced off. These were extruded into clean foil trays and immediately sealed ready for transport back to the laboratory. The remainder of each section was then sieved on deck using a 1 mm stainless steel sieve (Endcotts) to retain macrofauna which was fixed in 4% buffered formaldehyde until processing.

Sediment processing - Laboratory

Section 1 was washed through a 1 mm stainless steel sieve using MilliQ filtered water, into a clean glass dish, ensuring the sieve was well rinsed to wash through any plastic. Fauna >1 mm was fixed in 4% formaldehyde for a minimum of 48 hours before transferring animals to 70% ethanol and manually sorted ready for identification. The sediment and water was transferred from the glass dish to a clean foil tray, covered with a cardboard lid and dried (72 h; 50 °C) at the same time as the 30 mL subsamples collected during sampling from sections 2 and 3. Water content was estimated in sediment samples from each site (June 2016) by comparing sediment fresh and dry weight, after placing them in the oven at 60°C until weight remained stable. Sediment grain size was determined from a surface sediment sample collected at each site in June 2016, using a laser particle size analyser (Coulter LPS 230).

Microplastic extraction, characterisation and identification

Microplastics were extracted from sediment samples using Sediment-Microplastic Isolation (SMI) units as per Coppock et al., (2017). In brief, a density floatation technique was employed by means of a zinc chloride ($ZnCl_2$) solution of appropriate density (1.45 – 1.5 g cm^{-3}) which enabled the separation of denser sediment particles from floating plastic particles, including dense polymers such as PET (1.38 g cm^{-3}). Up to 50 g of dried sediment was gently broken up with a clean pestle and mortar and added to the zinc chloride solution, thoroughly mixed for 5 minutes using a magnetic stirrer and then allowed to settle gravimetrically overnight. The supernatant was then filtered through a 30 µm nylon mesh using a vacuum pump (Millipore) and rinsed filtering cups. All nylon meshes were visually inspected for microplastics prior to use to check for contamination. Samples were transferred immediately to new,

previously sealed, lidded Petri dishes (47 mm, Fisherbrand™) for later analysis. Prior to analysis, several drops of MilliQ water were added to each mesh to aid detection and minimise static, and then systematically visualised from top left to bottom right (Olympus SZX16 stereomicroscope; x25 magnification), agitating areas of thickened sediment residue with a clean, stainless steel needle. Any particles suspected to be anthropogenic (ie; no visible cellular structure, equally thick with 3 dimensional bending if a fibre; see Noren, 2007) were photographed and characterised, recording size, colour and type (fibre, fragment, film, bead). Isolated particles were chemically identified using Fourier Transform Infrared Spectroscopy (FT-IR; section 1 particles for June 2016 using Bruker Vertex 70 with Hyperion 1000 microscope, all other samples using Perkin Elmer Spotlight 400 FT-IR/NIR system; macroATR mode for particulates, µATR reflectance for fibres); all spectra obtained were visually inspected and compared with the Bruker or Perkin Elmer library databases to establish the best match. Matched spectra exceeding a confidence level of 70% were visually verified by the author and accepted. Matches between 60–70% prompted further consideration before accepting and anything falling below a 60% threshold was recorded as unknown. Extracted particles that were lost during the identification process were also recorded as unknown.

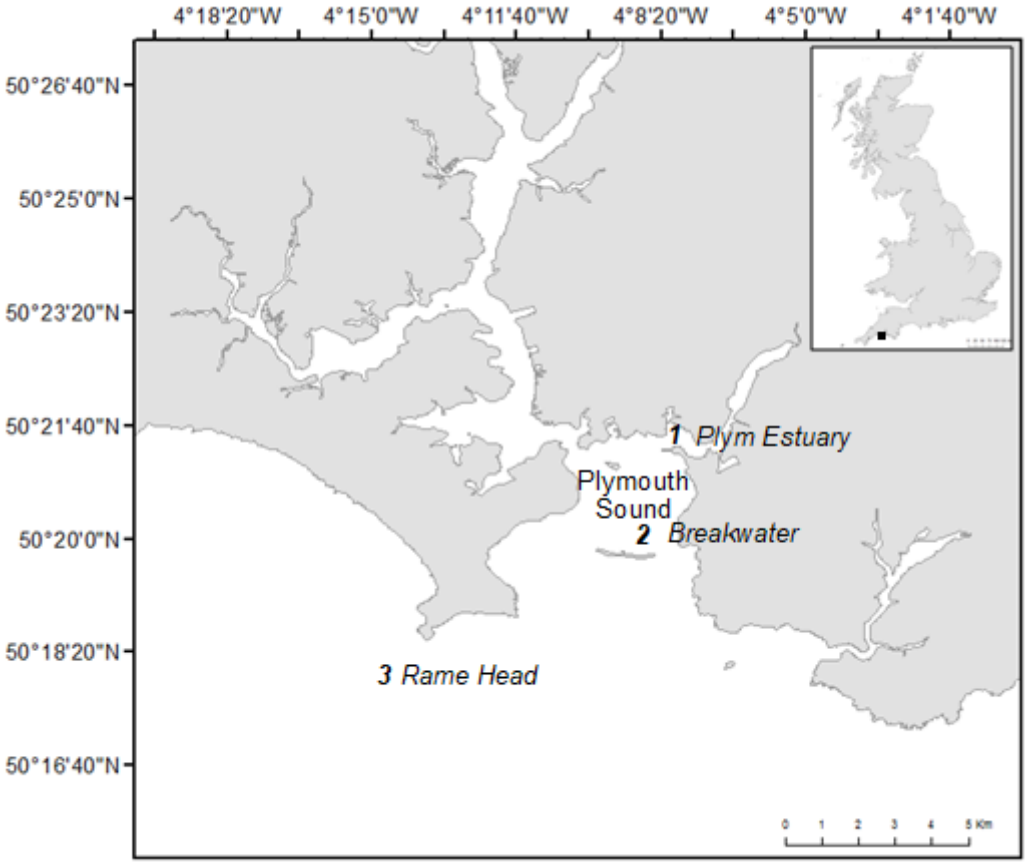

Figure 4.1. Site map detailing benthic sample locations from 1) entrance to Plym Estuary (N50°21.716'; W4°08.073'), 2) inside Plymouth Sound breakwater (N50°20.174'; W4°08.605') and 3) off Rame Head (N50°17.925'; W4°15.057').

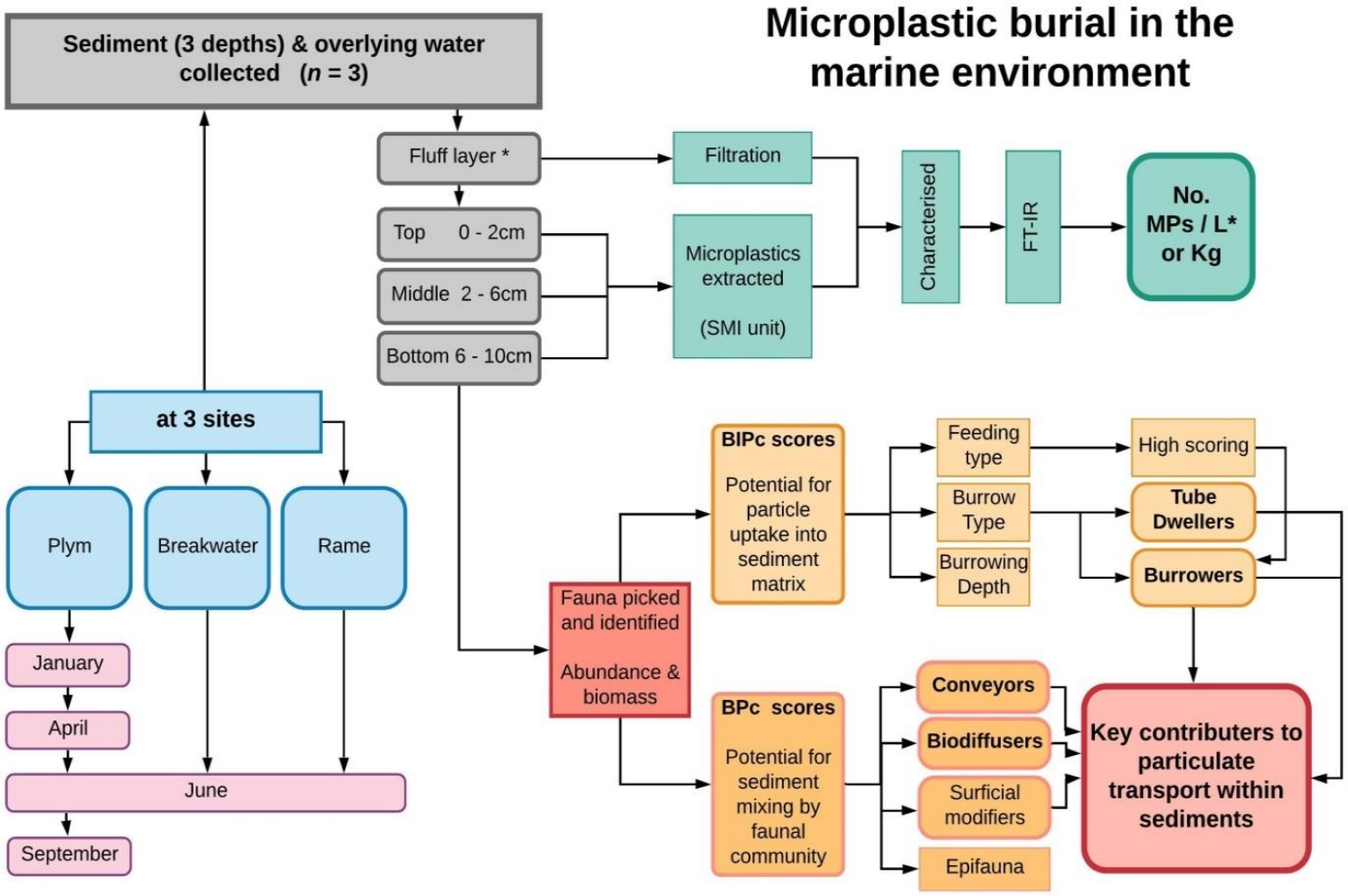

Figure 4.2. Flow chart illustrating sample collection on the left and onward sample processing to the right. Information in curved edged boxes was used in data analyses. (SMI: Sediment-Microplastic Isolation; FT-IR: Fourier Transform Infrared spectroscopy; BP_c: community bioturbation potential; BIP_c:community bioirrigation potential.

Fauna processing

All fauna in each sample were identified using a stereo microscope (Olympus SZX16) to the lowest taxonomic level, discarding any unrecognisable soft body parts. Taxa abundances were recorded and blotted fresh biomasses determined using a fine balance (Sartorius R200D). All species names were checked against the World Register of Marine Species (WoRMS).

Estimating faunal-mediated burial processes

Due to the complexities of benthic processes, it is not appropriate to use species biomass and abundance alone to describe fauna-mediated ecosystem processes such as bioturbation, and a functional trait approach is commonly used (Norling et al., 2007; Queirós et al., 2015; Solan et al., 2004). Metrics have been developed and widely adopted to estimate whole community bioturbation potential (BP_c; referring to particle mixing; (Queirós et al., 2013; Solan et al., 2004)) and bioirrigation potential (BIP_c; Renz *et al.*, 2018). Both provide a biomass and abundance weighted categorical scoring system, and include functional and life-history traits that are deemed important in calculating each. In both cases, the metric is summed for the whole community to estimate their potential effect on sediment mixing and bioirrigation.

- Community bioturbation potential (BP_c)

This trait-based approach was used to estimate faunal community bioturbation potential (BP_c) for each sample. The BP_c index (Queirós et al., 2013; Solan et al., 2004) characterises the abundance and biomass weighted effect of macro faunal community assemblages on sediment mixing. Scores are assigned (Table 4.1) to each taxon in each sample ($_i$) for sediment reworking mode (R_i) and mobility (M_i). Trait scores were attributed based on Queirós et al., (2013) and followed the scoring guidance based on the life history and ecology of the animal for additional taxa. 7 taxa were excluded as they were considered to bear no influence on plastic burial, including animals living fixed to hard substrata (ie; rocks or discarded shells) or in the water column.

Table 4.1. Trait scores and abbreviations used to calculate community bioturbation potential (BP_c), from Queirós et al., (2013).

$$BPc = \sum_{i=1}^{n} \frac{\sqrt{Bi}}{Ai} x\ Ai\ x\ Mi\ x\ Ri$$

***Mi* Score for traits**		***Ri* score for sediment reworking mode**		***Fti* code for reworking types**	
1	Organisms live in fixed tubes	1	Epifauna	E	Epifauna
2	Limited movement	2	Surficial modifiers	S	Surficial modifiers
3	Slow, free movement through sediment matrix	3	Upward/downward conveyors	UC/ DC	Upward/downward conveyors
4	Free movement, via burrow system	4	Biodiffusers	B	Biodiffusers
		5	Regenerators	R	Regenerators

- Community bioirrigation potential (BIP_c)

Community bioirrigation potential (BIP_c) for each sample was calculated per Renz et al. (2018), characterising the faunal community's potential for benthic-pelagic water (and solute) exchange, which has been shown to be highly important in the uptake of particulates into the sediment matrix (Kristensen and Kostka, 2004). Biomass (B_i) is calculated using individuals m^{-2}, abundance (A_i) converted to ash-free dry weight from wet weight (AFDW/WW conversions applied using Ricciardi and Bourget (1998) and scores assigned (Table 4.2) using ecologically driven faunal traits that affect ventilation and bioirrigation: feeding type (FT_i), burrow morphology (BT_i), and effective burrowing depth (L_{eff}; Table SI 1) of each species. BIP_c scores were assigned using a range of literature and online trait databases (Table SI 1). Where information for exact species was not available, scores were based on the closest related species or next taxonomic level. Effective burrowing depth (L_{eff}) was determined from the mean faunal environmental position from the data (ie; 2 cm, 6 cm or 10 cm). Where the population of a species was found in approximately equal

abundance at multiple depths, the effective depth was deemed the maximum of those depths.

Table 4.2. Trait scores and abbreviations used to calculated community bioirrigation potential (BIP_c), adapted from Renz *et al.*, (2018). L_{eff} was determined from the environmental position that each species was found.

$BIPc = \sum_{i=1}^{n} \frac{\sqrt{Bi}}{Ai} x\ Ai\ x\ FTi\ x\ BTi\ x\ Li$		
Trait	**Mode**	**Score**
FT (Feeding type)	Predator (P), Scavenger (S), Herbivore (H), Omnivore (O)	1
	Deposit feeder (DF)	2
	Facultative Deposit/ Suspension feeder siphon (fDF/SF I)	2
	Suspension feeder siphon (SFI)	3
	Facultative Deposit/ Suspension feeder (fDF/SF II)	2
	Suspension feeder (SF II)	4
	Subsurface deposit feeder (SDF)	5
	Funnel feeder (FF)	6
BT (Burrow type)	Attached, Epifauna	0
	Free living	1
	Living in a fixed tube	2
	Living in a burrow	3
	Epifauna	0
L_{eff} (effective depth; cm)	0 – 2 cm	2
	2 – 6 cm	6
	6 – 10 cm	10

Contamination controls

Strict contamination controls were implemented during field sampling and sample processing, as per Coppock et al., (2017). Laboratory sample preparation and analysis was conducted using either a laminar flow hood (Bassaire A4HF with Camfil HEPA filter), or positive pressure laboratory fitted

with HEPA filters, and cotton lab coats worn throughout. All laboratory equipment was thoroughly rinsed twice with MilliQ filtered watered (0.2 µm). Control glass fibre filters (Whatman GF/C) were left open to the air on board RV Quest, in the drying oven and in the laboratory and inspected at x10 magnification for any airborne contamination. Zinc chloride solution was filtered using a 25 µm nylon mesh before first use and between samples and blank procedural controls were carried out for every 3 uses (minimum) of each SMI unit. Samples open to the air were kept to a minimum, remaining covered at all other times.

Data analyses

Correction factors for each contamination risk (SMI procedural blanks and air contamination for boat, laboratories and drying oven, Table SI 2) and positive FT-IR identification were calculated and applied to all data prior to conducting analyses. FT-IR correction factors were calculated by subtracting lost and unidentified particles from the total particle count (Adjusted Total particles) and calculating the total FT-IR confirmed plastic as a percentage of the Adjusted Total. The maximum particle size considered for onward analysis was capped at 5 mm in any dimension. Functional classifications arising from BP_c and BIP_c index calculations were used to explore microplastic loading at each depth.

Statistical analyses

All data analyses were conducted using R statistical software (R Core Team, v3.4.1). Data were visually inspected for distribution and homogeneity of variances to determine whether data satisfied parametric *apriori* assumptions. To assess microplastic abundance in the fluff layer both spatially (microplastic abundance ~ site) and temporally (microplastic abundance ~ month), a Kruskal-Wallis test was performed as data distribution did not conform to normality, thereby failing to meet *apriori* requisites. To determine whether any relationship between microplastic abundance and sediment particle size existed, linear regression analysis (LM) was applied. To investigate microplastic abundance and distribution within the sediment, a one-way ANOVA was conducted and subsequent post-hoc analysis with a Tukey HSD pairwise comparison. To assess faunal contribution to microplastic burial, a linear regression analysis was conducted to investigate how the response variable (microplastic

abundance in deepest layer) was influenced by the explanatory variables (site, month, BP_c, BIP_c, conveyors, biodiffusers, mobility, tube dwellers, burrowers, feeding type). Collinearity, verified using pairwise plots and linear regression, occurred between BP_c and BIP_c overall, as well as between associated functional groups and were therefore modelled independently. The biodiffuser functional group and mobility scores >3 (BP_c) were highly collinear, as were burrowers and feeding type scores >4 (BIP_c). Mobility and feeding type were therefore removed from onward analyses. Hierarchical model selection was carried out using Akaike Information Criterion (AIC), beginning with a full model which included all associated predictors for BP_c (microplastic abundance ~ site + month + strict upward conveyors (UC) + strict downward conveyors (DC) + species that do both (UCDC) + biodiffusers (B) + surficial modifiers (S)) and BIP_c (microplastic abundance ~ site + month + tube dwellers + burrowers) and then performing both backward and forward stepwise model selection. Models producing the lowest AIC value were selected for analysis. Models were validated by visually inspecting error distributions and homogeneity of variances relative to linear model assumptions. As no effect of site or month was found when analysing faunal contribution to burial, all data collected for the bottom layer was considered together, improving model power to investigate any overarching trends. Removal of 2 outlying data points improved model structure and aided interpretation of the results.

Experimental study

Study species

Amphiura filiformis are brittlestars that burrow into soft sediment, reinforcing the burrow walls with mucus (Woodley, 1975). Their functional group within the sediment is “Gallery-diffuser”, a special case of “Biodiffuser” in that in addition to random mixing, they also transport particles vertically whilst forming and maintaining their burrows. The disc chamber is located 6–10 cm below the surface and aside from short rests, the animal is in continual motion, either maintaining its burrow, feeding or ventilating the burrow by undulating one or more of their long arms. Populations of *A. filiformis* live in muddy to fine sandy habitats and typically occur in aggregations of ~200 ind. m^{-2} (Queirós et al., 2015) but have been reported at >3000 ind. m^{-2} (Josefson, 1995). They live in semi-permanent burrows and are facultative deposit feeders, having the ability

to switch their feeding mode opportunistically between suspension and deposit feeding depending on the substrate type and current food availability, for example in shallow waters where the concentration of suspended food particles varies (Woodley, 1975). The activities of *A. filiformis* have considerable influence on ecosystem functioning; oxygenating sediments, nutrient recycling and creating niches for other macrofauna, earning them the reputation of 'key' species (Bowmer et al., 1986).

Animal and sediment collection

Sediment was collected from Cawsand Bay (50°19.81N 4°11.50W) on board RV Plymouth Quest using a Day grab in September 2018. The sediment was kept submerged with overlying bottom water and transported to Plymouth Marine Laboratory (PML) mesocosm laboratory (Findlay et al., 2008), where it was kept aerated in the dark at bottom water temperatures recorded at Cawsand Bay at the time of sampling (15°C). *A. filiformis* brittlestars were collected from the same site by hand in the following week using the same equipment. On deck, sediment was gently agitated by sluicing to minimise damage to individuals, which were then transferred to a shaded bucket of aerated local seawater at ambient temperature, and transported back to the laboratory within 2 hours of collection, where they were left in PML mesocosm (15°C) overnight, in the dark.

Sediment preparation

Collected sediment was defaunated within 48h of collection using a 1 mm stainless steel sieve to remove macrofauna, and homogenised using a wooden stick over the course of a week, leaving the sediment to settle between mixing. The overlying water was aerated using air stones, covered and maintained in the dark at 15°C matching the in-situ bottom water temperature at the time of sampling. The homogenised sediment was added to 12 aquaria (h:40 cm x w:12 cm x d:12 cm) to a depth of approximately 15 cm, topped with local seawater (salinity 35.5 psu), aerated and left to settle for 48 h before the addition of the brittlestars. Seawater was supplied to each aquarium via a re-circulating system consisting of seawater held in a 1000 L header tank and peristaltic pump (Watson Marlow 323), exchanging water at a rate of 11 mL min^{-1}. This did not cause sediment resuspension.

Microplastic preparation

Nylon microfibres were produced (as per Cole 2016): nylon 6,6 filaments (19 µm diameter, Goodfellows) were embedded in a glycol freezing solution (Neg 50™, Richard-Allan Scientific), frozen (10 min, −80 °C, New Brunswick U570 ultra low temperature freezer); and sectioned (343.5 ± 14.5 µm; mean ± SE) using a cryogenic microtome (Leica CM1950). The resultant rod-shaped microfibres were recovered via filtration and thoroughly rinsed with MilliQ water. In order to recover the fibres post-experiment, Nile Red was used to fluorescently stain the fibres using a solvent-extraction protocol (Cole, 2016). Nile Red stain has been shown to penetrate deep tissues of live zebrafish larvae without exerting toxic effects (Jones et al., 2008), thus any effect of fibres can be confidently attributed to the plastic and not the stain. Recovered fibres were suspended in MilliQ water and quantified using a Sedgwick Rafter counting cell and stereo microscope (x20 magnification; Wild, M5-49361). Fibre length was quantified using scaled photographs and ImageJ.

Experimental set up

The blotted fresh weight of individual brittlestars was recorded using a fine balance (Ohaus AX223) prior to assembly of experimental units. Ensuring even biomass distribution across replicates, five intact brittlestars were introduced to each of the 12 aquaria; one placed at the edge of each side plus one placed centrally to a density of 357.14 ind.m^{-2}, in line with natural field densities (Queirós et al., 2015; Solan and Kennedy, 2002). Brittlestars were fed Instant Algae® Marine Microalgae Shellfish Diet 1800, 8% dry-weight every second day at dusk. Dilutions were prepared at 20% of estimated dry-weight of brittlestar abundance based on appropriate husbandry conditions for invertebrates and wet weight/dry weight conversion (Ricciardi and Bourget, 1988). Animals were left to acclimate to experimental aquaria for 5 days before the addition of nylon microfibres at a concentration of 10,000 MP Kg^{-1} of sediment, equivalent to a mass concentration of 0.001 g kg^{-1}. The fibres were then suspended in the same seawater in a glass beaker, continually mixed to avoid settling, and delivered to the sediment surface of 6 treatment tanks using an electric pipette, ensuring even coverage. Aeration and circulation was halted for 15 mins to allow microplastics to settle. All aquaria were kept covered and maintained at

15.0 ± 0.07 °C (mean ±SE), salinity 35.9 ± 0.03 psu in the dark throughout the experiment.

Bioturbation

After 7 days of microplastic exposure, burial behaviour was quantified in experiment aquaria using 2D particle tracing methods (Mahaut and Graf, 1987) and the setup described in Queiros et al. (2015). 0.10 g cm^{-2} of fluorescent sediment tracer particles ("luminophores", Partrac Ltd) were added to each aquarium to form an even layer of approximately 0.2 cm on the sediment surface. Luminophores had been custom made to match the sediment particle distribution at the Cawsand sampling site. Aeration and water circulation were interrupted for 1 hour to allow the luminophores to settle on the sediment surface. Individual aquaria were placed at one end of a black box (h:90 cm x w:35 cm x d:64 cm) which allowed for images to be recorded under UV light (Bailey TL 8W G5 d:1.6 cm x l:28.8 cm tubes), using a digital SLR camera (Canon EOS 500D; 15.1 MP) mounted at the opposite end of the box. Two images were taken per side of each core; the first with just the single UV light above the tank, the second with both UV lights on. This enabled adequate contrast to distinguish luminophores touching the front plane of the aquarium only (image with single overhead light) whilst still capturing the luminophores at depth (image with both lights on). Images were captured using a 10s exposure, f = 5.6, ISO100 and remotely controlled via a PC using GB Timelapse software (V3.6.1). All four sides of each aquaria were imaged within 3 hours of luminophore addition to capture the initial luminophore profile at the sediment surface, and then again after 8 days to capture the tracer burial profiles. The total exposure to microplastics at the time of capture of the last image was therefore 15 days. Each set of four images were stitched together in ImageJ for each time point, resulting in one image per replicate, per time point. Luminophore burial was estimated from the stitched images using image segmentation methods described in Queirós et al., (2015), using the R statistical software (R Core Team, v3.4.1) and ImageJ (v1.46). Luminophore profiles were calculated from a flat sediment surface (Maire et al., 2006; Figure 4.6). Bioturbation was estimated from profiles via a number of parameters; 1) maximum burial depth, 2) overall bioturbation activity, quantified by calculating the percentage of luminophore tracer left at the sediment surface in the final

compared to initial image (ie; 100% - % remaining, Queirós et al., 2015) and 3) luminophore profiles (count per depth) were then binned at 2.5 cm intervals to compare burial activity at different depths with plastic burial, which result from sediment slicing (see below).

Oxygen uptake

Sediment community oxygen consumption was measured to establish potential implications of plastic loading to sedimentary function mediated by brittlestar bioturbation. The water in each aquarium was gently siphoned down to a 5cm water layer and an oxygen optode sensor disc (5mm, World Precision Instruments) was glued onto the inside of the tank using low toxicity silicon adhesive (World Precision Instrument, KWIK-SIL™). Each aquarium was then carefully refilled with the same water and topped up to the brim. An initial temperature compensated dissolved oxygen reading was taken immediately using the Oxy-mini fibre optic logger (World Precision Instruments). Custom made Perspex lids with motorised vanes were used to create a gentle flow (13.1 ± 0.1 L min^{-1}; mean ± SE) and then sealed onto each aquarium using non-toxic aquarium silicon sealant (Geocel). Incubations were carried out in sealed tanks and maintained in the dark at 15°C. Further oxygen measurements were taken after 6 hours. Sensors were batch calibrated using the manufacturer 2 point calibration method, using 0% (0% Oxygen solution, Hannah Instruments) and 100% oxygen saturation using air stones, then corrected for salinity and temperature. Percent oxygen measurements were converted into concentration as mg L^{-1} and then scaled to *A. filiformis* biomass per aquarium as mg^{-1} L^{-1} g^{-1}.

Quantifying plastic burial

Triplicate syringe cores (6 cm^2) were taken from burrows in each treatment tank to a depth of 10 cm and immediately frozen at -20 °C. They were then sliced at 0–2.5 cm, 2.5–5 cm, 5–7.5 cm and 7.5–10 cm and fibres extracted using Sediment-Microplastic Isolation (SMI) units, using the same method employed in field sample analysis. Fibres were collected as before onto 20 µm nylon meshes and were enumerated by observation under a microscope (Olympus IMT2 inverted microscope, x 40 magnification) using fluorescence (G fluorescence block, 480 - 550 nm). Each mesh was inverted in the Petri dish with a few drops of MilliQ water and a glass disc placed on top to facilitate

inspection via inversion. Quantified fibre abundances were then scaled up to represent total abundance in each slice.

Quantifying plastic ingestion

After sediment sampling, *A. filiformis* were recovered from experimental aquaria, rinsed with seawater and preserved in 10% buffered formaldehyde. Only wholly intact brittlestars were used to quantify fibre ingestion. To eliminate potential external fibre adherence, brittlestar arms were removed and the central disc was rinsed with water prior to dissection. The discs were then placed onto a Petri dish pre-rinsed with MilliQ, dissected to reveal the gut and flushed with water. The presence of nylon fibres in the gut were quantified using a stereo microscope (Olympus SZX16, x25 magnification).

Statistical analyses

All data analyses were conducted using R statistical software (R core Team, v3.4.1). All data were graphically inspected for distribution and homogeneity of variance to assess *apriori* assumptions for parametric test suitability. To assess the differences in rates of oxygen consumption, a Kruskal Wallis test was performed as data distributions failed to meet parametric assumptions. The effect of plastic on maximum burial depth was assessed using a one-way ANOVA. To assess the difference in the overall proportion of burial activity from luminophore counts (unbinned), a generalised linear model (GLM) with binomial family was conducted and the model was assessed by visually inspecting error distributions and homogeneity of variances relative to linear model assumptions. To further assess the effect of plastic on burial, and to facilitate comparison with plastic burial, luminophore counts were binned at 2.5 cm intervals and a generalised linear model (GLM) was performed, using the negative binomial family (“MASS” package; Venables and Ripley, 2002) to account for overdispersion in the data. A post-hoc pairwise comparison was then conducted using Estimated Marginal Means (EMM) joint-test function (“emmeans” package; Lenth, 2019).

Results

Environmental study

Sediment microplastic abundance and characterisation

Overall, microplastic abundances, adjusted for contamination and FT-IR corrections ranged 0-314 kg^{-1} of dry sediment with a mean abundance of 109 ± 8.7 (± SE) kg^{-1}. Fibres or bundles of fibres constituted 73% of the particles extracted, 18% were fragments, 16% films and a single bead was observed. The dominant polymer types were polyester, polyethylene, polypropylene, acrylic, nylon, and the semi-synthetic rayon, however we also found polyethylene terephthalate (PET), plasticised paint particles, rubber and epoxy resin film. Particle sizes ranged 80 µm-5 mm in length, with a mean length of 1.6 ± 0.08 mm and mean width of 0.19 ± 0.03 mm. The plastics varied in colour, with blue and black contributing to 52.3% of all particles. Transparent (15.9%), red (11.8%), green (6.8%), white (3.6%) and pink (3.2%) made up the bulk of the remainder, but grey, orange, yellow and mixed colours were also found.

Fluff layer microplastic abundance and characterisation

Microplastics were present in the fluff layer, representing a viable method for entry into the sediment matrix (Figure 4.3, Figure 4.4; Figure 4.). Overall, microplastic abundances varied between 0–13.1 particles L^{-1}, with a mean abundance of 5.2 ± 1 (± SE) MPs L^{-1}. There was a mean abundance of 2.9 ± 0.8 MPs L^{-1} across the 3 sites in June, whereas the mean abundance at the Plym site was 6.9 ± 1.3 MPs L^{-1} across the year. There was no difference in microplastic abundance in the fluff layer between the three sites (Kruskal-Wallis; H = 2.526, *df* = 2, *p* = 0.283) or between seasons at the Plym site (Kruskal-Wallis; H = 2.408, *df* = 3, *p* = 0.492). Particle sizes mirrored those found in the sediments, ranging from 80 µm-5 mm in length, with a mean length of 1.6 ± 0.17 mm, but a smaller mean width of 69 ± 35 µm. A higher proportion of particles found in the fluff layer, compared to the sediment, were fibrous in form (90%) with the remaining 10% fragments. Again, the majority of the colours noted were blue (44%), black (22%), red (16%) and transparent (10%) with white, pink and grey also found. As found in the sediments, polyester, polyethylene, nylon, acrylic and rayon were identified from the fluff layer.

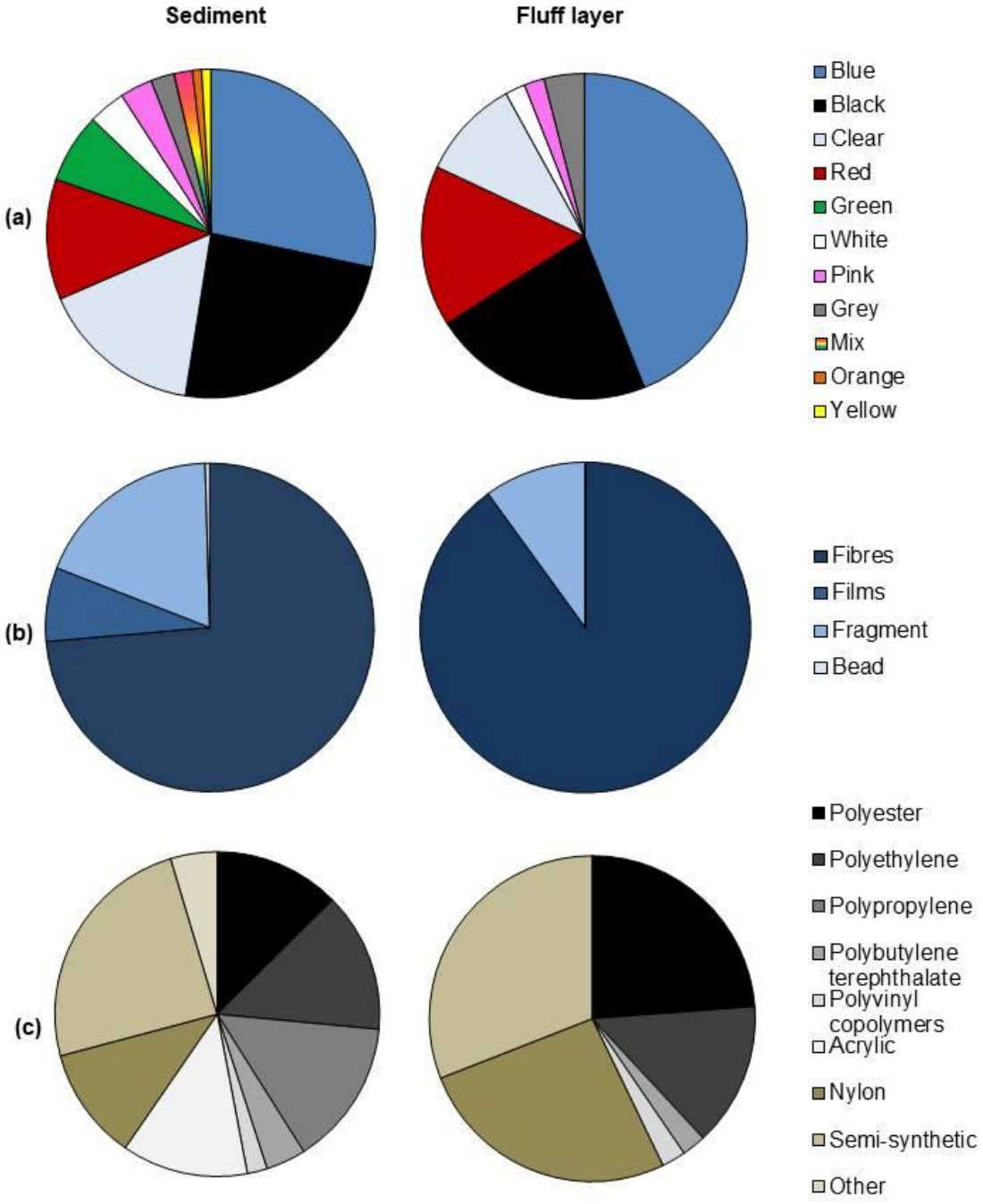

Figure 4.3. Composition of plastic particles identified in sediment (left column) and fluff layer (right column) samples. Breakdown of particles by colour (a), shape (b) and polymer (c).

Sediment characteristics

Sediment at the Plym site was categorised as fine clay/silt with a particle size of 10.25 ± 3.02 µm (mean ± SD). At the breakwater site, sediment particle size was 20.78 ± 3.05 µm and categorised as fine, silty mud and sediment at the Rame site was also categorised fine, silty mud with a particle size of 21.33 ± 3.29 µm. There was no relationship between microplastic concentration and particle size ($F_{25,1,}$ = 1.144, p = 0.295, R^2_{adj} = 0.005).

Microplastic burial

Overall, microplastics occurred ubiquitously at all depths and were found in greater numbers in the deepest layer compared to the top (ANOVA; $F_{51,2}$ = 3.815, p = 0.029; TukeyHSD p = 0.026).

Spatial analysis

Microplastics occurred at all three depths at all sites sampled in June; concentrations were highly variable and no significant difference was found between sites or depths (MP abundance ~ depth; ANOVA $F_{24,2,}$ = 1.641, p = 0.215; Figure 4.4).

Temporal analysis

Microplastics were present throughout the year at the Plym site and were found in significantly greater numbers at depth compared to the surface layer (microplastic abundance ~ depth; ANOVA $F_{33,2}$ = 3.696, p = 0.036; TukeyHSD p = 0.041; Figure 4.5). Microplastic abundance was highly variable but model selection revealed there was no significant variation throughout the year.

Overall faunal contribution to microplastic burial in deepest layer

As we found no difference between sites or between seasons at the Plym site, all data in the deepest layer were aggregated to analyse faunal contribution to burial.

When exploring whole community effect on microplastic loading in the deepest layer, we found that neither the BP_c (MP abundance ~ BP_c; F16,1 = 1.093, p = 0.311, R^2_{adj} = 0.005) or BIP_c (MP abundance ~ BIP_c; F16,1 = 0.376, p = 0.548, R^2_{adj} = -0.038) indices had any overall effect. When refining the parameters to investigate the contribution of each functional type to microplastic abundance at depth, we found no significant BIP_c predictors (MP abundance ~ Tubes +

Burrowers), however both upward and downward conveyors (“UC/DC”) were found to have a positive influence on microplastic loading in the deeper layer, whilst strict upward conveyors (“UC”) and biodiffusers (“Biodiffuser”) had negative effects (MP abundance ~ 303.202 +94.32 * UCDC -555.312 * UC -30.867 * Biodiffuser + site; $F_{10,5} = 6.7$, $p = 0.005$, $R^2_{adj} = 0.655$).

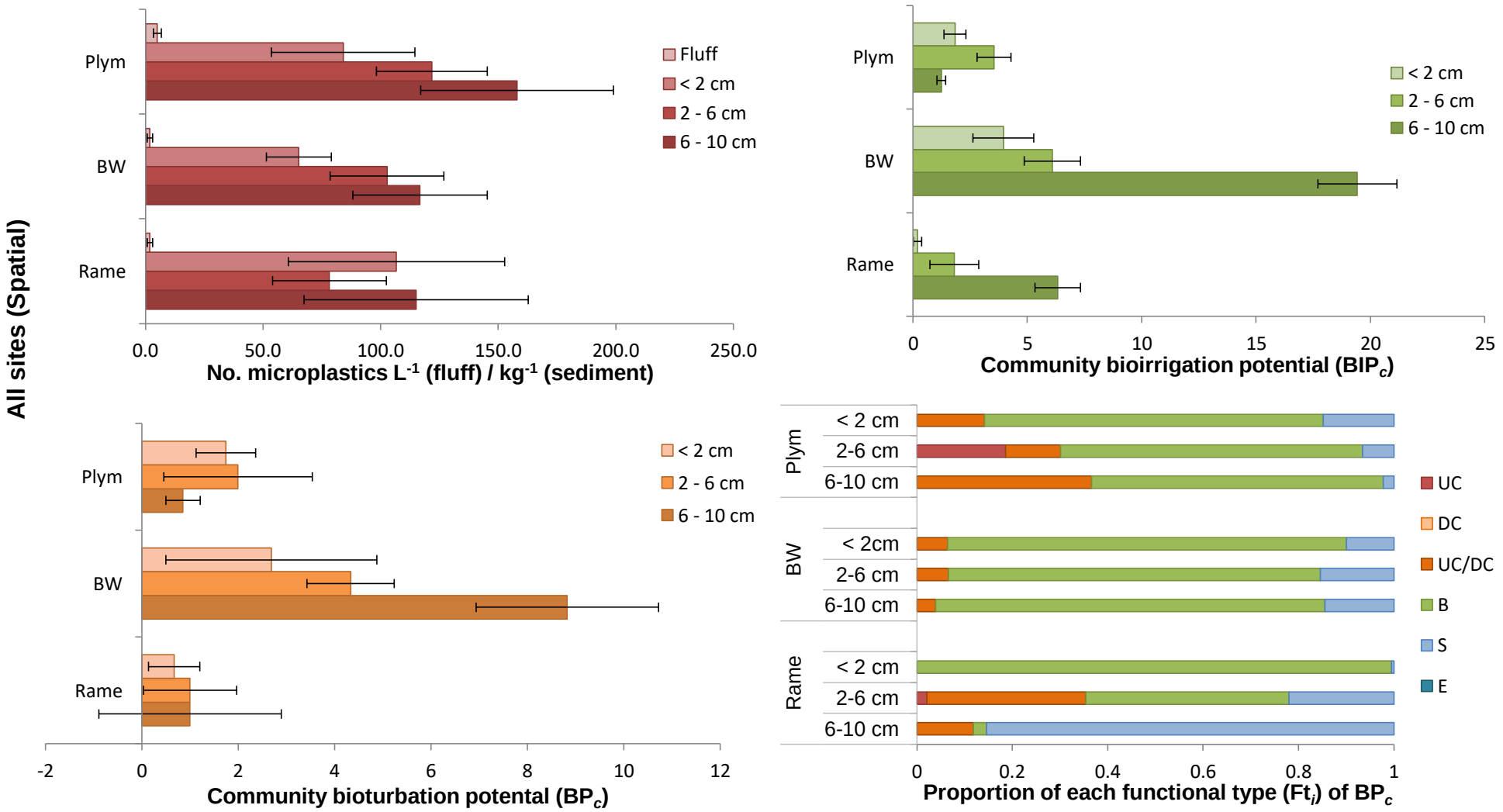

Figure 4.4. (a) mean (± SE) microplastic loading in fluff layer and each depth at all sites in June, community (b) bioirrigation potential (BIP_c), (c) bioturbation potential (BP_c) and (d) proportion of each functional group of the whole BP_c at each depth. UC: strict upward conveyors; DC: strict downward conveyors; UC/DC: both upward and downward conveyors; B: biodiffusers; S: surficial modifiers; E:

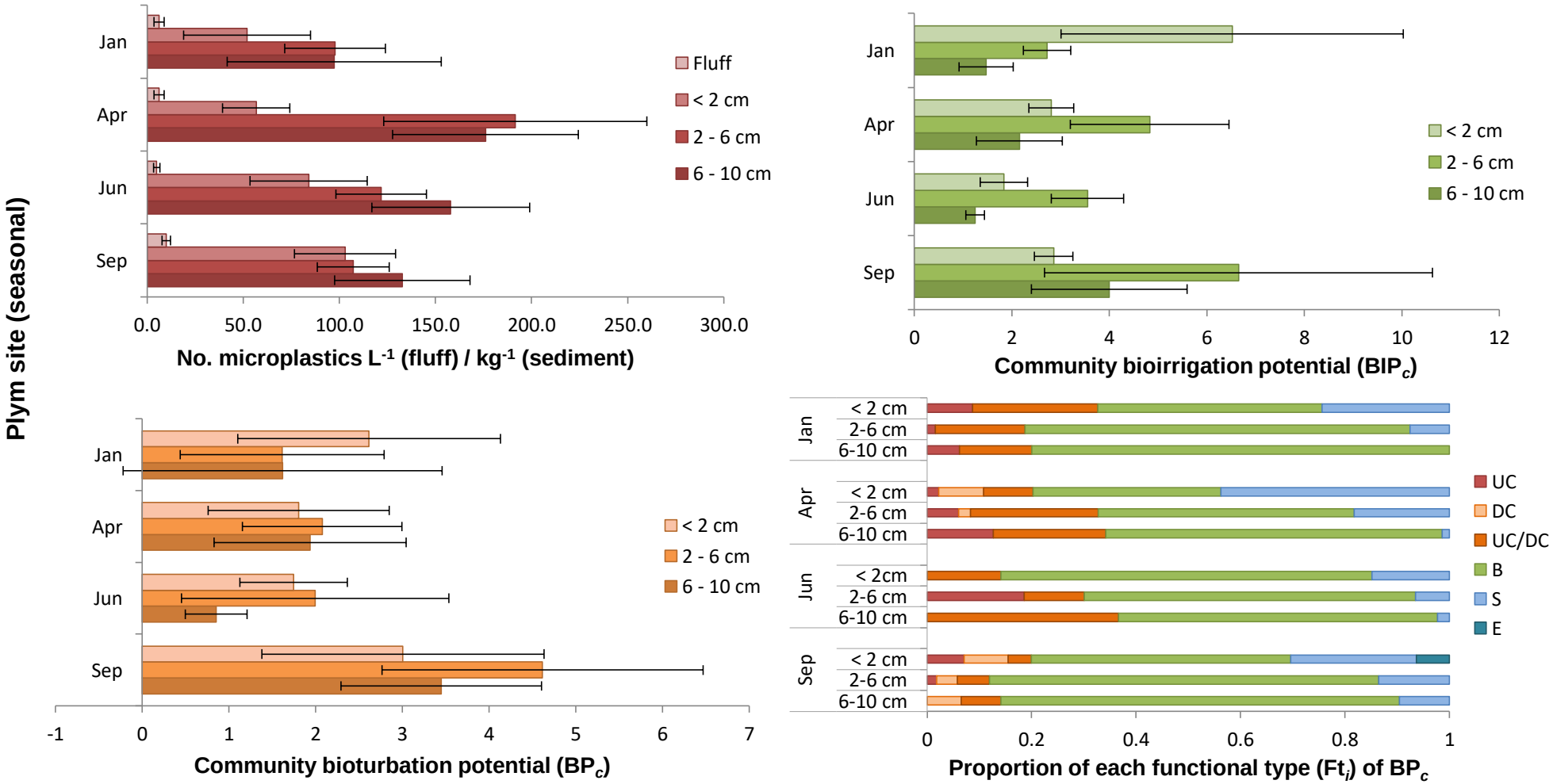

Figure 4.5. (a) mean (± SE) microplastic loading in fluff layer and each depth throughout the year at the Plym site, community (b) bioirrigation potential (BIP_c), (c) bioturbation potential (BP_c) and (d) proportion of each functional group of the whole BP_c at each depth. UC: strict upward conveyors; DC: strict downward conveyors; UC/DC: both upward and downward conveyors; B: biodiffusers; S: surficial modifiers; E: epifauna

Experimental study

Bioturbation activity

There was no overall difference in the burial activity of *A. filiformis* between the plastic treatment and control ($F_{10,1}$ = 0.01, p = 0.921; Figure 4.6). When assessing burial by binned luminophore counts at 2.5 cm intervals to compare alongside plastic burial, there was an effect of plastic on burial activity in the deepest layer (7.5-10 cm; count ~ depth + treatment + depth*treatment; $F_{40,3}$ = 3.378, p = 0.027; EMM joint-test, p = 0.007). There was no difference in the maximum burial depth between treatment and control conditions ($F_{10,1}$ = 2.676, p = 0.133). Full (unbinned) luminophore profiles indicated that maximum burial depth in control tanks was 9.24 ± 0.45 cm (mean ± SE). In comparison, maximum burial depth for nylon fibre exposed brittlestars was 8.29 cm ± 3.66.

Sedimentary community oxygen consumption

Oxygen consumption in the fibre-exposed aquaria was higher than that of controls at the end of incubations. Brittlestars exposed to nylon fibres for 14 days (ie; fibres introduced 7 days prior to luminophore addition) consumed oxygen at a rate of 0.105 ± 0.012 mg L^{-1} h^{-1} g^{-1} biomass (mean ± SE) compared to controls at 0.088 ± 0.011 mg L^{-1} h^{-1} g^{-1} biomass, however this was not statistically different (H = 1.32, *df* = 1, p = 0.251).

Plastic burial

Nylon fibres were buried in all treatment tanks and found at all depths down to 10 cm. 55.6% of fibres were recovered from the top 2.5 cm of sediment, with numbers reducing to 8.3% in the deepest layer (7.5–10 cm). The plastic distribution matched that of the luminophore profile (Figure 4.6).

Plastic ingestion

Of the 25 (out of 30) brittlestars that were wholly intact post exposure in the plastic treatment, 48% had nylon fibres in their discs at time of dissection. Of that 48%, the number of fibres recovered ranged 1-6 per individual, with a mean of 1.9 ± 0.29 (± SE) fibres per individual.

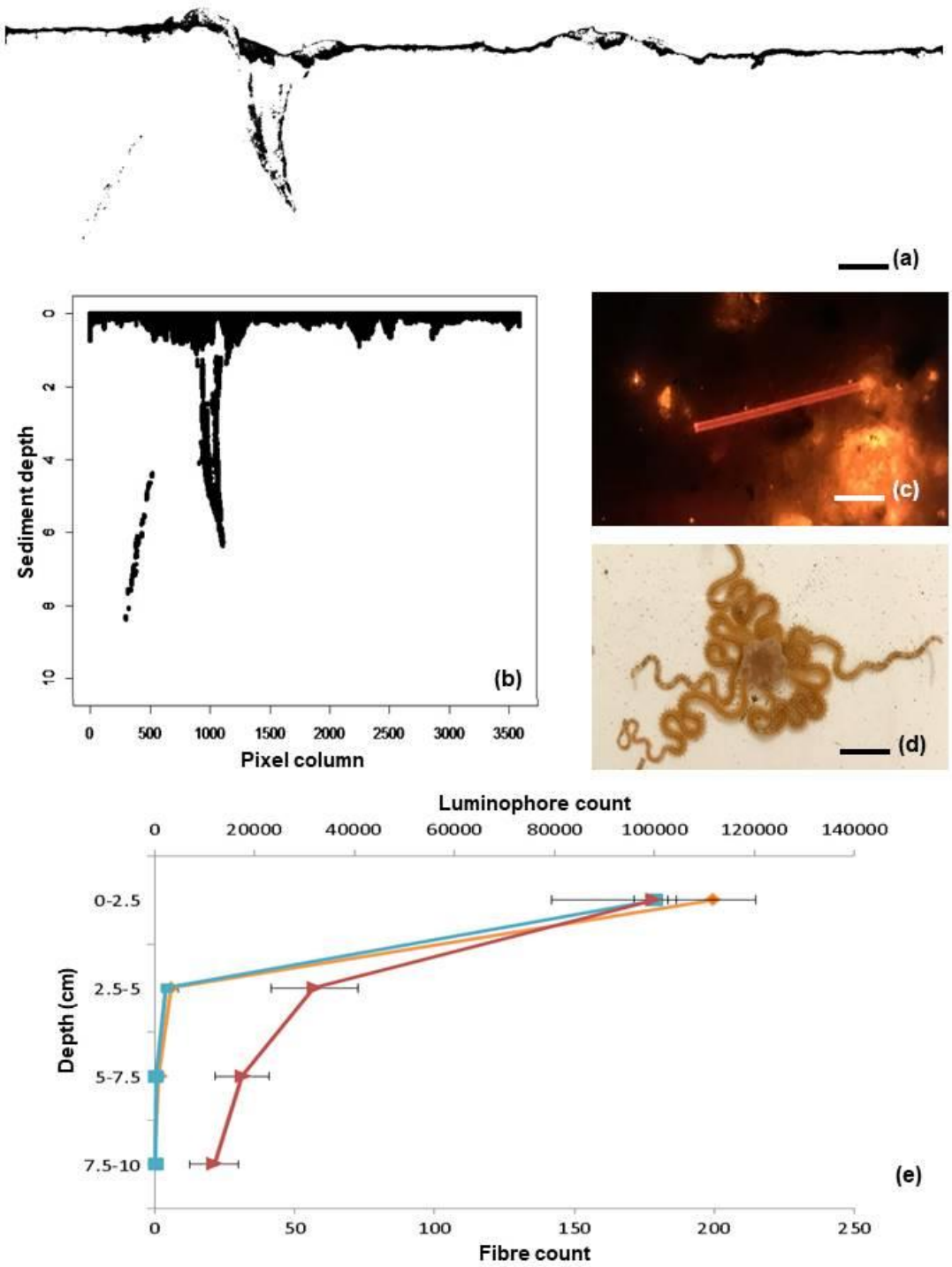

Figure 4.6 (a) Threshold set for luminophores touching front of aquarium, at sediment-water interface buried (scale bar = 2 cm) and (b) XY coordinates plotted when surface flattened to quantify burial activity and depth from luminophore profiles. (c) Images of a fluorescing nylon fibre dyed with Nile Red (scale bar = 100 µm) and (d) a specimen of Amphiura filiformis (scale bar = 5 mm). (e) Plot *profiling mean* (±SE) luminophore (blue square = control, orange circle = plastic treatments) and fibre (red triangle) burial at 2.5 cm intervals.

Discussion

Environmental study

This study demonstrates that microplastics are being buried in the marine environment and faunal trait associations indicate that benthic faunal activity plays a role. We identified that the fluff layer is a consistent reservoir of microplastics for uptake into the sediment matrix and microplastic loading at depth is stable across sites and throughout the year. Furthermore, we have identified functional trait based mechanisms underpinning faunal-driven microplastic burial. This is the first time that biotic-driven microplastic burial has been investigated under environmental conditions.

Published literature has reported that waterborne microplastic abundance is highly variable, due in part to the natural heterogeneity of water bodies but also to differences in sampling, methods used to extract and quantify plastics and variability in reporting between studies (see Shim et al., 2018 and references therein). Microplastic abundance increases with decreasing size (Enders et al., 2015; Lenz et al., 2016), adding to the complexity of generalising microplastic concentrations. We observed a mean concentration of 5.2 microplastics L^{-1} (5.2 $x10^{3}$ m^{-3}) in the fluff layer which is higher than the global mean average for marine surface waters at 2.4 $x10^{3}$ m^{-3} (Shim et al., 2018) but the same order of magnitude. The plastics isolated from the fluff layer were similar in size and composition to those extracted from the adjacent sediments, albeit a higher percentage of fibres were recorded in the fluff layer compared to within sediments, suggesting microfibres may be less prone to burial or more prone to resuspension and lateral movement than particulates. This is in line with a study of 42 sites around the south-east Australian coast, which reported a strong positive correlation between fibres and increasing wave exposure, suggesting a strong influence of hydrodynamic forces in the settling of microplastics (Ling et al., 2017). We did not observe any microplastic concentration gradient from nearshore to offshore, as may be expected considering such a gradient has been observed in surface waters off Plymouth (Steer et al., 2017) and in surface waters in the Mediterranean (Pedrotti et al., 2016). However, our findings are in keeping with microplastic abundances reported in sediments around the Belgian coast, where stable abundances were found from near shore to 20 km offshore

(Claessens et al., 2011). One explanation for the stable loading in this present study, may be the disposal of dredged material near our site at Rame Head, which co-occurred during our sampling period. Modelled spatial distribution of dredged material primarily from the dockyard and River Tamar, indicate our site may be affected by these deposits (Okada et al., 2009). Another explanation may be that according to hydrodynamic modelling of the locality, all three sites in our study are predicted accumulation zones (Uncles et al., In Press), potentially diluting the expected gradient from land seawards.

Sediment microplastic concentrations are also highly spatially and temporally variable, with the wide array of methodologies available making data comparisons challenging. Given this heterogeneity, it was perhaps surprising that no significant difference in microplastic loading between sites or throughout the year was observed, albeit variability was high. Our data however, compares favourably with the study of Ling et al., (2017), which also found no difference in microplastic abundance between sites but did report a positive relationship between particles and fine (63 µm) sediments. We found no relationship between microplastic abundance and grain size, however all of the sites in the current study are characterised by fine sediment (maximum mean grain size of any site is 21 µm), and the smallest microplastic size was limited by the ability to detect, lift and identify particles, thereby potentially obscuring a relationship with such fine sediment.

Microplastic abundance was elevated at the Plym site in April and this slight increase might be related to an increased phytoplankton and zooplankton faecal flocculation, sinking down to the seabed at this time of year (Zhang et al., 2015). There was a sharp increase in chlorophyll *a* fluorescence recorded at a depth of 10 m at station L4 (Western Channel Observatory long-term time series) three days prior to sampling. These organic rich aggregates can transport high concentrations of microplastics, relative to the ambient seawater, down through the water column to the fluff layer at the sediment-water interface and enhance microplastic bioavailability to benthic organisms (Porter et al., 2018). Evidence of strong benthic-pelagic coupling in a temperate, coastal system was reported at the Western Channel Observatory site L4 (Tait et al., 2015), where the composition of settled material during a spring bloom indicated vertical transport of detritus and phytoplankton to the benthos. However, given that this site is

subject to riverine input, it is also plausible that the observed increase in microplastic abundance is due to increases from terrestrial sources, or spring rainfall flushing out microplastic from riverine systems (Hurley et al., 2018).

Once deposited onto the seabed, different biotic and abiotic processes may influence whether a particle is re-suspended into the water column or taken up into the sediment matrix. Benthic macrofaunal communities can dominate sediment stability and erosion thresholds (Montserrat et al., 2008; Sgro et al., 2005) and depending on the dominant species, can completely alter the sediment structure, cohesion and biogeochemistry (Montserrat et al., 2009). Bioturbation and bioirrigation (faunal mediated movement of particulates and pore-waters within sediments) activity is vitally important in ecosystem functioning, facilitating benthic-pelagic processes such as nutrient cycling (Volkenborn et al., 2007). Different sediment reworking modes, or functional groups, have different effects on the vertical distribution of particulates within the sediment matrix (Kristensen et al., 2012). Biodiffusers, such as the common cockle *Cerastoderma edule* and cat worms, *Nephtys* sp. randomly move particulates through burrowing activity, whereas particle conveyors such as the lugworm, *Arenicola marina* can both transfer surface sediments to deeper layers or significantly contribute to the resuspension of sediment and nutrients to the water column (Kristensen et al., 2012). Collectively, these reworking modes substantially alter the chemical, physical and biological environment within the sediment, generating a highly dynamic and heterogeneous environment which depends on the species composition of the resident faunal community and varies both spatially and temporally. When investigating the effect of the benthic community on microplastic burial, we found no relationship between microplastic abundance at depth and overall community bioturbation potential (BPc) or bioirrigation potential (BIPc). However, when we refined the parameters of our model to investigate the contribution of different bioturbator functional groups to microplastic burial, we identified that strict upward conveying fauna and biodiffusers had a negative effect on microplastic abundance in the deepest layer (6-10 cm) whereas animals that contributed to both upward and downward conveying had a positive effect. An *in-situ* study in South Africa of the sand prawn *Callianassa kraussi*, found chlorophyll *a* in greater concentrations at depths of 15–25 cm than at the sediment surface, due

to the conveying and bioirrigation activities of these animals burying benthic surface diatoms (Branch and Pringle, 1987). In contrast, sediment ejection from burrows can re-suspend particles, presumably including microplastics, as indicated by the negative effect of upward conveying animals in our results. *In situ* observations of the echiuran worm, *Maxmuelleria lankesteri* in a Scottish sea loch, found a mean sediment ejection rate of 2.75 kg burrow^{-1} year^{-1} (Hughes et al., 1999), substantially contributing to sediment resuspension. These actions could also re-suspend microplastics back into the water column. However, from our results, the stable microplastic loading at depth both seasonally and spatially, indicate that the cumulative effect is permanent burial of microplastics at our study sites.

In this novel field observational study, we have demonstrated that microplastics are buried in coastal sediments and that benthic fauna influence the microplastic loading. These results indicate that in upward and downward conveyor dominated benthic habitats, the bioturbation activities of these animals may be an important determinant of microplastic burial. Further effort is now required to determine whether bioturbation and bioirrigation activities contribute to the uptake of microplastic in a wider range of sediment matrices.

Experimental Study

The role sediment-dwelling biota play in the burial of microplastics was confirmed using a targeted exposure study. Our experimental data revealed that the brittlestar, *Amphiura filiformis*, buried nylon fibres up to 10 cm deep, following the same profile as the sediment tracer particles (Figure 4.6). This same trend was also reported in a study investigating microplastic transport using the lug worm, *Arenicola marina* (Gebhardt and Forster, 2018). Whilst *A. marina* are conveyors and *A. filiformis* are gallery-diffusers, both construct and maintain burrows, moving particles vertically within the sediment. Whilst we detected no overall change to bioturbation activity, we found that sediment mixing, driven by *A. filiformis*, was significantly impeded in the deepest parts of their burrows in the microplastic treatment compared to control. Similarly *A. filiformis* have shown reduced sediment reworking activity when exposed to North Sea oil drilling cuttings (Trannum, 2017) and in an experiment utilising nylon filaments as seagrass mimics (Valdemarsen et al., 2011), the authors

noted an unexplained high number of inactive *A. marina* than was expected. Reduced burrowing depth is a common stress response of benthic macrofauna to changes in environmental conditions, such as low oxygen environments (Diaz and Rosenberg, 1995), fluctuating salinities (Haider et al., 2018), high temperatures and reduced food availability (Przeslawski et al., 2009). Given that *A. filiformis* can reach densities of >3000 ind. m^{-2} (Josefson, 1995), such changes in faunal behaviour could result in substantial impacts on sediment characteristics and mediated biogeochemistry (Przeslawski et al., 2009; Volkenborn et al., 2007). *A. filiformis* are an active and dominant member of benthic macrofauna and the bioturbating and bioirrigation activities of such key species significantly increases sediment oxygenation around the burrow (Woodley, 1975). These indirect anthropogenic impacts may uncouple species interactions within the seabed. Inhibition of deeper burial activity may reduce the mediated flux of oxygen rich water and nutrients at these depths, enhancing sediment hypoxia and reducing their facilitating effect on macrofauna diversity (Solan et al., 2004).

In this experiment, we used microplastic concentrations of 10,000 fibres kg^{-1}, and whilst these concentrations were an order of magnitude greater than found at our study sites, they remain very much environmentally relevant. For example, Ling et al. (2017) reported a regional average of 3400 microplastics L^{-1} across 42 coastal sites around Australia, with the highest individual concentration reported at 12,500 microplastics L^{-1}, while 6600 microplastics kg^{-1} have been reported for Arctic sediments (Bergmann et al., 2017). At our experimental exposure concentrations, we did not detect any change in oxygen consumption by *A. filiformis* at the end of a 6 hour incubation. This is in keeping with prior studies using *A. marina* which only resulted in significantly greater oxygen consumption and altered bioturbation activity when exposed to high concentrations (10% sediment volume) of polyvinyl chloride (PVC), but no effect was observed for lower doses or for high density polyethylene (HDPE) and 'biodegradable' polylactic acid (PLA; Green et al., 2016). We did however, demonstrate that *A. filiformis* ingested micro-fibres; at the time of dissection, almost half of the brittlestars had fibres in their guts, indicating that these animals do not just passively pass the microplastic downwards with their arms whilst feeding or maintaining their burrows, but also actively through ingestion

and, potentially, egestion. Whilst not quantified here, other experimental studies have reported adverse health effects after exposure to microplastics; *A. marina* suffered a reduction in feeding and energy reserves were depleted by 50% when exposed to PVC for 4 weeks (Wright et al., 2013a), energy that is required for important functions such as reproduction and growth. *Amphiura filiformis* undergo frequent arm regeneration following loss of limbs owing to predatory behaviour of demersal fish and invertebrates (Sköld and Rosenberg, 1996). Regeneration occurs at a rate of 65 to 104 mm yr^{-1} (Salzwedel, 1974), with an adult regenerating an average 22% of their total biomass annually (Loo and Rosenberg, 2003), constituting a substantial proportion of energy allocation. Furthermore, nylon particulates (mass concentration 90g kg^{-1}) significantly reduced reproduction in terrestrial worms (Lahive et al., 2019), illustrating the need for further research to better understand the physiological and ecological implications of microplastic ingestion in key benthic, invertebrate species.

Coastal sediment as a plastic sink

Collectively, data from this two-component study indicates coastal benthic sediments are permanent sinks for microplastic pollution. Microplastic burial was ubiquitous at both spatial and temporal scales, with the fluff layer being a consistent source of microplastic for uptake into the sediment matrix. Microplastic burial was readily apparent from the environmental data, with the highest concentration in the deepest sediment layer. This is in contrast to other studies in other areas of the world (Wang et al., 2019, Martin et al., 2017), but these differences may be related to shallower sedimentary sampling and coarser depth resolution in those studies. However, no large scale bioturbation events were noted in one of those studies (Martin et al., 2017), contrasting with the high biological activity recorded at all sedimentary depths sampled in the present work. This difference highlights that faunal contribution towards the elevated microplastic loading at depth within sediments may thus be especially important in highly biologically active coastal benthic environments, as also noted in previous work (Wang et al., 2019). Previous experimental studies have also demonstrated faunal contribution to plastic sequestration in the laboratory. At high bioturbation rates, *A. marina* removed all microplastics from sediment surface layers (Gebhardt and Forster, 2018) and sediment reworking from the same species led to a downward displacement of nylon filaments promoting

burial (Valdemarsen et al., 2011). Similarly to the present work, sediments in the Northern Baltic Sea have also been proposed to be potentially serving as sinks for microplastics, after an experimental study found plastic fragments buried at a depth of 5 cm were rarely brought back to the surface by common Baltic benthic fauna, the clam *Limecola balthica*, polychaete worm *Marenzelleria* spp. and amphipod *Monoporeia affini* (Näkki et al., 2019).

Physical factors, including weather, hydrodynamics and sediment characteristics, are hugely important in the movement and deposition of particulates in coastal systems. Because hydrodynamics vary considerably between summer and winter, we would expect to see variation in the microplastic loading seasonally, but this was not evident from our data. Large volumes of freshwater input after heavy rain is most prevalent in winter, and whilst not significantly different to the rest of the year, we found the lowest quantity of plastic loading at the Plym in January. Heavy rainfall occurred the week prior to sampling, recording a maximum mean flow in one day of 125 m^3 s^{-1} from records at Gunnislake in the larger River Tamar (UK Environment Agency). The slightly lower plastic loading during this time is an indicator that the plastic particles may be washed out towards the sea during high flow events. This is in keeping with evidence provided from 40 sites across urban, suburban and rural catchment areas in northwest England, which found that flooding over the same time period as our winter sampling, exported 70% of the riverine microplastic loading (Hurley et al., 2018).

If current plastic production continues to increase and global waste infrastructure remains unchanged, an estimated 100–250 million tonnes of plastic waste is projected to enter the oceans annually by 2025 (Jambeck et al., 2015). This being the case, microplastic accumulation in coastal sediments is likely to substantially increase. If, as our results suggest, microplastics are being permanently buried in coastal sediments, due to the lack of any thermal or photo degradation of plastic within sediments (Andrady, 2011), once buried, microplastics could remain with little degradation for millennia, contributing irrevocably to the geological age of the “Anthropocene” (Zalasiewicz et al., 2016).

Conclusion

We have demonstrated for the first time under environmental conditions that microplastics are being buried and sequestered in UK coastal sediments and that benthic faunal activity contributes to this microplastic loading. What has not been so clear is the determination of a causal link, as the interactions between hydrological dynamics, natural sediment variation and faunal activity are complicated, differ between sites, and inherently cannot act in isolation of one another. However, through our targeted experimental study, we have confirmed that conveyor and biodiffuser bioturbators such as *Amphiura filiformis*, play a role in plastic sequestration in sediment. To gain a deeper understanding of the residence times of microplastic in marine sediments, longer term studies encompassing whole communities are needed, with burial and resuspension rates qualified at that scale. There is also currently a paucity of research into the physiological effects of microplastics on benthic animals. Elevated microplastic loading in sediments will invariably increase encounter rates by benthic fauna, posing a heightened potential health risk. In already fragile ecosystems, this additional anthropogenic stressor, set to increase annually, means it is vital to gain a deeper understanding of potential health risks to enable inclusion in multi-stressor evaluations.

Chapter 4: Supplementary Information

Table SI 4.1. All fauna found in environmental samples, listing trait scores for BP_c (Queirós et al., 2013; Solan et al., 2004), BIP_c trait scores (from Renz et al., 2018) and references used to determine BIP_c scores. L_{eff} determined from the environmental position each taxa found.

				BP_c			BIP_c				
Phylum	**Family**	**Genus**	**Species**	R_i	M_i	Ft_i	**Feeding Mode**	FT_i	BT_i	L_{eff}	**Reference** *
Cnidaria	Edwardsiidae	Edwardsia	claparedii	2	2	S	P	1	3	10	1
Cnidaria	Edwardsiidae	Edwardsiella	carnea	2	2	S	SFII	4	0	10	2
Crustacea	Ampeliscidae	Ampelisca	brevicornis	2	1	S	fDF,SFII,SDF	2	2	2	1,2,3
Crustacea	Ampeliscidae	Ampelisca	spinipes	2	1	S	fDF,SFII,SDF	2	2	6	1,2,3
Crustacea	Ampeliscidae	Ampelisca	tenuicornis	2	1	S	fDF,SFII,SDF	2	2	2	1,2,3
Crustacea	Ampeliscidae	Byblis	gaimardi	2	1	S	fDF,SFII,SDF	2	2	2	1,2,3
Crustacea	Amphipoda	-	-	2	1	S	fDF,SFII,SDF	2	2	2	1,2,3
Crustacea	Apseudidae	Apseudopsis	latreillii	2	2	S	P,S	1	1	2	4
Crustacea	Bodotriidae	Bodotria	scorpioides	2	3	S	DF/SDF	2	1	2	5
Crustacea	Caprellidae	Pariambus	typicus	2	2	S	P,S	1	0	2	5,6
Crustacea	Caprellidae	Phtisica	marina	2	2	S	P,S	1	0	2	6

Crustacea	Corophiidae	Leptocheirus	pilosus	2	4	S	fDF/SFII	2	2	2	7
Crustacea	Gnathiidae	Gnathia	oxyuraea	2	3	S	P,S,H	1	0	2	8
Crustacea	Harpacticoida	-	-	2	3	S	DF	2	1	2	1
Crustacea	Isaeidae	-	-	2	3	S	SFII	4	1	6	9
Crustacea	Ostracoda	-	-	2	3	S	S,H	1	0	2	10
Crustacea	Photidae	Photis	sp.	2	1	S	SFII	4	1	2	9
Crustacea	Phoxichilidiidae	Anoplodactylus	sp.	1	4	E	P,S	1	0	2	9
Crustacea	Phoxocephalidae	Metaphoxus	simplex	2	3	S	DF	2	1	2	9,11,12
Crustacea	Porcellanidae	Pisidia	longicornis	1	3	E	SFII	4	0	2	9
Crustacea	Tanaopsidea	Tanaopsis	graciloides	2	2	S	P,S	1	1	2	4
Echinodermata	Amphiuridae	-	-	4	3	B	fDF/SFII	2	3	10	1
Echinodermata	Amphiuridae	Amphipholis	squamata	3	4	B	fDF/SFII	2	3	2	1
Mollusca	Corbulidae	Corbula	gibba	2	2	S	SF(l)	3	1	2	13
Mollusca	Montacutidae	-	-	2	2	S	SFI	3	1	2	1
Mollusca	Montacutidae	Kurtiella	bidentata	2	2	S	DF,SF(l)	2	1	10	13
Mollusca	Montacutinae	Tellimya	ferruginosa	2	2	S	SFI	3	1	10	1

Mollusca	Nuculidae	Nucula	hanleyi	2	3	S	DF	2	1	2	9
Mollusca	Semelidae	Abra	nitida	2	2	S	DF	2	3	6	1
Mollusca	Thyasiridae	Thyasira	sp.	3	2	DC	fDF/SFI	2	3	2	1
Mollusca	Turritellidae	Turritella	communis	4	3	B	fDF/SFI	2	1	2	1
Nematoda	-	-	-	2	2	S	DF	2	1	2	14
Nemertea	-	-	-	4	3	B	P,S	1	1	6	15
Oligochaeta	-	-	-	4	3	B	SDF	5	3	6	1
Phoronida	Phoronidae	Phoronis	sp.	2	1	S	SFII	2	0	10	1
Polychaeta	Ampharetidae	-	-	3	2	UC/DC	DF	2	2	10	13,16
Polychaeta	Ampharetidae	Melinna	palmata	3	1	UC/DC	DF	2	2	6	1,16
Polychaeta	Ampharetinae	Ampharete	lindstroemi	3	2	UC/DC	DF	2	2	6	1,16
Polychaeta	Ampharetinae	Amphicteis	gunneri	3	2	UC/DC	DF	2	2	6	13,16
Polychaeta	Aphroditidae	Aphrodita	aculeata	4	3	B	P,S	1	1	2	16
Polychaeta	Capitellidae	-	-	3	2	UC	SDF	5	3	6	13
Polychaeta	Capitellidae	Heteromastus	filiformis	3	2	UC	SDF	5	3	6	1
Polychaeta	Capitellidae	Mediomastus	fragilis	3	2	UC	SDF	5	2	2	1,9

Polychaeta	Maldanidae	Euclymene	oerstedii	3	1	UC	FF	6	2	6	9,16,19
Polychaeta	Maldanidae	Praxillella	affinis	3	1	UC	FF	6	2	6	9,16,19
Polychaeta	Nephtidae	Nephtys	incisa	4	3	B	P, S	1	1	6	13
Polychaeta	Nephtidae	Nephtys	juv.	4	3	B	P, S	1	1	2	13
Polychaeta	Nephtidae	Nephtys	kersivalensis	4	3	B	P, S	1	1	2	13
Polychaeta	Nephtidae	Nephtys	sp.	4	3	B	P, S	1	1	10	13
Polychaeta	Nephytidae	Nephyts	hombergii	4	3	B	P, S	1	1	6	13
Polychaeta	Pectinariidae	Lagis	koreni	3	1	UC	SDF	5	2	2	13/16
Polychaeta	Pholoidae	Pholoe	baltica	2	2	S	P,S	1	1	2	1,9
Polychaeta	Phyllodocidae	Eulalia	bilineata	4	3	B	P,S	1	0	2	9
Polychaeta	Phyllodocidae	Eumida	sanquinea	4	3	B	P,S	1	0	2	18,16,19
Polychaeta	Polynoidae	Harmothoe	imbricata	4	3	B	P,S,H	1	0	10	9,16
Polychaeta	Polynoidae	Harmothoe	impar	4	3	B	P,S,H	1	0	2	9,16
Polychaeta	Polynoidae	Malmgrenia	arenicolae	4	3	B	S,P,G,DF	1	1	10	1
Polychaeta	Sabellidae	-	-	2	1	S	SFII	2	2	2	16
Polychaeta	Sabellidae	Chone	sp.	2	1	S	fDF/SFII	2	2	2	1,16

Polychaeta	Sabellidae	Dialychone	usticensis	2	1	S	fDF/SFII	2	2	2	1,16
Polychaeta	Scalibregmidae	Scalibregma	inflatum	4	4	B	SDF	5	3	6	1
Polychaeta	Spionidae	-	-	3	2	UC/DC	fDF/SFII	2	0	10	1
Polychaeta	Sternaspidae	Sternapsis	scutata	4	3	B	SDF	5	3	6	18
Polychaeta	Syllidae	Exogone	sp.	4	3	B	S,P	1	0	2	1
Polychaeta	Terebellidae	-	-	3	1	DC	DF	2	2	10	16,19,20
Polychaeta	Terebellidae	Pista	cristata	3	1	DC	DF	2	2	2	16,19,20
Polychaeta	Tubificidae	Tubificoides	sp.	4	3	B	DF	2	3	2	1,9
Sipuncula	Golfingiidae	Golfingia	margaritacea	4	3	B	DF	2	3	6	1
Sipuncula	Golfingiidae	Nephasoma	minutum	4	3	B	SDF	5	3	6	9

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Table SI 4.2. Correction factors applied to all particles < 5 mm isolated from (a) sediment cores and (b) fluff layer after calculating potential exposure risk to samples at all stages of collection and processing.

Location	Stage	No. samples	Mean sample exposure time (h)	Mean filter exposure time (h)	Mean contamination per filter	Mean particles contamination per sample		
(a)								
Boat	Collection	54	0.25	3	0.00	0.00		
Lab 1	Top section sieve	54	0.5	2	0.02	0.00	**Mean contamination**	
Drying Oven	Drying	54	48	48	0.00	0.00	SMI	0.03
Lab 2	Picking	27	0.5	4	0.44	0.05	Picking	0.03
Lab 3	SMI air 36+ hr	27	0.5	36	2.27	0.03	Other	0.00
Lab 1/ Lab 3	SMI air 1hr	27	0.5	1	0.07	0.03	**Sediment Correction Factor**	
Lab 3	Picking	27	0.5	4	0.00	0.00		**0.06**
(b)								
Boat	Collection	18	0.1	3	0.00			
Lab 1	Filtering	18	0.5	1	0.00			
Lab 2	Picking	9	0.5	1	0.00		**Fluff layer Correction Factor**	
Lab 3	Picking	9	0.5	2	0.00			**0.00**

Table SI 4.3. Model outputs and interpretation for environmental data; non-parametric Kruskal Wallis test (Microplastic (MP) abundance in fluff layer), ANOVA (MP abundance at depth) and after model simplification for Linear Models (LM; Response variable; MPs Kg-1, Explanatory variables; Grain size, Community Bioturbation Potential (BPcSum), Community Bioirrigation Potential (BIPcsum), BPc functional guilds (Up and downward conveyors (BPcUCDC), Upward only conveyors (BPcUC), Biodiffusers (BPcBio)) and BIPc functional traits (Tube Dwellers (BIPcTube) and Burrowers (BIPcBurrow)). Separate models were conducted to avoid collinearity.

Model	**Treatment factors**	**Output**	**Interpretation**
Kruskal.test (fluff ~ month, data=PlymTop)	month	H = 2.408, df = 3, *p* = 0.492	No seasonal effect on MP abundance in fluff layer
Kruskal.test (fluff ~ site, data=AllSitesTop)	site	H = 2.5263, df = 2, *p* = 0.283	No effect of site on MP abundance in fluff layer
Model1 <- aov (MPs/Kg ~ depth, data = AllData)	depth - full model output	ANOVA; $F_{51,2}$ = 3.815, *p* = 0.029	Depth is a significant predictor of MP abundance overall
Tukeys post hoc test for Model1	middle-bottom	adj *p* = 0.709	No difference in MP abundance between middle and bottom layers
	top-bottom	adj *p* = 0.026	MP abundance greater in bottom layer compared to top
	top-middle	adj *p* = 0.150	No difference in MP abundance between top and middle layers
Model2 <- aov (MPs/Kg ~ depth, data = AllSites)	depth - full model output	$F_{24,2}$ = 1.641, *p* = 0.215	No significant difference found between sites or depths
Model3 <- aov (MPs/Kg ~ depth, data = Plym)	depth - full model output	$F_{33,2}$ = 3.696, *p* = 0.036	MP abundance at the Plym site differs with depth
Tukeys post hoc test for Model3	bottom-top	adj *p* = 0.041	MP abundance significantly greater in bottom layer compared to top
	middle-top	adj *p* = 0.104	No difference in MP abundance between top and middle layers

	middle-bottom	adj p = 0.901	No difference in MP abundance between middle and bottom layers
Model4 <- lm (MPs/Kg ~ grain, data = AllSites)	full model output	$F_{25,1}$ = 1.144, p = 0.295, R^2_{adj} = 0.005	No relationship between MP abundance and particle size at all sites
Model5 <- lm (MPs/Kg ~ BPcSum, data = AllBottom)	full model output	$F_{16,1}$ = 1.093, p = 0.311, R^2_{adj} = 0.005	No overall effect of community bioturbation potential (BPc) on MP burial
Model6 <- lm (MPs/Kg ~ BIPcSum, data = Allbottom)	full model output	$F_{16,1}$ = 0.376, p = 0.548, R^2_{adj} = -0.038	No overall effect of community bioirrigation potential (BIPc) on MP burial
Model7 <- lm (formula = MPsKg ~ BPcUCDC + BPcUC + BPcBio + site, data = AllBottom-2Obs)	full model output	$F_{10,5}$ = 6.7, p = 0.005, R^2_{adj} = 0.655	Individual functional guilds influence MP abundance in bottom layer
	BPcUCDC effect	Intercept 303.202 + 94.321 (est)	Up+downward conveyor guild positively influences MP abundance in bottom layer
	BPcUC effect	Intercept 303.202 - 555.312 (est)	Upward conveyor guild negatively influences MP abundance in bottom layer
	BPcBio effect	Intercept 303.202 - 30.435 (est)	Biodiffuser guild negatively influences MP abundance in bottom layer
Model8 <- lm (MPsKg ~ BIPcTube + BIPcBurrow + site, data = AllBottom)	No significant predictors for BIPc functional traits found during model simplification, therefore nothing to report		

Chapter 5:
General discussion

Microplastics are a pervasive marine pollutant, acknowledged globally as a cause for concern owing to the risk they pose to marine life and ecosystems. In this thesis, I set out to understand how microplastics are transported from “top to bottom”, tracking their passage through the water column, and into marine sediments, and determine the roles biota may play in the transport, burial and permanent sequestration of plastics. My aim was also to extend current knowledge on the impacts that microplastics have on the health and functioning of coastal marine biota and ecosystems.

Transfer through the water column

Just prior to starting my PhD, suggestions were emerging of a mismatch between the expected microplastic abundance in surface waters from modelled predictions (Cózar et al., 2014; Eriksen et al., 2014) and those being reported. It wasn’t clear whether those mismatches were as a result of sampling constraints, ie; the difficulties in sampling very small size fractions in surface waters (such as nets clogging with biological material; Song et al., 2014), differing methodologies between studies, or whether the missing fraction was sinking out into the ocean interior and seabed. Resolving the question of this missing plastic was deemed a research priority (Cózar et al., 2014; Law and Thompson, 2014). At the same time, deep sea sediments were proposed as a final sink for microplastics (Woodall et al., 2014), reporting microplastic abundances up to four orders of magnitude higher in deep ocean seamounts and sediments than in surface waters. Relatively recent thinking has suggested that biota may be a vector for microplastic transport, presenting another cause of mismatch between the types and concentrations of microplastics used in experiments and those measured in the marine environment. Early microplastic experiments used polystyrene spherical beads, as this was all that was commercially available, coupled with concentrations far higher than those reported in the natural environment as, at that time, there was very little knowledge of actual plastic abundances. I was motivated to investigate biotic driven vertical microplastic transport, using the types of plastic more commonly found in the marine

environment, such as fibres and irregularly shaped fragments, combined with a variety of polymer types at more realistic concentrations (Burns and Boxall, 2018). Reports were emerging in both experimental (Cole et al., 2013) and environmental (Desforges et al., 2015) studies, of pelagic biota ingesting microplastics, and observations of polymers that are predominantly buoyant in seawater being found in ocean sediments (Bergmann et al., 2017; Ling et al., 2017). Collectively, this prompted the question of whether biota had any influence over the transport of microplastics through the water column and into the seabed.

In Chapter 2 (Coppock et al., 2019), I demonstrate through experimental exposures that microplastics are readily encapsulated in copepod faeces, facilitating movement through the water column. Zooplankton faecal pellets, along with the faeces of other pelagic biota, play an important role in the biological pump, which transfers fixed carbon out of photic waters to the deep sea and seabed (De La Rocha and Passow, 2007; Turner, 2015). Specifically, my results demonstrate that microplastics of varying density can alter the sinking rate of faecal pellets of the widely distributed *Calanus helgolandicus*. Changes to the vertical flux of this organic matter could potentially impact on carbon and nutrient deposits in the water column or seabed. A prior study demonstrated that polystyrene spheres caused a 2.25 fold reduction in the sinking rate of faecal pellets (Cole et al., 2016). I built upon this study and used plastic polymers of different densities and forms that are commonly found in the marine environment (eg; fibres and irregular shaped fragments) to investigate their influence on the sinking rates of *C. helgolandicus* faecal pellets. Polyethylene (PE), a low density (0.91 g/cm^3) polymer and the most commonly manufactured polymer globally (Plastics Europe 2015), significantly reduced the sinking rates of contaminated faecal pellets by 27% compare to controls, whilst polyethylene terephthalate (PET), a high density polymer (1.38 g/cm^3) and the main constituent of single use plastic bottles, significantly increased sinking rates by 23%. Whilst it is unknown how significant this might be in the natural environment, impacts are most likely to occur in productive, coastal waters where interactions between biota and microplastic sources are most prevalent (Clark et al., 2016). For example, regions of coastal upwelling play a crucial role in oceanic nutrient transport and support highly productive fisheries, such as off the continental shelf in the South China Seas (Jing et al., 2009). The densely populated coasts of China are home to 263 million

people and 1.3 - 3.5 million tonnes of plastic marine debris was estimated to enter the seas off China as a result of mismanaged waste in 2010 (Jambeck et al., 2015). Biotic/microplastic interactions here are likely to play a role in the downward flux of microplastics from surface waters, potentially impacting carbon transport and sequestration and promoting plastic ingestion by marine biota, such as filter feeding zooplankton (Vroom et al., 2017) and mussels (Porter et al., 2018). Rivers are a prominent source of plastic emission into the world's oceans and the top 20 most polluting rivers account for more than two thirds of annual global input (Lebreton et al., 2017). Asian rivers contribute an estimated 86% of marine plastic waste, with high population densities, episodes of heavy rainfall and poor waste management all contributory factors. My findings help elucidate the mechanisms governing the fate and movement of microplastics through the water column to the seabed. The contribution of zooplankton faecal pellets to vertical particulate organic carbon (POC) export flux is highly variable, both spatially and temporally (Turner, 2015). In the Northeast Pacific, the proportion of zooplankton faecal pellets to total POC export ranged between 3.3—47.7% (Wilson et al., 2013). If we consider the clearance rates observed in Chapter 2 of approximately 50 mL per copepod per day, with a microplastic concentration of 9,200 m^{-3} reported in the Pacific Ocean (Desforges et al., 2014), 0.46 microplastics would be ingested per copepod per day. However, in the natural environment clearance rates can be much higher than this; for example, in the Celtic Sea *C. helgolandicus* showed clearance rates of between 626—1347 mL per copepod per day when feeding upon ciliates (Fileman et al., 2007). At these higher clearance rates, we can postulate that 5.8—12.4 microplastics may be ingested per copepod per day in the Northeast Pacific. An average of 14.4 faecal pellets are produced by *C. helgolandicus* daily (Jansen et al., 2006), therefore each faecal pellet may contain between 0.4—0.9 microplastics, constituting a potentially major pathway for microplastic vertical flux in the ocean. Future research should seek to establish whether faecal pellets are a substantial route of microplastic transport in natural settings, potentially through deployment of sediment traps in a range of pelagic environments. The transfer of microplastics to the benthos is likely to differ between different water bodies and most experimental studies, including those conducted here, have quantified faecal sinking rates in still water. Shallow, dynamic coastal waters may be more likely to transport microplastic laterally or be

more prone to re-suspension, whereas deep, slow moving water bodies may lend themselves to more constant rates of microplastic sedimentation.

Method development

Advancement of sedimentary microplastic abundances has been hindered in part by methodological techniques. Early in my PhD, I conducted a wide literature search to find the most appropriate and effective way of extracting microplastics from the fine sediment samples collected from my study sites (Chapter 4). Some of the drawbacks associated with existing methods included expense (Imhof et al., 2012), complicated or convoluted methodology (Nuelle et al., 2014), incompatibility with fine sediments (Claessens et al., 2013) or inefficient at extracting the plastics, requiring multiple repetitions for each sample (see Table 3.1 in Chapter 3). Therefore, the method developed in Chapter 3 was not planned at the start of the PhD, but evolved out of necessity. Based on my literature search, I trialled several methods but found them inadequate for efficiently extracting microplastics from my fine, silty sediments. The final method I developed resulted in the Sediment-Microplastic Isolation (SMI) unit (Figure 5.1).

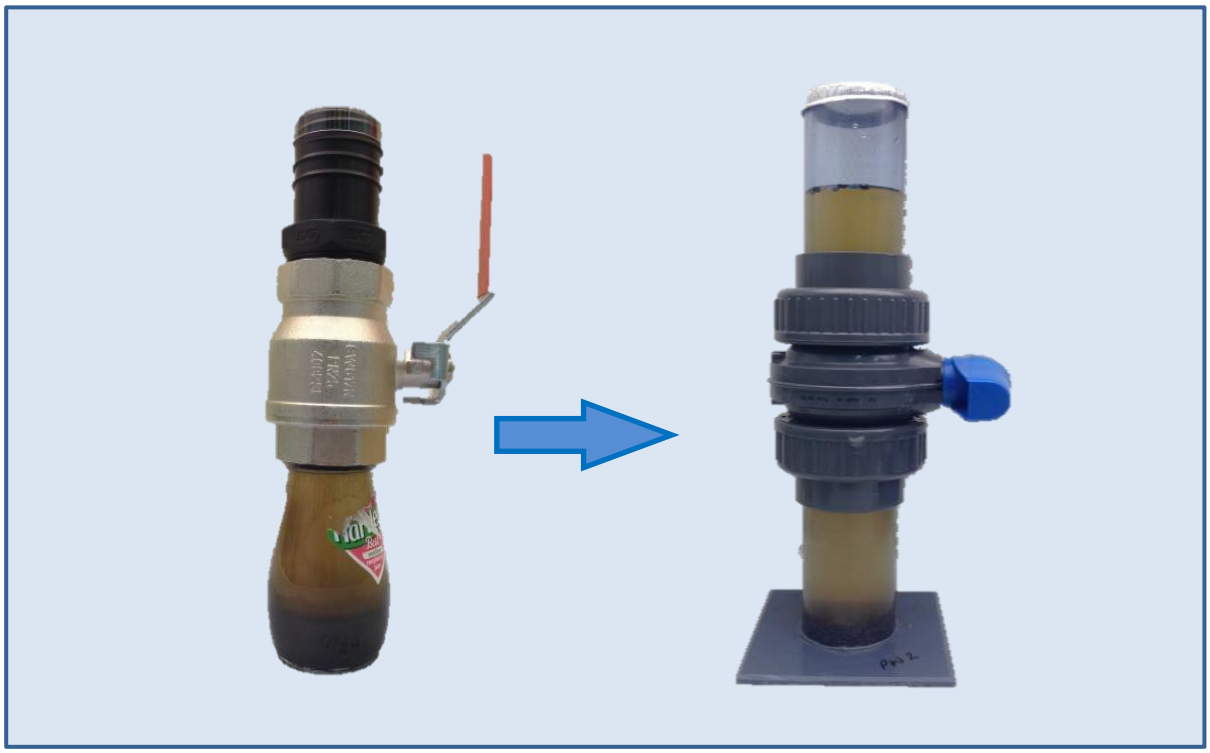

Figure 5.1. Sediment-Microplastic Isolation (SMI) unit, from first prototype to finished design.

The SMI unit proved to be excellent in extracting microplastics from fine sediments and coarse sands alike, with a 96% success rate in a single step. The unit is cheap to produce, enabling its use with most budgets and thereby promoting increased compatibility between studies. Whilst constructing the unit from plastic (PVC) isn't ideal, it is 10 times cheaper than stainless steel, with the benefits being that the unit can be used by many research facilities, including those with limited funds. Potential self-contamination from the units can be controlled by including spectra in the FT-IR/Raman library database. The method, coupled with a "do-it-yourself" instruction guide was published in 2017 (Coppock et al., 2017) and has received global attention (Figure 5.2). I am aware (from personal communications) that the SMI units are being used for monitoring purposes by the Environment Agencies in Norway, the US and also the UK, highlighting the potential for this design to gain a much better understanding of the types and amount of microplastic polluting coastal systems. Regular monitoring at predicted hotspots may highlight potential sources and therefore work towards identifying and eliminating the source. I have personally received a high volume of correspondence and interest regarding this method; an SMI unit was made for the BBC for their Blue Planet Live series and the method is also advocated on an online hub for citizen science by Arizona State University (Scistarter.org). It has been particularly

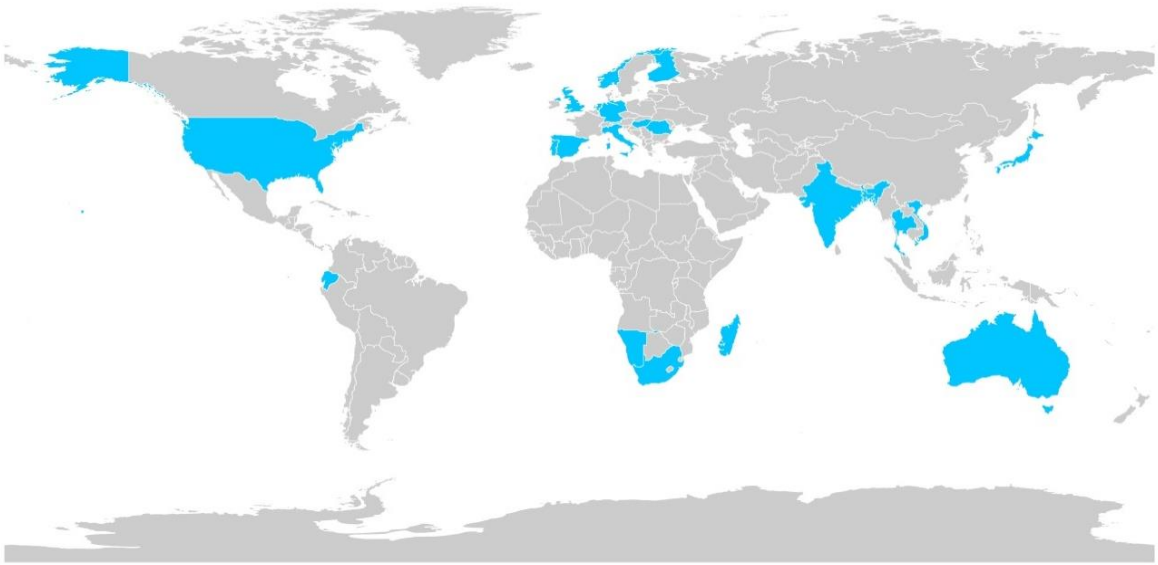

Figure 5.2. World map indicating known countries (coloured blue) that have used SMI units to extract microplastics from marine, fresh water or terrestrial environments, for research or monitoring (map produced by Dr. Sarah Nelms).

gratifying to receive emails from other researchers who are using the method; notable is a German researcher who had built an 'Army' of 12 SMI units (Figure 5.3) to investigate the effects of soil erosion on microplastic deposits and another from a researcher in Madagascar who wholeheartedly thanked me for publishing a method that they could use with their very limited budget. As a result of this method development, I have contributed to the harmonisation of the estimation of sedimentary plastic concentrations globally, which will accelerate our understanding of the prevalence of sediments as a sink for plastic.

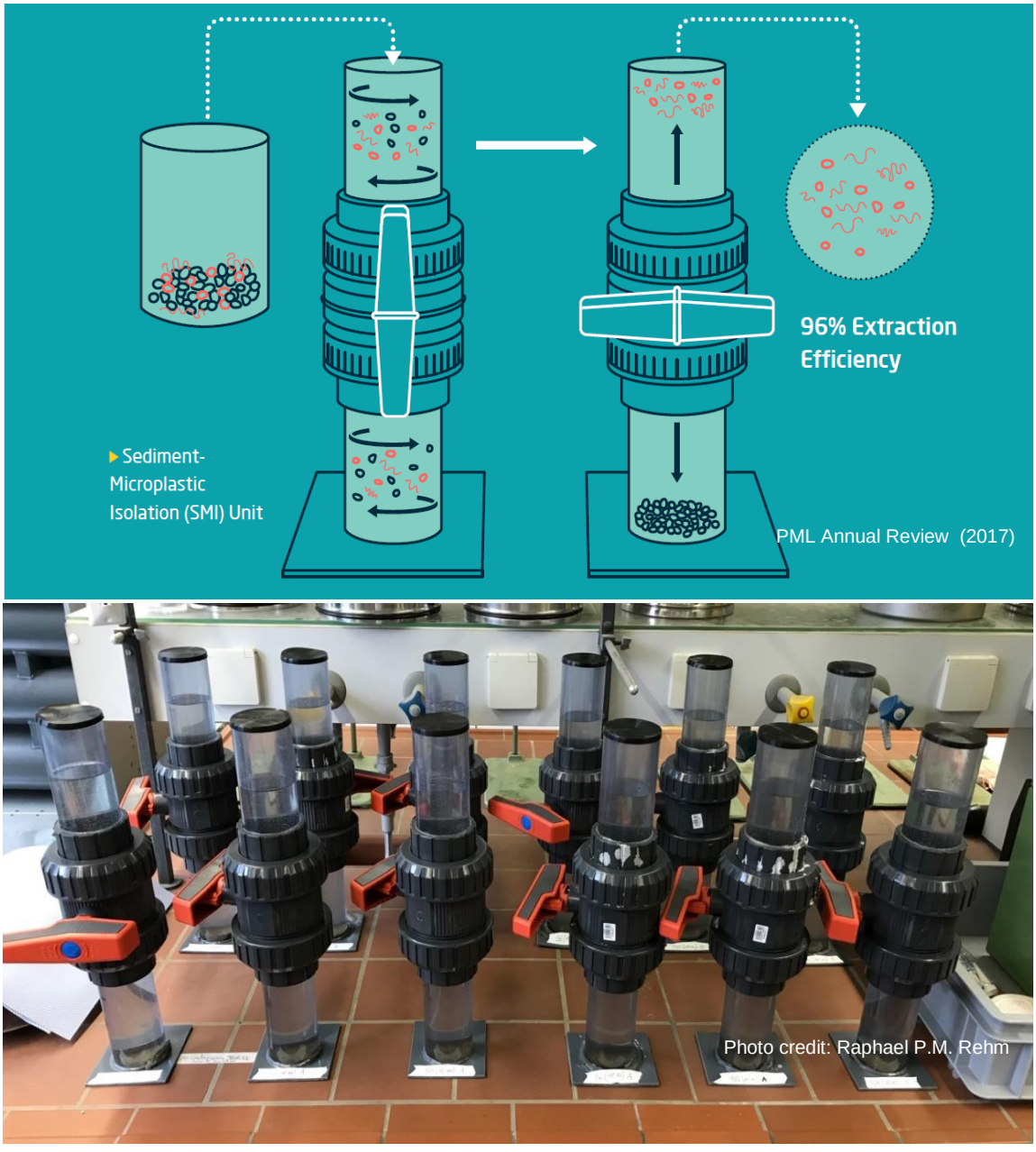

Figure 5.3. (a) Graphical demonstration of the SMI unit process and results and (b) an 'Army' of SMI units at the University of Augsburg, Germany investigating microplastic behaviour in soil erosion and run-off.

Microplastic burial in marine sediments

Whilst there is now a growing body of evidence reporting microplastic accumulation in marine sediment, there is little information pertaining to microplastic burial. To my knowledge, only one other study has investigated the vertical profile of microplastics in a marine system, finding that plastic pollution on Belgian beaches reflected the global increase in plastic production (Claessens et al., 2011). If we are to understand the impacts of microplastic pollution on marine biota and ecosystems, we need to better understand where the plastic is accumulating and identify potential permanent stores. In Chapter 4, I found that microplastics were present in the fluff layer; the overlying water adjacent to the sediment surface where organic and inorganic detritus accumulates (Queirós et al., 2019). The fluff layer was found to be a consistent source of microplastics to the seabed, highlighting for the first time in natural conditions that this is a reservoir of microplastics persistent across the sites I sampled and throughout the year, and thus indicating that this may be a finding common to wider regions of the coastal ocean. I further demonstrated for the first time in sub-tidal, coastal marine sediments that not only is microplastic being buried, but that sediment dwelling fauna also play a major role, identifying mechanisms behind this. My data suggest that microplastic distribution in sediment can be influenced by the bioturbation (sedimentary particle mixing and pore water flux exchanges (i.e. bioirrigation) mediated by burrowing fauna (Kristensen et al., 2012)) activities of infaunal organisms, determined using a functional biodiversity classification. Whilst I was unable to detect any contribution to microplastic burial when investigating the effect of the whole community bioturbation potential (BP_c index; Queirós et al., 2013; Solan et al., 2004), when further exploring specific mechanisms within the community assemblage I identified a number of relationships explaining microplastic abundance in the deepest layer (6-10 cm). I have demonstrated that fauna which randomly mix sediment through burrowing activities (“biodiffusers”) and those employing a strict upward conveying of particulates had a negative effect on microplastic abundance at depth in the sediment, whereas fauna conveying particles both upwards and downwards had a positive effect. These results are in line with the wider understanding of the effect of fauna on sedimentary particle movement (Kristensen et al., 2012; Kristensen and Kostka, 2004).

The flushing and oxygenation of burrows via bioirrigation results in potentially high volumes of water being pumped into the sediment (Kristensen and Kostka, 2004), drawing in water and particulates from the bottom water and fluff layer. In view of this, I would expect dominant contributors to community bioirrigation potential (BIP_c index; Renz et al., 2018) to be an important determinant of microplastic uptake into sediments. It was therefore surprising not to find any relationships between microplastic burial and bioirrigation parameters in my environmental data, highlighting the difficulty in detecting trends in such heterogeneous environments. Whilst technically challenging to study faunal traits in isolation, studies specifically targeting mechanisms associated with bioirrigation, such as faunal burrow ventilation, may reveal a relationship between bioirrigation and microplastic uptake into sediments. To further explore the role of benthic fauna in the burial of microplastic, I conducted a targeted experiment under controlled conditions using a key benthic species, *Amphiura filiformis*. My results confirmed that the normal bioturbation activities of *A. filiformis* contribute to the burial of microfibres, following the same depth profile as particulate burial from the surface. Longer term experimental exposures are likely to capture potential toxicity effects of microplastic contamination and their impacts on benthic-pelagic coupling. Through further experimentation of faunal mediated microplastic movement in sediments, a trait-based index could be formulated to quantify and predict the Plastic Burial Potential (PBP_c) of a community assemblage. This may then be used in combination with other parameters, such as the level of risk of microplastic input (eg. near waste water outlets), microplastic abundance and habitat type (eg. ecosystem modifiers such as mussel beds) to facilitate microplastic impact assessments of benthic communities.

Impacts of microplastic exposure on marine life and ecosystems

Microplastic ingestion has been documented in a wide range of taxa and experimental studies have reported negative effects in lower trophic organisms such as reduced food intake, reproductive output and energy reserves (Cole et al., 2015; Sussarellu et al., 2016; Wright et al., 2013a). During my research, I investigated impacts of microplastics on two key, marine species and their respective ecosystems.

In Chapter 2, I sought to further understand the interaction between microplastics and a dominant member of marine zooplankton communities, the copepod *Calanus helgolandicus*. Calanoid copepods are highly abundant, important members of marine trophic webs, at times constituting up to 90% biomass of the total zooplankton community (Bonnet et al., 2005). They provide both a food source for higher level organisms and a link between carbon fixed at the sea surface by phytoplankton and its export out of photic waters (Turner, 2015). In an experimental study, I found that the shape or size of the microplastic in the ambient system influences algal prey selection by the copepods. When exposed to nylon microfibres, copepods ingested less chain forming diatoms that resembled the fibres and similarly, when exposed to nylon fragments, ingested less of the unicellular algae that was similar in size and shape to the fragments. From my results, it was not possible to determine whether it was the size or shape of the plastic that was most influential in copepod prey selection, however it was clear that the presence of plastic was a significant factor; similar results were found in an experiment using the boreal copepod, *Calanus finmarchicus* (Cole et al., 2019), a study on which I was 2nd author. Another recent study found that size was more important than shape when exposing planktivores and fish to PET fragments versus spheres (Lehtiniemi et al., 2018). However, further studies may reveal different results if fibres are included, as their dimensions are very different to either fragments or spheres. In my study, I found that fibres, rather than fragments, significantly reduced copepod feeding and as fibres are the most prevalent type of microplastic reported from the marine environment (Burns and Boxall, 2018), they are therefore the most likely type of microplastic that zooplankton will encounter. We know from previous studies that in addition to reduced feeding, microplastic ingestion by copepods can impair fecundity and energy (Cole et al., 2015) available to carry out different life history traits, including the timing of developmental stages such as moulting (Cole et al., 2019). Copepods have previously been documented exhibiting selective feeding behaviours; a subtle downwards shift in algal cell size preference was detected when exposed to polystyrene (PS) microplastics (Cole et al., 2015); nutritious phytoplankton cells were selected over PS beads (Fernández, 1979) and live food was preferentially selected over detritus (Paffenhöfer and Sant, 1985). It is possible that the copepods in my study were attempting to avoid eating the plastic. This

is a very interesting concept and further studies should seek to understand the mechanisms involved in this change in prey preference and to further assess potential impacts on these keystone species.

At current reported microplastic abundances, impacts to whole ecosystems are unlikely. However, the projected increase of marine litter into the ocean will only serve to elevate microplastic concentrations, adding to whole ocean plastic loading (Eriksen et al., 2014; Jambeck et al., 2015), with plastic continuing to fragment into smaller and smaller pieces in surface waters where mechanical and UV degradation predominantly occurs (Andrady, 2011). Currently, technological hindrances prevent capture, detection and identification of the very small microplastic fraction (< 10 µm) and we therefore simply do not know how much of this size class is in the environment. It is becoming apparent however, that microplastic abundance increases with decreasing size (Enders et al., 2015; Lenz et al., 2016), therefore this unreported size fraction, coupled with future plastic estimates, is likely to increase encounter rates with low trophic animals such as copepods, potentially resulting in population decline. Such a decline may result in a trophic cascade shift, with potential impacts to higher trophic organisms that rely on energy rich copepods as a food source. As well as impacts to pelagic organisms, microplastic contamination is also likely to have significant impacts on benthic organisms and processes.

In Chapter 4, I used nylon microfibres at environmentally relevant concentrations (Bergmann et al., 2017; Ling et al., 2017) to explore their effect on the normal bioturbation behaviour and respiration rate of a key benthic species, the brittlestar *Amphiura filiformis*. I found that the brittlestars had significantly reduced activity at the lower reaches of their burrows compared to the control. I also found that *A. filiformis* ingested the microfibres, which were present in the guts of 48% of intact individuals. Whilst exposure to the fibres at my experimental concentrations did not adversely affect respiration rates after a 6 hour incubation period, the fact that fibres were being ingested suggests that there may be potential impacts that were not tested for, or that longer term exposures may uncover. For example, it has been documented that microplastic ingestion can cause deleterious effects in other key benthic organisms. Exposing the lugworm, *Arenicola marina* to sediment containing polyvinyl chloride (PVC) at 5% by weight resulted in reduced feeding, energy

reserves and bioturbation activity (Wright et al., 2013a), whilst polyethylene microplastics resulted in a shift in ecosystem functioning when exposed to the oyster, *Ostrea edulis* (Green et al., 2017). Furthermore, in an experiment utilising nylon filaments as seagrass mimics, the authors noted an unexplained higher number of inactive *A. marina* than was expected (Valdemarsen et al., 2011); it could therefore be hypothesised that this inactivity was due to the presence of the nylon. A reduction in the normal bioturbation activities of benthic fauna as a result of contaminated sediments could lead to a shift in benthic-pelagic exchanges and ecosystem function (Snelgrove et al., 2018). Longer term experimental exposures are likely to capture potential toxicity effects of microplastic contamination and their impacts on benthic-pelagic coupling.

To predict long term consequences of microplastic exposure on key benthic species and communities, it is important to conduct further experiments using potential future scenario concentrations in sediments. Whole community studies are needed to better understand their role in microplastic accumulation and re-suspension in sediments. Depending on the dominant functional type within a community, which can vary both spatially and temporally, microplastics may either get locked away deep in the sediments or become re-suspended back into the water column. Further studies investigating the rates of microplastic burial and resuspension are needed to commence and validate environmental modelling computations, thus enabling a better understanding of the extent and rate at which faunal mediated benthic-pelagic coupling may contribute to microplastic loadings or resuspension. If the overall net effect leads to permanent microplastic burial, as indicated by my results, the plastics are likely to form part of the strata and remain buried, potentially for millennia (Andrady, 2011), in the geological age of the “Anthropocene” (Zalasiewicz et al., 2016).

Plastic waste entering the marine environment is predicted to rise by an order of magnitude in 2025 from an estimated 4-12 million tonnes per annum in 2010 (Jambeck et al., 2015). Continual fragmentation will increase microplastic abundance and as such, understanding and mitigating the long term consequences on the health of individuals, populations and ecosystems must be a research priority to both aid targeted solutions and inform policy decisions.

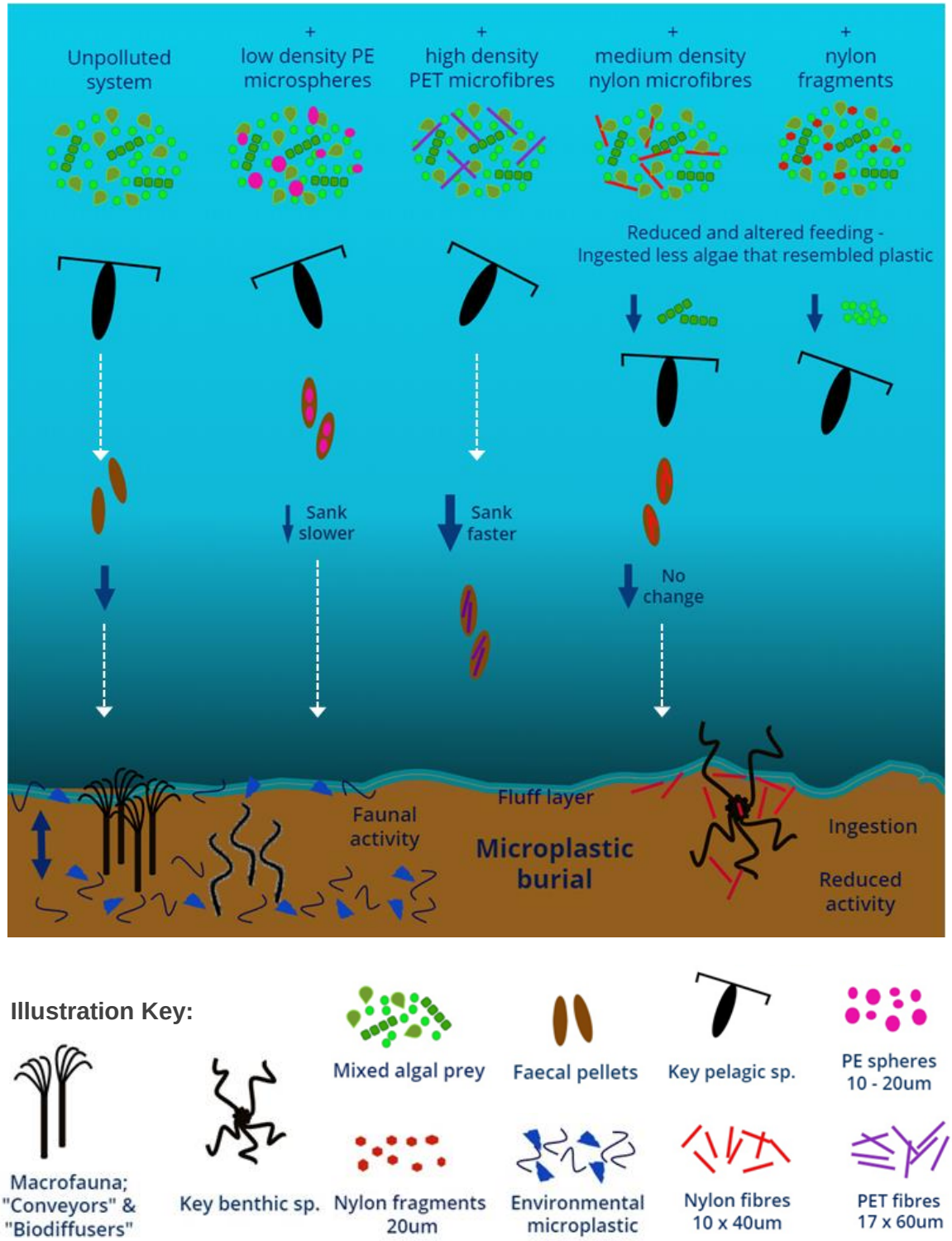

Figure 5.4. Illustration summarising main findings from my research; different polymers altered sinking rates of copepod faecal pellets (PE 27% slower, PET 23% faster). Nylon had no effect on sinking rate but shape influenced copepod prey selection (ate less algae resembling plastic). Microplastics are buried in coastal sediments and burrowing macrofauna contribute via benthic-pelagic coupling processes, the rates of which are modified by plastic exposure.

In addition to other environmental stressors such as hypoxia, ocean acidification and loss of biodiversity, microplastic pollution may tip the balance of species tolerances and ultimately, alter the benefits that humans derive from marine ecosystems (Cardinale et al., 2012) such as climate regulation, storing and cycling of nutrients, atmospheric composition and shoreline protection (see Snelgrove et al., 2014).

Recommendations for further work

As a result of the findings from this thesis (Figure 5.4), recommendations for further work are presented in the following research questions;

From Chapter 2:

- Are copepods altering their choice of algal prey in an attempt to avoid eating plastic?
- Would we see the same results if the plastic were weathered/biofouled?
- What is the mechanism leading to the altered prey selection?
- What are the implications on individual fitness and ecosystem functioning in both pelagic and benthic realms as a result of microfibre (as opposed to microplastic sphere) ingestion, considering both current and future microplastic abundances?
- Is there evidence of contaminated faecal pellets in the natural environment? Does this vary between water bodies?
- How does water flow influence faunal mediated microplastic distribution in the water column?

From Chapter 3:

- Can regular monitoring of benthic sediments at predicted accumulation zones help understand the drivers of microplastic accumulation?
- Is it possible to pinpoint the sources of microplastics into sediments?
- Is it possible to formulate and effectively disseminate standardised methodologies and practices in microplastic research?

From Chapter 4:

- What are the rates of burial and resuspension of microplastics in benthic sediments?
- How much of the burial/resuspension is due to faunal activity?
- Do some animals have much more influence over microplastic burial than others?
- What are the key faunal characteristics to predict burial? Can these be formulated into a metric; eg. community Plastic Burial Potential (PBP_c)?
- Can data be collected for computational modelling to predict which areas or community assemblages (eg. based on known communities and biotopes) might be more prone to high microplastic accumulation?

I hope that through my research, I have made a significant contribution to our understanding of the movement of microplastic through the water column and in sediments, and identified risks posed to the health and functioning of key marine invertebrate species. The results from my work in Chapter 2 may contribute to the development of a flux model, providing robust data to validate models seeking to understand the vertical movement of microplastics in the ocean. The development in Chapter 3 of a method to extract microplastic from sediments (SMI units) has been adopted globally and promotes harmonisation of sedimentary microplastic estimates between studies, which will accelerate our understanding of microplastic prevalence in benthic habitats. In Chapter 4, I showed that microplastic burial occurs in coastal sediments and that individual invertebrate functional traits may contribute to this burial. It is clear from my research that both pelagic and benthic fauna are inexorably linked with the movement and fate of microplastic in the marine environment and the onus is upon us to understand and mitigate the risks posed to marine life and ecosystems.

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Appendix 1:

CHAPTER 11

Microplastics in Marine Food Webs

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11.1 INTRODUCTION

A multitude of food webs exist in the world's oceans, are made up of a wide variety of organisms that occupy distinct niches, and possess different behavioral and feeding strategies. So far, only a small fraction of these taxa have been included in studies concerning microplastic debris in marine ecosystems. Microplastics (microscopic plastic debris, 100 nm to 5 mm diameter) are now widely recognized as a pollutant of international concern (Galgani et al., 2013; GESAMP, 2016). Understanding the potential impacts this prolific contaminant can have on marine life and food webs has become of intense interest, with an exponential increase in research being conducted in recent years. In this chapter, we explore how microplastics enter marine food webs and consider the complex, iterative relationship between microplastics, biota, and biologically mediated ecological processes. Microplastic ingestion has been documented in animals throughout the marine food web, including zooplankton (Desforges et al., 2014), fish (Bellas et al., 2016; Lusher et al., 2013), marine mammals (Lusher et al., 2015a; Bravo Rebolledo et al., 2013), turtles (Nelms et al., 2016), and seabirds (Tourinho et al., 2010). We explore the factors affecting microplastic consumption and infiltration into marine food webs, with consideration given to spatial overlap, predator-plastic ratios, the properties of microplastic debris, and the life history and feeding strategies of biota demonstrated to consume plastic. At the individual level, microplastics pose a risk to the health of the organism; indeed, a growing number of experimental studies have demonstrated that at critical concentrations, microplastics can adversely affect feeding, energetic reserves, reproduction, growth, and survival in invertebrate and vertebrate species, including calanoid copepods (Cole et al., 2015; Lee et al., 2013), polychaete worms (Wright et al., 2013b; Green et al., 2016), fish (Rochman et al., 2015), and oysters (Sussarellu et al., 2016). The latest evidence suggests that microplastics could also affect higher levels of biological organization, with population shifts and altered behavior impacting upon the ecological function of keystone species (Galloway et al., 2017). While the risks microplastics pose to individual biota are explored in greater detail in other chapters of this book, here, we focus on how plastics have the potential to affect food webs and marine

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ecosystems as a whole. Furthermore, we consider how trophic interactions and ecological processes can change the microplastics themselves.

11.2 THE OVERLAP BETWEEN PLASTICS AND BIOTA

Perhaps the most important variable affecting the flux of microplastic particles into marine food webs is their abundance and distribution in the environment and physical overlap with biota.

11.2.1 Geographical Overlap

In recent years, there has been a concerted effort to identify the different habitats polluted with plastic debris and ascertain the concentrations of microplastics across a wide range of aquatic ecosystems. Microplastics are ubiquitous in the world's oceans, and their presence in remote locations, including the Arctic (Lusher et al., 2015b), Antarctic (Waller et al., 2017), mid-oceanic atolls (Do Sul et al., 2014), and oceanic depths (Woodall et al., 2014), have highlighted their widespread distribution. However, accurately determining the concentrations and type of microplastics present in seawater and sediments has proved a challenge. Adaptations to traditional sampling techniques (e.g., trawls and sediment grabs; see review by Hidalgo-Ruz et al., 2012) have proved invaluable for collecting samples; however, isolating and identifying microplastics have required a more novel approach (see Box 11.1). In recent years, a wide range of methodologies have been

BOX 11.1 Methodological Approach

Although microplastics are a relatively new topic in the environmental sciences, researchers have been able to learn from the experimental approaches and understanding gleaned from the fields of ecotoxicology, marine biology, and aquatic chemistry. Basic mechanisms of feeding and energy transfer in marine food webs are well understood, and this knowledge has been useful in understanding observed interactions between microplastics and biota. Lessons learnt from nanoparticle research have been of particular relevance to microplastic exposure studies, particularly with respect to uptake mechanisms and mechanisms underpinning observed health effects and developing sound ecological risk assessment (Syberg et al., 2015; Hüffer et al., 2017). In contrast, collecting field data on the distribution and quantity of microplastics in different ecological compartments (water surface, water column, seafloor habitats, and strandline) has turned out to be a significant challenge, requiring novel approaches, method development, and optimization (Hidalgo-Ruz et al., 2012; Lusher et al., 2017). An ongoing issue facing microplastic researchers is the absence of harmonized sampling or sample analysis protocols, and a forward challenge for the field is to work toward methodological standardization.

suggested for extracting and analyzing plastics (see reviews by Lusher et al., 2017; Miller et al., 2017); however, the variety of methods employed can often result in incomparable datasets. Analyzing such data is further confounded by the heterogeneous distribution and temporal variability in microplastic concentrations.

Global sampling efforts have helped to identify "hot spots" of plastic (Eriksen et al., 2014; Cózar et al., 2015; Van Sebille et al., 2015). For example, the North Pacific, South Pacific, and North Atlantic subtropical oceanic gyres, which amass flotsam from throughout the oceanic basins, have all been highlighted as accumulation zones for microplastic debris (Moore et al., 2001; Law et al., 2010; Eriksen et al., 2013). Oceanic gyres are largely oligotrophic and therefore relatively devoid of marine life; however, for biota that can survive in the gyres, interactions with microplastic will be commonplace. For example, in the North Pacific gyre, Moore et al. (2001) observed a 6:1 plastic-to-plankton ratio, and Goldstein and Goodwin (2013) identified that 33% of gooseneck barnacles (*Lepas* spp.) had consumed between 1 and 30 items of microplastic. However, our understanding of the numbers and distribution patterns of microplastics in marine environments is far from complete. This was pointed out already in the study dataset of >330 μm particles from surface water tows, which showed smallest particles to be most prevalent, but only down to a certain size group (1 mm) after which the concentrations decreased (Cozar et al., 2015). This absence of smaller plastic may result from difficulties in identifying very small particles or might be explained by biotic or abiotic degradation or movement of these plastics.

Enclosed and semienclosed seas like the Mediterranean Sea and the Baltic Sea have also been noted for their high microplastic concentrations (Collignon et al., 2012; Setälä et al., 2016b; Gewert et al., 2017) and thus have been proposed to accumulate plastic debris in greater amounts than open oceans (Fossi et al., 2016). As increasing concentrations inevitably increase the exposure of organisms at the base of the food webs, this may be the case also at higher trophic levels. In the Mediterranean Sea, stomach analyses from large pelagic predators (swordfish and tuna) revealed that 18.5% of the fish examined contained microplastics. The reported concentrations of microplastics from the surface waters of another highly polluted semienclosed sea basin, the Baltic Sea, show how the microplastic concentrations in surface waters may significantly differ spatially (Setälä et al., 2016b; Gewert et al., 2017) and may reach high concentrations (up to $4.7 \times 105\ km^{-2}$) close to highly populated urban areas with low water exchange, or as was found by Gorokhova (2015), in deep water layers separated by a halocline. In the Baltic Sea, the field observations of microplastics in the food web have mainly related to fish, herring being the most studied fish species. Bråte et al. (2017) analyzed the data from various studies on microplastics in fish from these Nordic waters; in the analyzed dataset consisting of 1425 individuals of Atlantic and Baltic herring, microplastic ingestion varied between 0% and 30%. Ogonowski et al. (2017) reported that approximately 50% of herring individuals had ingested plastics along the Swedish coast in the Baltic Sea,

although the numbers of microplastics on individual fish were low (0–1 per fish), reflecting great variability between samples. In comparison, very low numbers of particulate microplastics (fibers were excluded) were also found in a recent study containing over 500 herring individuals from the open sea areas of the northern Baltic Sea (Budimir et al., 2018). The reported share of herring with ingested microplastic particles varies greatly between these studies and may at least partly be explained by spatial differences in the overlap of microplastics and herring. Differences in methods used for extracting microplastics from fish tissue makes comparisons between studies difficult and conclusions vague.

A recent study predicts the greatest overlap between microplastics and marine life will occur in coastal regions (Clark et al., 2016). Coastal waters and estuaries have relatively high biological productivity owing to their shallow, protected waters and fresh nutritional inputs from rivers, which are valued by aquaculture and fisheries, and encompass important nursery grounds for commercially exploited marine taxa. It is postulated that their proximity to sources of anthropogenic pollution (e.g., maritime industry, urban areas, and riverine inputs) puts them at high risk of microplastic pollution. Microplastic sampling in coastal regions is problematic owing to the density of organic material in these waters (Cole et al., 2014); nevertheless, recent studies have highlighted the overlap between plastics and biota in coastal waters. In the English Channel, a 36.5% incidence of microplastic ingestion in demersal and pelagic fish species has been observed (Lusher et al., 2013), while 70% of brown shrimps (*Crangon crangon*) sampled from the coastlines of European countries along the English Channel have been shown to consume microplastic (Devriese et al., 2015). More recently, Steer et al. (2017) identified the ratio of microplastics to fish larvae ranged from 27:1 nearest Plymouth (United Kingdom) to 1:1 35 km from the shoreline.

11.2.2 Habitats

Microplastics consist of a wide range of polymers which have their own special characteristics that affect their distribution in the water, and thereby which organisms and habitats are prone to plastic exposure. Local wind conditions, water currents, and geomorphology all affect the distribution of microplastics in water and their spatial accumulation (Barnes et al., 2009). The vast amounts of anthropogenic debris washing up on beaches across the globe (Browne et al., 2011) provide visual evidence of the efficiency with which floating plastic debris can be transported on the sea surface. Approximately half of marine plastic debris is initially buoyant (e.g., polystyrene, polyethylene, and polypropylene), while denser plastic (e.g., polyvinylchloride and nylon) readily sinks in seawater. As observed from numerous sampling campaigns, microplastics can permeate throughout the water column, with plastic and microplastic debris, including low-density polymer plastic, widely evident in benthic ecosystems (Miller et al., 2017).

Laboratory exposures have been used to demonstrate that biotic interactions including biofouling (Fazey and Ryan, 2016; Kaiser et al., 2017), egestion (Cole et al., 2013, 2016), and bioturbation (Näkki et al., 2017), as well as physical processes such as fragmentation (Andrady, 2017), can affect the properties and movement of plastics; it is hypothesized that these processes could result in changes to the distribution of microplastics within marine ecosystems where biota and plastics overlap (Fig. 11.1; Clark et al., 2016). In these waters, we might expect a downward flux of plastic debris, resulting in an accumulation of microplastics on the seafloor (Barnes et al., 2009; Woodall et al., 2014). However, it is important to recognize that vertical flux should be considered a redistribution of plastics, and not a "removal" mechanism. Benthic ecosystems can be highly biologically productive habitats, supporting a diverse array of life that play vital roles in the oceanic carbon pump (Turner, 2015), reef formation (Beck et al., 2011), and bioturbation (Cadée, 1976). Environmental sampling has identified plastic pollution in every benthic habitat investigated, including highly remote areas such as both Arctic (Bergmann et al., 2017) and Antarctic (Munari et al., 2017) polar regions and the deep sea (Woodall et al., 2014; Bergmann et al., 2017). Plastic concentrations in sediments are highly variable, due in part to different sampling and extraction methodologies and also to the natural heterogeneity of sediments. Concentrations of up to 6600 microplastics per

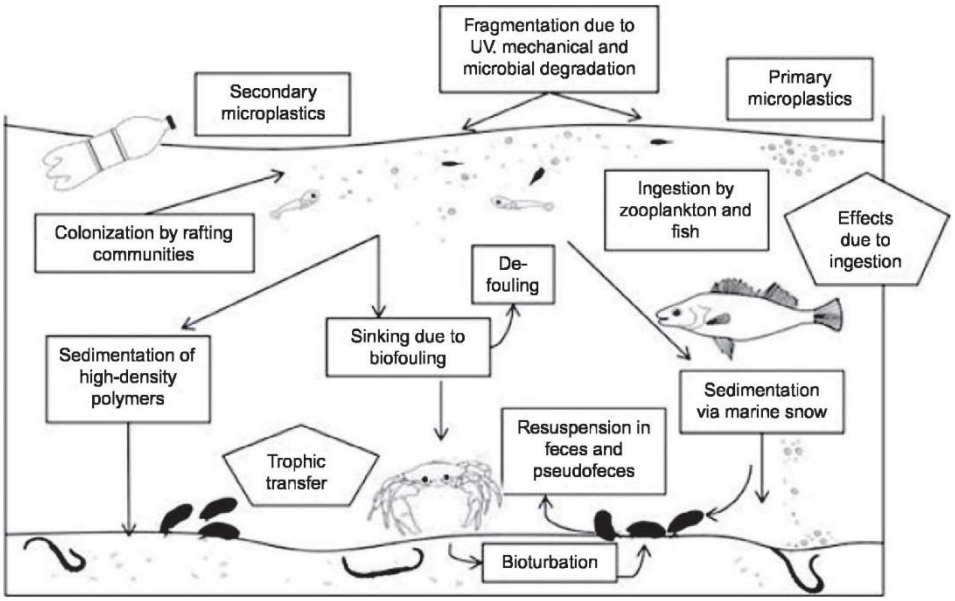

Fig. 11.1 Potential pathways for the transport of microplastics and its biological interactions. *Courtesy of Wright, S.L., Thompson, R.C., Galloway, T.S., 2013a. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.*

kg have been reported in Arctic sediments (Bergmann et al., 2017), and in a study of 42 sites around the Australian coastline (Ling et al., 2017), a regional average of 3400 microplastics per L was reported, with the highest individual sample yielding 12,500 plastics per L. Laboratory exposures have shown that benthic invertebrates readily consume plastic, and this can have a detrimental impact on their health and functionality. A reduction in energy reserves (Wright et al., 2013a), reproduction (Sussarellu et al., 2016), metabolism, and bioturbation activity (Green et al., 2016) has been reported in benthic organisms, with potential impacts to ecosystem functioning (Volkenborn et al., 2007).

11.3 ENCOUNTERING AND DETECTION OF MICROPLASTICS

Compared with the dynamic interactions between a predator/grazer and their natural prey, the relationships between an animal and microplastic are somewhat simplified. The feeding mode and life history of an organism will affect both its encounter and ingestion rate of microplastic. Organisms may actively select microplastics from the environment in search of prey, or they may ingest them accidentally while feeding on food particles or animals that contain plastic.

11.3.1 A Passive Particle

Microplastics are passive: freely floating on the water surface, suspended or slowly sinking in the water column, or deposited on or within the seabed. Encounter rate (i.e., the commonality with which a predator comes into contact with its prey) is a crucial factor affecting the ingestion rate of that prey (e.g., Evans, 1989). Primarily, encounter rate is influenced by the relative abundance of predator/grazer and prey; for microplastic ingestion to occur, there would need to be a significant spatial overlap between biota and plastic and a substantial amount of plastic present for a likely encounter to occur.

Classic work on feeding efficiencies has shown how changes in prey density affect the ingestion rates of predators. Ingestion increases with an increasing prey density up to a saturation point, whereby the predator cannot process more prey even though the prey density still increases, as described by Solomon (1949) and Holling (1959). This has also been shown in laboratory studies with virgin microplastics and various invertebrate taxa: the more particles the organisms were offered, the more they were ingested, even when working with the relatively high concentrations used in laboratory settings (Fig. 11.2) (e.g., Cole et al., 2013; Setälä et al., 2016a). Gelatinous organisms (e.g., jellyfish and ctenophores) may feed without reaching a saturation level. This means that even in very high concentrations of prey, they continue capturing them but start to egest/vomit prey that they are unable to process. However, it has been observed that jellyfish ingested relatively low numbers of microplastics compared with other filter feeders (e.g., copepods)

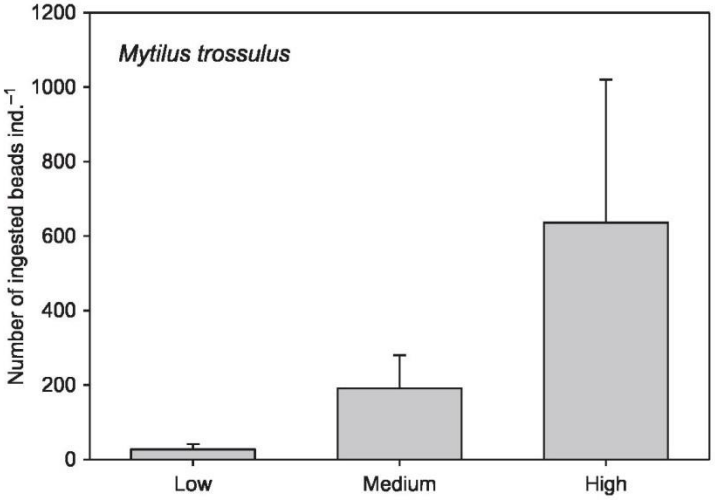

Fig. 11.2 The number of ingested 10 μm spheres (mean ± SD) in *blue* mussel (*Mytilus trossulus*) at three different bead concentrations (low, 5; medium, 50; and high, 250 beads mL^{-1}). *Data from Setälä, O., Norkko, J., Lehtiniemi, M., 2016. Feeding type affects microplastic ingestion in a coastal invertebrate community. Mar. Pollut. Bull. 102(1), 95–101.*

in the South China Sea (Sun et al., 2017). The classical Holling-type ingestion patterns may also be affected by clogging of feeding appendages. In such cases, a high concentration of microplastics (fibers) may decrease feeding activity, resulting in lower ingestion rates.

Active, motile predators (e.g., cruising predators) will encounter prey, and we therefore assume plastic, more readily as they move through the water or sediment. Nonmotile animals will encounter microplastics the same way they come into contact with suspended or deposited prey (i.e., water currents bringing particles close enough for capture or generating localized currents to draw suspended particles to the organism). Sessile organisms are also not able to avoid exposure to microplastics and are subjected to all particles present in the suspension they are feeding in. However, passively floating and sessile organisms and ambush predators can compensate for reduced encounter rates through high efficient filtering activity (Green et al., 2003).

11.3.2 Detecting Microplastics

Animals detect prey using visual or chemical cues or hydromechanical signals when identifying motile prey moving through the water. Organisms relying upon visual detection may mistake microplastics as prey. For example, ocean-foraging Fulmars travel vast distances across the North Atlantic, relying on visual cues to select prey floating near the ocean surface; dissections of Fulmars beached along European coastlines have routinely

identified that the seabirds' stomachs are full of plastic (Van Franeker et al., 2011). Researchers often note that microplastic debris comes in a wide range of shapes, size, and color; however, it is currently unclear whether these attributes have any influence on its likelihood of being consumed by animals relying on visual detection.

The swimming activity and speed of motile prey affect their encounter rate, with numerous studies establishing that actively moving prey are detected more frequently and encountered more often (Gerritsen and Strickler, 1977; Gerritsen, 1984; Tiselius et al., 1993). As microplastics are passive particles, they cannot be detected using hydromechanical signals, and we would therefore expect them to be encountered less frequently than motile prey at similar concentrations. For example, in pelagic communities, the swimming activity of the predator is affecting the encounter rate of microplastic particles in addition to their density and overall distribution. However, as plastic particles are nonmotile, they make easy targets for predators and may therefore be ingested (if not actively rejected) more readily than natural prey that can incite escape responses (e.g., Green et al., 2003) and may require an active capturing process.

Chemical cues play a significant but variable role in the prey selection of marine organisms from invertebrates to mammals. For example, fish have diversely developed olfactory organs (Hara, 1975) for detecting signals related to reproduction and feeding. Some marine species possess highly developed chemosensory organs (e.g., sharks), while in some others, they may be poorly developed (e.g., visual predators like pike) (Hara, 1975). Crustaceans, such as copepods are generally considered to be selective feeders that display flexibility in their feeding behavior (Koehl and Stickler, 1981); discrimination between prey can be based on size (Frost, 1972), motility (Atkinson, 1995), or chemical signals (Cowles et al., 1988). Not all chemicals are sensed; what is important is that in order for an organism to receive chemical stimuli, the chemical itself should be soluble in water. Chemical signals can assist in the selection for high-quality food, determined by protein content (Cowles et al., 1988), or be used to avoid unsuitable prey (e.g., harmful algae containing toxic compounds like saxitoxin). However, active avoidance of unsuitable or toxic prey by copepods is most likely a result of a common history, that is, coevolution of the prey and predator (Colin and Dam, 2002).

Field-collected data and exposure experiments show that plastic particles floating in the water and embedded in the sediment are rapidly colonized by rich microbial communities comprising prokaryotic and eukaryotic organisms, like bacteria and algae (Oberbeckmann et al., 2014; Harrison et al., 2014). So far, there is very little information on how the formation of biofilm actually affects the ingestion of microplastics. Recent studies show that the effects of biofouling are most likely taxon- or even species-specific. Vroom et al. (2017) identified that biofouling of polystyrene beads promoted ingestion by planktonic crustaceans, although this was somewhat dependent on taxon, size, and stage of the grazers. For two of the three copepod species studied (*Acartia longiremis* and *Calanus finmarchicus*, excluding the adult females of the latter), it was shown that

BOX 11.2 Experimental Work

Most of the information that has so far been produced on the parameters affecting microplastic ingestion by marine organisms come from simplified laboratory experiments. Results from experimental work should not be directly applied to natural conditions where confounding factors exist. When conducting environmentally relevant experimental work on ingestion and effects of microplastics in food webs, the concentration, size, and type of the used particles should be adjusted to correspond to natural conditions. At the moment, there is still a mismatch between "reality" and laboratory experiments. So far, most experiments are run with microplastic concentrations higher than those commonly found in the environment and with virgin particles of uniform size and shape that fail to accurately represent the conditions in the field (Phuong et al., 2016). This inconsistency is likely to influence our understanding of the marine microplastic problem as Ogonowski et al. (2016) showed in laboratory experiments comparing the effects of primary and secondary microplastics. They showed that secondary microplastics have more negative effects on feeding in a cladoceran, *Daphnia magna*, compared with primary microplastics commonly used in the previous studies. The reason why experimental laboratory studies have not used microplastic concentrations commonly observed in marine environment is not only their "low" concentrations but also the uncertainty in assessing their concentrations. Microplastic concentrations found in marine environments vary significantly between areas and habitats but seem to be low when compared with the numbers of the real prey, which makes environmentally relevant exposure studies difficult. Long-lasting exposure experiments in mesocosms mimicking natural conditions would be needed to more accurately assess the relationships between microplastics and their potential predators.

in most cases, the fouled microplastics were ingested by more individuals and at higher rates than the unfouled plastics. However, one copepod species, *Pseudocalanus* spp., did not ingest any of the microplastic particles offered. Contradictory results were reported by Allen et al. (2017) who studied the ingestion of weathered, fouled, and unfouled pre-production pellets (polystyrene (PS), low-density polyethylene (LDPE), and high-density polyethylene (HDPE)), by a scleractinian coral species known to use chemosensory cues for feeding. Their results showed that the corals ingested different types of plastics, consuming significantly more unfouled than fouled microplastics that were taken up (Box 11.2).

11.4 INTO THE FOOD WEBS

The ingestion, entanglement, or inhalation of microplastic by marine organisms can be viewed as an entry point into marine food webs. Owing to their small size, microplastics are bioavailable to a wide range of marine organisms and can be both selectively and accidentally ingested (Schuyler et al., 2012). The ingestion of microplastic particles is affected

by their concentration, size, shape, distribution, and chemical character (i.e., density and chemical signal) and the animal's feeding habits. In animals with developed organs for prey detection, plastic polymers may thus not be selected, or they may be rejected if they are recognized as being unfavorable or if a more preferable prey is available.

11.4.1 Filter Feeding

Filter-feeding organisms are prevalent throughout marine food webs, from small planktonic invertebrates and benthic taxa to megafauna, where they feed on suspended organic material, such as algae, zooplankton, fish larvae, and detritus. The size range of particles that can be ingested by a grazer depends on the feeding mode (e.g., filter feeding or raptorial), gape size, and specific feeding mechanisms of the grazer/predator. For filter feeders, the actual size limits for the ingested prey are set by the structure and function of the filtering apparatus used for trapping particles from the suspension (Riisgård and Larsen, 2010). Filtering devices in suspension-feeding organisms are not simple sieves that mechanically clean the water from suspended particles. The structures of filtering apparatus found in unicellular, invertebrate, or vertebrate organisms differ greatly, both between and among taxa, with varying levels of adaptability and sensory capability. Particle capture depends on particle type (e.g., shape, size, and density), particle concentration, water viscosity, the quantity of water that is filtered, and filtering efficiency. Besides direct contact, the capturing mechanisms may also involve other factors, such as chemo- and mechanoreception (Riisgård and Larsen, 2010). Moreover, experimentally measured clearance rates of plankton have been found to vary also depending on temperature, salinity, and the type of prey that has been offered (e.g., Kiørboe et al., 1982; Garrido et al., 2013). Daily clearance rates of marine invertebrates can vary from microliters (unicellular organisms, like ciliates) to milliliters (copepods), liters (bivalves), hundreds of liters (gelatinous zooplankton), or more (baleen whales).

Two parameters are commonly used to estimate the efficiency and outcome of filter feeding: ingestion and clearance rate. The ingestion rate denotes the number of prey particles ingested per predator in a time unit. Ingestion rate can be experimentally estimated directly, through observations of ingested prey particles inside the organism, or indirectly, as the disappearance of prey from the experimental media over time. In the past, inert plastic particles (spheres) have been used as surrogates for natural prey to estimate feeding parameters in planktonic organisms (Huntley et al., 1983; Borsheim, 1984; Nygaard et al., 1988). These historical studies with *Calanus* and related copepod genera have demonstrated a preference for algae over polystyrene beads, alongside size selectivity (Fernandez, 1979; Donaghay and Small, 1979; Huntley et al., 1983). However, observations for such preferences do not necessarily hold for all developmental stages, which further complicates things, that is, when exposure studies are being conducted. Clearance rate is a derivative of ingestion rate and is calculated by dividing the latter by prey

concentration. The clearance rate thus measures the water volume that an individual organism can clear of food particles in a time unit. To understand the probability of any suspended particle to be ingested by a filter-feeding organism, both the clearance rate and the concentration of suitable prey should be taken into account.

From the viewpoint of a small filter-feeding organism under natural conditions, microplastic concentrations may be too low for routinely encountering a plastic particle. However, in waters containing high concentrations of microplastics, the situation is different even for a small organism with a relatively low clearance rate and efficiency, such as a copepod. As an example, the experimentally defined daily clearance rates of common copepods may vary between ~10 and <200 mL (Frost, 1975; Engström et al., 2000; Setälä et al., 2009). In theory, a copepod feeding, for example, with a high clearance rate of 144 mL/day (Frost, 1975), at a concentration of 9200 plastics per m^3 as has been observed from the Pacific Ocean (Desforges et al., 2014), a single microplastic would be ingested by every 0.7 copepods, assuming all particles are edible and the animals are solely undertaking passive ingestion without rejection of plastic. Assessments based on animals collected from the field have also confirmed the role of zooplankton as entry points for microplastics to food webs. The study of Desforges et al. (2015) which was based on the analysis of the number of ingested microplastics from subsurface-collected zooplankton and the overall distribution of these species from the Northeast Pacific Ocean, identified encounter of microplastics by zooplankton as 1 particle per every 34 copepods and 1 particle per every 17 euphausiid. The authors further estimated that both the juvenile salmon and adult returning fish would be affected daily with ingested microplastics through their zooplankton prey.

Invertebrates with a capacity for filtering larger quantities of water and with a longer life span (e.g., bivalves) or large filter feeders (such as whales) may encounter microplastics far more frequently than zooplankton. Bivalves are one of the key organisms when entry points of microplastics to marine food webs are assessed. They are efficient suspension-feeding animals that form links between the pelagic and benthic ecosystems and are a key source of prey for many marine fish, birds, and mammals. In the Baltic Sea, it has been assessed that within 1 year, the blue mussel beds would, in theory, filter a water volume equivalent to the whole sea basin (Kautsky and Kautsky, 2000). The numbers of microplastics found in bivalves vary significantly ranging from <0.5 particles (Eastern Atlantic and Baltic Sea) to over 100 particles (Western Atlantic) per animal (Mathalon and Hill, 2014; Vandermeersch et al., 2015; Railo, 2017). Exposure of large filter feeders to microplastics has been shown by Fossi et al. (2014) after examining concentrations of phthalates and organochlorine compounds of a basking shark and a baleen whale. The authors concluded that microlitter is ingested by these large filter feeders together with their neustonic prey. A comparative study carried out in two semienclosed basins, the Mediterranean Sea and the Sea of Cortez in the Gulf of California (Fossi et al., 2016), gives supporting information indicating that fin whales in highly polluted areas are

BOX 11.3 Microplastics, an Issue of Size

"Microplastic" is typically used to describe plastic particles smaller than 5 mm in diameter, with a lower size limit of 100 nm; plastics larger than 5 mm are considered "macroplastics," while plastics smaller than 100 nm in size are termed "nanoplastic" (Cole et al., 2011). Using these size classifications, the largest microplastic particles (5000 μm) have a diameter 50,000 times larger than the smallest microplastic (0.1 μm). Moreover, when we consider volume and surface area, these differences become even more apparent. Imagine a spherical shaped microplastic particle, like the ones used in experimental studies, or the plastic microbeads commonly used in exfoliating personal care products: a 5 mm-diameter bead is 1.25×10^{14} times greater in volume and 2.50×10^{9} larger by area than a 100 nm-diameter bead. Of course, most of the weathered microplastic particles that are found in the marine environment are not uniform in shape, with fibrous, planar, and irregularly shaped plastic being most prevalent. Nevertheless, differences in a particle's dimensions will have a significant impact on the risk they pose to marine life. For example, microplastics of different sizes may differ in their behavior under marine conditions (i.e., buoyancy), biological availability, and capacity to incite biological effects. Furthermore, the larger surface-area-to-volume ratios associated with smaller particles greatly increase the plastic's capacity for adsorbing (and potentially desorbing) waterborne pollutants (e.g., persistent organic pollutants and hydrophobic organic contaminants) (Koelmans et al., 2016), up to 1 million times greater than that found in the surrounding seawater (Mato et al., 2001).

exposed to major health hazards due to microplastics and their cocontaminants. Considering the vast amounts of water these animals filter (5893 m^3 day^{-1}; Fossi et al., 2014), this conclusion is more than relevant (Box 11.3).

11.4.2 Respiratory Intake

Ventilation has also been identified in exposure experiments as a means by which microplastics can be concentrated from the surrounding water. Watt et al. (2014) identified that the shore crab (*Carcinus maenas*) was able to respire polystyrene microbeads, which accumulated on the surface of their gills. Blue mussels (*Mytilus trossulus*) and Baltic clams (*Macoma balthica*) have also been shown to accumulate microplastic particles to their gills after 24 h incubations; however, the bead concentrations were much higher in the digestive tracts of the same animals (Setälä et al., 2016a).

11.4.3 Entanglement

Numerous organisms have been shown to entangle with fibers or larger plastics (e.g., Laist, 1997; Cole et al., 2013; NOAA, 2014; Taylor et al., 2016). They may be found in the swimming or feeding appendages of invertebrates and in the valve gapes of bivalves or entangled

around larger animals. Entanglement with fibers in field-collected animals has been observed even in remote areas such as the deep seas, where fibers were found on sea pens and hermit crabs (Taylor et al., 2016). When these organisms are eaten by higher trophic level predators, the plastics adhered to external surfaces of the organisms will be eaten as well.

11.4.4 Trophic Transfer

Once ingested, microplastics will be either egested or retained by the organism. If a predator consumes an organism that has retained microplastic, the predator will be indirectly consuming this plastic as part of its diet, in a process referred to as "trophic transfer." The trophic transfer of plastic has been documented in predatory Norway lobsters (*Nephrops norvegicus*) that consumed polypropylene rope fibers embedded in fish (Murray and Cowie, 2011), shore crabs (*C. maenas*) that indirectly ingested fluorescent polystyrene 0.5 and 10 μm microspheres present in common mussels (*M. edulis*) (Farrell and Nelson, 2013; Watt et al., 2014), mysid shrimps (*Neomysis integer*) that consumed fluorescent polystyrene 10 μm spheres previously taken up by mesozooplankton (Setälä et al., 2014), and fish (*Gasterosteus aculeatus*) that consumed an insect larvae containing microbeads in a mesocosm experiment (Lehtiniemi and Setälä, unpublished). The trophic transfer of microplastics and associated POPs from *Artemia* nauplii to zebra fish (*Danio rerio*) was also verified in a laboratory experiment (Batel et al., 2016), and microplastic debris found in fecal pellets of predatory seabirds (great skuas, *Stercorarius skua*) was greatest when correlated with the remains of surface-feeding Northern fulmars (*Fulmarus glacialis*) (Hammer et al., 2016).

For trophic transfer to occur, microplastic must be consumed alongside the prey. This includes plastic adhered to algae (Bhattacharya et al., 2010; Gutow et al., 2015) or the external surfaces of an animal (e.g., entrapped in the setae of a copepods' appendages; Cole et al., 2013) or retained indefinitely within the organism itself. Plastics are commonly observed in the intestinal tract of marine animals, including seabirds (Van Franeker and Law, 2015), fish (Lusher et al., 2013), invertebrates (Murray and Cowie, 2011), and turtles (Nelms et al., 2016); this occurs where larger plastics or coalesced polymeric fibers cause a gut blockage, preventing the plastic from being shifted via peristaltic action. In the common shore crab (*C. maenas*), polystyrene microspheres have been observed to lodge between the microvilli that line the stomach, resulting in prolonged gut retention times. In copepods, starvation has been observed to increase gut retention times, with 10 μm polystyrene microspheres remaining in the intestinal tracts of *C. helgolandicus* for up to 7 days, far exceeding the typical gut passage times of just 2 h (Cole et al., 2013). In the common mussel (*M. edulis*), 3.0–9.6 μm polystyrene microspheres have been demonstrated to translocate into the circulatory fluid (hemolymph), where they can remain for in excess of 48 days (Browne et al., 2008; von Moos et al., 2012). Owing to their small size, nanoplastics (<100 nm diameter) have the capacity to cross epithelia and therefore have the capacity to enter tissues and circulatory fluids, for example, in dendritic cells that transport small particles (e.g., bacteria) across gut epithelial cell walls (Rescigno et al., 2001). Microplastic transfer has also

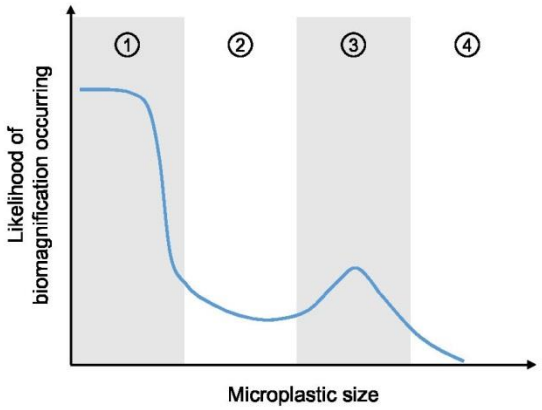

Fig. 11.3 Considering how microplastic size might influence the probability of biomagnification of plastics occurring in a food chain. (1) Very small (i.e., nano) plastics are readily absorbed by the gut and are retained within the circulatory fluid and/or tissues; (2) moderately sized plastics are ingested, are present within the organism during gut transit, and are then readily egested; (3) larger and fibrous plastics are ingested but, owing to their size, remain in the intestinal tract; (4) the largest microplastics are inedible to organisms at the base of the food chain.

been documented in a top marine predator, where the presence of microplastics in captive gray seal scats was attributed to trophic transfer from the wild-caught mackerel they were fed upon (Nelms et al., 2018).

In numerous aquatic ecosystems, persistent chemical pollutants (i.e., PCBs, PAHs, and methyl mercury) have been shown to biomagnify as they pass up the food chain (reviewed by Blais et al., 2007). The increasing body burdens of such pollutants in higher trophic organisms arise from the hydrophobicity of these chemicals, resulting in their accumulation within fatty tissues of prey species. So far, there have been no quantitative measures of microplastics passing up the food chain, and it therefore remains unclear whether plastics will biomagnify in marine food webs. Biomagnification will largely depend upon the transience of plastics in an organism, with biomagnification only occurring where plastics are readily ingested and retained. Retention of plastics can be influenced by food availability (Cole et al., 2013; Watt et al., 2014) and shape (Murray and Cowie, 2011) but will be predominantly governed by the size of the plastic (Galloway, 2015). In Fig. 11.3, we predict how the size of a plastic particle is likely to relate to the probability of that microplastic biomagnifying up the food chain.

11.5 ALTERATION, REPACKAGING AND TRANSPORT OF MICROPLASTICS WITHIN MARINE FOOD WEBS

In this section, we consider how marine organisms, trophic dynamics, and biologically mediated ecological processes can alter the fate of a microplastic and highlight how microplastics might impinge on biota, food webs, and marine ecosystems.

11.5.1 Biological Transport of Microplastic

Microplastics consumed, respired, or adhered by an organism will be subject to passive, biologically mediated transportation, with both vertical and lateral movement to be expected across a variety of habitats (e.g., water column and sediments). The distances by which microplastics can be transported via a biological vector will largely depend on the movement, migratory routes, and gut transit times of the individual organism (Fig. 11.4).

Diel vertical migrations, a synchronous daily migration of a wide range of taxa, have been highlighted as a potential route by which microplastics could be transported from the sea surface to deeper waters (Cole et al., 2016; Clark et al., 2016). Organisms may ingest plastics while feeding at the surface at night, which can then be egested hundreds of meters below the surface. For example, a large (2–3 mm) copepod swimming at speeds of between 30 and 90 m h^{-1} (Enright, 1977), with a gut evacuation time of approximately 2 h (Cole et al., 2013), could vertically transport microplastic to depths of 60–180 m. Lusher et al. (2016) identified that 11% of mesopelagic fish caught in the Northeast Atlantic had microplastics in their digestive tracts, and although it was unknown at what depth these plastics were consumed, the majority of the species identified undergo diel vertical migration and follow their zooplankton prey to the surface to feed; it is therefore plausible to suggest that ingestion of the microplastics may have occurred at the surface while feeding and egested at depth.

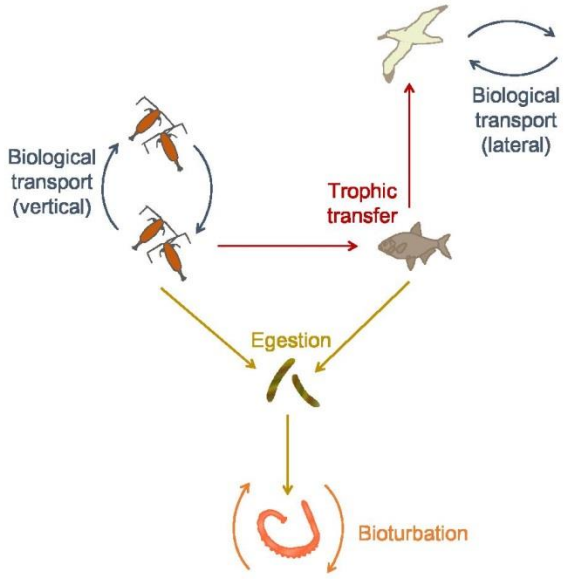

Fig. 11.4 How biota transport microplastics within marine ecosystems. *Image by Matthew Cole (original content).*

The geographic distribution of marine plastic has largely been considered from a physical perspective, with abiotic processes (i.e., wind, rivers, and oceanic currents) expected to be the dominant factors in distributing this pervasive pollutant (Sherman and Van Sebille, 2016). We consider that migratory species could also facilitate the transport of plastics. Migratory species have been widely demonstrated to play a vital role in the long-range transport of persistent pollutants (e.g., PCBs, DDT, and methyl mercury; Blais et al., 2007). For example, migratory fish (e.g., trout and salmon) have been shown to accumulate persistent organochlorines in their tissues while feeding in marine habitats, which are released in their eggs during spawning at otherwise pristine freshwater sites (Krümmel et al., 2003; Mu et al., 2004). Numerous migratory species, including turtles (Nelms et al., 2016), ocean-foraging seabirds (Van Franeker and Law, 2015), and cetaceans (Lusher et al., 2015a), are routinely sampled with plastics in their intestinal tracts. These animals undertake large-scale annual migrations; for example, the gray whale (*Eschrichtius robustus*) travels 6000 km annually from the coast of Mexico to the Chukchi Sea, and the Arctic tern (*Sterna paradisaea*) migrates 19,000 km from Greenland to the Antarctic each year (Alerstam et al., 2003). The egestion of plastic within feces, scat, or guano; the regurgitation of plastics by seabirds when feeding their young (Sileo et al., 1990); or the death of the animal will all contribute to the deposition of plastic in terrestrial, freshwater, or marine habitats far from the waters where such plastic was ingested.

11.5.2 Incorporation of Microplastics Into Biological Matrices

Within the marine environment, microplastics are rapidly colonized by "biofilms," made up of microorganisms, plants, and epibionts that attach and grow on substrates. The characteristics of the biofilm that forms on a plastic will be influenced by the polymer and the biological or ecological matrix through which it has passed; as such, the microbial complex that forms on the surface of plastics may act as a tracer of the journey of a microplastic within marine compartments (Galloway et al., 2017). The development of a biofilm can change the characteristics of the plastic polymer, for example, by increasing their mass (Lobelle and Cunliffe, 2011; Zettler et al., 2013; Rummel et al., 2017) and altering their chemical signal (see Section 11.3.2). It has been postulated that biofilm formation could be enough to cause otherwise buoyant plastics to sink or oscillate within the water column, depending on the size and density of the plastic (Ye and Andrady, 1991; Kooi et al., 2017).

In bivalves, feeding or rejection of particles that are suspended in the water is the outcome of passive and active selection. The size of the particles that may be ingested depends on the filtration apparatus of the particular species. In Pacific oyster (*Crassostrea gigas*) larvae, uptake of polystyrene microbeads was size-dependent, with microplastics larger than the oral groove unable to be ingested, while smaller plastics were readily consumed (Cole and Galloway, 2015). If the size is right and prey is directed to the

specialized feeding organs (ctenidium), it may still be rejected as pseudofeces if considered unpalatable. Studies made with blue mussels have shown that the identification of unsuitable particles and their sorting in suspension-feeding bivalves take place in the lectin-containing mucus that covers feeding organs, where interaction with carbohydrates from suspension takes place (Espinosa et al., 2010). Mussels (*M. edulis*) have been visualized rejecting nanopolystyrene (Ward and Koch, 2009) and microplastic polyvinylchloride in their pseudofeces (personal observations of authors). The fate of microplastics incorporated into pseudofeces remains unclear.

Ingested microplastics will typically be passed along the intestinal tract through peristaltic action. Within the intestinal tract, microplastics will either be adsorbed across the gut lining, become entrapped in the gut (i.e., intestinal blockage causing retention of plastic), or become incorporated into the animal's feces and egested. Microplastics have been identified in the fecal pellets of copepods (Cole et al., 2013), and it is assumed that most animals that consume plastics will then egest them. Microplastics have been observed in commercially caught fish (e.g., Lusher et al., 2013), and while there are currently no data to explain the fate of plastic post ingestion, it could be assumed that the majority would pass through the gut and get packaged in fecal pellets. The repackaging of plastic into the feces of an animal will alter the properties (i.e., relative buoyancy) of the plastics within the water column (Cole et al., 2015) and represent an alternate route by which plastics can be transferred within marine ecosystems (Clark et al., 2016).

Sinking feces and marine aggregates play a vital role in the biological pump, whereby carbon and nutrients in the euphotic zone are repackaged and transported to the ocean depths (Turner, 2015). Feces from anchovies in the productive upwelling system off the coast of Peru were observed as a key contributor to downward flux in sediment traps, with fecal sinking rates averaging >1 km day^{-1} (Staresinic et al., 1983). In this scenario, any microplastics contained within these pellets may reach benthic sediments within a very short space of time. However, experimental work has documented that the incorporation of microplastics into fecal pellets (Cole et al., 2016) and marine aggregates (Long et al., 2015) will alter the buoyancy of the biological matrix. Many carbon flux studies have concluded that slowly sinking feces are unlikely to reach the seabed, instead becoming repackaged through coprophagy (i.e., the consumption of fecal matter) by larger zooplankton species (Turner, 2002), or broken down through microbial action. In feces containing microplastic, coprophagy would therefore represent a route by which plastics can reenter the marine food web. This has been demonstrated with copepods, when polystyrene microplastics ingested by the small copepod, *Centropages typicus*, were egested in their fecal pellets and subsequently ingested by the larger copepod, *C. helgolandicus* (Cole et al., 2016). The study further highlighted that microplastic-laden pellets were more prone to fragment, making them more bioavailable to detritivores during their descent through the water column.

11.5.3 The Fate of Microplastics in Benthic Ecosystems

Benthic sediments have been identified as an important sink for microplastics, including high-density plastics, which readily settle out of the water column, and lower-density plastics whose movement to the benthos is facilitated by biological matrices. Highly polluted coastal sediments may comprise 3% microplastics (Carson et al., 2011), while estimates of 4 billion bioplastic and polymer fibers per km^2 are reported in Indian seamount sediments (Woodall et al., 2014). Within sediments, microplastics become bioavailable to benthic dwelling fauna, including important commercial species such as Norwegian lobster, *N. norvegicus* (Murray and Cowie, 2011), and shellfish (Rochman et al., 2015). A number of papers having highlighted the capacity for benthic organisms, including bivalves (Sussarellu et al., 2016), echinoderms (Graham and Thompson, 2009), and polychaetes (Wright et al., 2013b; Besseling et al., 2012; Green et al., 2016) to ingest microplastics, with the potential to incite negative health effects with repercussions for their functionality (i.e., reduced bioturbation activity and reduced energetic reserves). As with pelagic organisms, it is hypothesized that benthic taxa can alter the properties of microplastics and through bioturbation move plastics from the sediment–water interface deeper into sediments. This has been evidenced in polychaetes and clams that transported microplastic fibers (polyethylene fishing line <1 mm) to depths of 1.7–5.1 cm during a 3-week mesocosm experiment (Näkki et al., 2017). However, determining the capacity for sediment-dwelling biota to redistribute plastic under natural conditions remains unknown, and it is unclear whether bioturbation can result in the permanent burial of this plastic.

11.6 CONCLUSIONS

Microplastics are under extensive research, and their complex interactions with marine food webs are becoming increasingly evident. Microplastics are pervasive, environmentally persistent particles, which have the potential to flux between the water column, seabed, and biota. Nano- and microplastics can enter marine food webs via a number of entry points and can subsequently be cycled through different biotic compartments; these biotic processes can result in changes to the properties and movement of the microplastic. Parameters governing the entrance of microplastics into food webs include the spatial overlap of microplastics and biota, the feeding strategy and motility of the organism, and the characteristics of the plastic. From the studies carried out so far, we have learned that different taxa, species, and developmental stage of a species will each process, handle, and react to microplastics in a myriad of ways. Some organisms have mechanisms that protect them from consuming anthropogenic contaminants, while others readily ingest large numbers of microplastic particles together with their natural prey. With microplastic pollution in the marine environment becoming a growing threat, the numbers of both

primary and secondary microplastics are increasing. There may therefore come a time when the exposure experiments that are carried out today and that have been criticized because of their high microplastic concentrations will be considered as "historic" research with environmentally relevant concentrations.

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FURTHER READING

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Appendix 2:

Environmental Science & Technology

Article

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Effects of Nylon Microplastic on Feeding, Lipid Accumulation, and Moulting in a Coldwater Copepod

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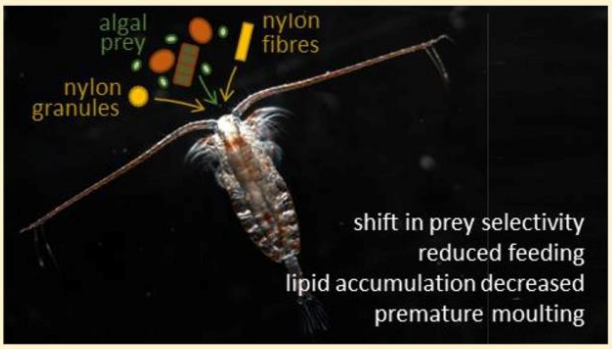
Supporting Information

ABSTRACT: Microplastic debris is a pervasive environmental contaminant that has the potential to impact the health of biota, although its modes of action remain somewhat unclear. The current study tested the hypothesis that exposure to fibrous and particulate microplastics would alter feeding, impacting on lipid accumulation, and normal development (e.g., growth, moulting) in an ecologically important coldwater copepod *Calanus finmarchicus*. Preadult copepods were incubated in seawater containing a mixed assemblage of cultured microalgae (control), with the addition of ∼50 microplastics mL^{-1} of nylon microplastic granules (10−30 μm) or fibers (10 × 30 μm), which are similar in shape and size to the microalgal prey. The additive chemical profiles showed the presence of stabilizers, lubricants, monomer residues, and byproducts. Prey selectivity was significantly altered in copepods exposed to nylon fibers (ANOVA, $P < 0.01$) resulting in a nonsignificant 40% decrease in algal ingestion rates (ANOVA, $P = 0.07$), and copepods exposed to nylon granules showed nonsignificant lipid accumulation (ANOVA, $P = 0.62$). Both microplastics triggered premature moulting in juvenile copepods (Bernoulli GLM, $P < 0.01$). Our results emphasize that the shape and chemical profile of a microplastic can influence its bioavailability and toxicity, drawing attention to the importance of using environmentally relevant microplastics and chemically profiling plastics used in toxicity testing.



INTRODUCTION

Microplastics (1 μm to 1 mm) are a pervasive and persistent environmental contaminant, impinging on freshwater, terrestrial, and marine ecosystems across the globe.[1,2] These synthetic particulates and fibers are either directly manufactured (e.g., exfoliates in personal care products), or derive from fragmentation of larger plastic debris.[3,4] It is conservatively estimated that over 4.75×10^{12} plastic particles (in the size range of 0.3−4.5 mm) are floating in the global ocean.[5] As complete mineralization of plastic debris is estimated to range from tens to hundreds of years, and with plastic inputs expected to rise for the foreseeable future, marine microplastic concentrations are likely to increase.[6,7] Owing to their small size, microplastics can be directly or indirectly (i.e., via trophic interactions) ingested by a range of marine organisms across trophic levels, including zooplankton,[8] shellfish,[9] fish,[10,11] and megafauna.[12,13] Microplastics contain additives, plasticizers, and monomers (e.g., bisphenol A, polybrominated diphenyl ethers) incorporated during their manufacture to provide a wide range of functions including as emollients, stabilizers, and flame retardants.[14] Furthermore, they may carry persistent organic pollutants (POPs), metals, and pathogens that adsorb or adhere to their surface in the marine environment.[15,16] Where equilibrium has not been reached (i.e., there is a chemical gradient), there is evidence that POPs and metals (e.g., copper, zinc) can transfer from microplastics into biota potentially enhancing their toxicity.[17,18] Laboratory testing has highlighted the negative impacts microplastic ingestion can have on marine organisms, including zooplankton, mussels, oysters, crustaceans, and fish, with effects including reduced feeding, fecundity, growth, and survival.[19−24] These effects can cascade through levels of biological organization, resulting in

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impacts on the ecological functionality of keystone species (e.g., bioturbation, nutrient cycling).[25]

Copepods are an abundant class of marine zooplankton that provide an essential trophic link between primary producers and secondary consumers, and contribute to ecological processes such as marine nutrient cycling.[26] Laboratory exposures have demonstrated the capacity for a range of pelagic and benthic copepods, including *Acartia* spp., *Calanus* spp, *Centropages* spp., *Limnocalanus* spp., *Temora* spp., and *Tigriopus* spp., to ingest polystyrene microplastic beads and fragments.[27−30] Furthermore, wild copepods sampled from the South China Sea[31] and Northeast Pacific Ocean[32] have been shown to ingest microplastic fibers and particulates in the natural environment. Exposure studies have highlighted that polystyrene microspheres can negatively affect copepod feeding and health. Exposed to a monoalgal diet and polystyrene beads (20 μm; 65 microplastics mL^{-1}), the temperate calanoid copepod *Calanus helgolandicus* showed significant reductions in their dietary intake of carbon owing to a shift in feeding, with a preference for smaller, less nutritious algae.[22] After 3 days exposure, Cole et al.[22] observed microplastic exposed copepods produced significantly smaller eggs with reduced hatching success, which was attributed to reduced energetic intake. There is relatively little data currently available to determine whether microplastics with different physical properties (e.g., shape, size, density)[33] will exhibit altered bioavailability or toxicity, and there are few published microplastic exposure studies focused on early life stages. Incorporation of microplastics more representative of those found in the environment (i.e., irregularly shaped, fibers) and consideration of impacts on early life stages have been encouraged within the scientific community.[34,35]

In this study, we investigate the impact of fibrous and particulate microplastic exposure on feeding, lipid accumulation, growth, and moulting in preadult *Calanus finmarchicus*, a boreal (coldwater) copepod that is widely distributed in the northern hemisphere and the dominant mesozooplankton species in the North Sea and Norwegian Sea.[36] The energetic reserve of *C. finmarchicus* takes the form of a large oil sac, comprising wax esters with long-chain fatty acids and fatty alcohols, built-up during their juvenile life stages in periods of high food availability.[37,38] These lipid stores make *C. finmarchicus* nutritionally valuable to higher-trophic organisms (e.g., fish, whales), and facilitate "diapause", whereby the copepods descend to deeper waters and enter a state of dormancy over winter.[39,40] We hypothesized that reductions in algal feeding stemming from microplastic exposure would result in reduced lipid accumulation in developing *C. finmarchicus* with consequences for the normal development of the copepod. In our experiments, cohorts of preadult *C. finmarchicus* (copepodite stage, CV) were exposed to mixed algal assemblages with the addition of either nylon microplastic fibers or granules at a concentration of 50 microplastics mL^{-1} for 6 days, with sublethal end points including: algal ingestion rates, growth, lipid mass and profiles, and moulting. Our results provide evidence of the risks fibrous and particulate microplastics pose to the energetics and development of a keystone species.

■ MATERIALS AND METHODS

Copepods and Microalgae. Juvenile *Calanus finmarchicus* (CV) were subsampled from copepod cultures maintained at the Norwegian University of Science and Technology (NTNU). Copepods were fed a mixed assemblage of three microalgal species of different size and shape (Supporting Information, SI, Figure S1), which are part of the natural diet of *Calanus* sp.: (i) the chlorophyte *Dunaliella tertiolecta* (9 × 13 μm); (ii) the chain-forming diatom *Thalassiosira rotula* (19 × 24 μm); and (iii) the dinoflagellate *Scripsiella trochoidea* (29 × 34 μm). Algae were cultured with F/2 media, with addition of silica for *T. rotula*, and maintained at 18 °C at a 16:8 light/dark cycle. Copepods were fed a nonlimiting concentration of microalgae, comprising ∼200 cells mL^{-1} of *D. tertiolecta*, ∼50 cell mL^{-1} of *T. rotula*, and ∼15 cells mL^{-1} of *S. trochoidea*. *D. tertiolecta* were quantified using a Coulter Counter (Beckman Multisizer 3), while *T. rotula* and *S. trochoidea* were quantified using a Sedgewick rafter chamber. *C. finmarchicus* were acclimated to their algal prey for 48 h prior to experiments. The carbon biomass of algal prey was estimated using a literature derived conversion factor of 5 nL biovolume ≈ 1 μg C.[41] To calculate algal biovolume, microalgae were imaged under an inverted microscope (Nikon TE2000S), cellular dimensions determined using ImageJ, and the formulas for a volume of an ellipsoid (*D. tertiolecta* and *S. trochoidea*) or cylinder (*T. rotula*) applied.

Microplastics. Nylon fibers (10 × 30 μm), of a similar shape and size as the chain-forming microalgae *T. rotula*, were prepared by sectioning polyamide nylon-6,6 polyfilament line (Goodfellow; AM325705) per the method of Cole (2016).[42] In brief, the polyfilament line was wrapped continuously around a custom spool, embedded in TissueTek cryogenic solution, and then sectioned at 30 μm intervals using a cryogenic microtome (LEICA CM1950). Nylon granules (10−30 μm), of a similar shape and size distribution as the unicellular microalgae *D. tertiolecta* and *S. trochoidea*, were prepared by size fractionating polyamide nylon-6 powder (Goodfellow; AM306010) with 30 μm nylon mesh and 10 μm polycarbonate membrane filters. Prior to use, microplastics were rinsed with ethanol and copious amounts of ultrapure water, suspended in ultrapure water and quantified using a Sedgewick rafter chamber. For imaging purposes, a subsample of the fibrous and granular microplastics were dyed with Nile Red (500 μg mL^{-1}).

Chemical Profiling. To ascertain what compounds (e.g., monomers, additives) were present in the microplastics, samples of fibers (∼20 mg) and granules (∼50 mg) were extracted using either 4 mL of dichloromethane (DCM, Rathburn; n = 3) or 4 mL of ethyl acetate (EtOAc, Fluka; n = 3). Solvent was added to each sample and then the sample sonicated for 30 min (Bandelin Sonorex Super RK 510H ultrasonication bath, 640W, 35 kHz) at either room temperature (DCM) or 65 °C (EtOAc). The solvent extract was filtered through a pipette packed with Bilson cotton and a small amount (∼50 mg) of anhydrous Na_2SO_4 to remove particulates, and then concentrated by solvent evaporation (40 °C under a gentle flow of N_2) to an approximate volume of 500 μL prior to analysis by GC-MS (Agilent 7890A GC equipped with an Agilent 5975C Mass Selective Detector). Here, the inlet was set to 250 °C, the transfer line to 300 °C, the ion source to 230 °C and the quadrupole to 150 °C. The carrier gas was helium, at a constant flow of 1.1 mL/min. One μL of sample was injected by pulsed splitless injection (Agilent DB5-MS ultrainert GC column; 30 m, 0.25 μm film thickness, 0.25 mm internal diameter). The GC oven was held at 40 °C (2 min), ramped by 6 °C min^{-1} to 320 °C (20 min hold). Mass spectra were recorded after 12 min of hold time (50−500

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m/z). Chromatograms and mass spectra were recorded using Chemstation software, investigated in Masshunter Qualitative Navigator B.08.00, further processed using Masshunter Unknowns Analysis ("Unknowns") followed by export to csv format using Python and data processed in R. After initial inspection of chromatograms, peaks were deconvoluted using Unknowns algorithms and best hits from the NIST 2017 library were extracted. Compounds were filtered based on observed presence in at least 3 of the 6 total replicates and >80% match to NIST 2017 library mass spectra.

Exposure. Treatments comprised: (i) controls, (ii) nylon fibers, and (iii) nylon granules. Exposure media consisted of 0.22 μm filtered natural seawater containing mixed algal prey and 20 mL of Guillard's F/2 media to ensure water remained nutrient replete, plus 50 microplastics mL^{-1} of nylon fibers or granules as applicable. Stocks were prepared daily, thoroughly mixed with a perforated plunger, and then carefully poured into 1 L glass bottles (n = 10 per treatment). Ten preadult copepods (copepodite stage CV) were added to each bottle, and exposure media used to fill all bottles to the brim (total volume 1150 mL), thereby eliminating air bubbles. To account for the natural growth of the algae (see *Algal ingestion rates*), exposure media in 500 mL bottles (n = 5) was also incubated without copepods on Day 3−4. Bottles were secured to a rotating plankton wheel (<5 rpm) submerged in a water bath for temperature stability, and the setup maintained at 8.7 ± 0.1 °C in the dark for a total of 6 days. Water changes were conducted daily by gently pouring the contents of each bottle through a partially submerged 500 μm mesh to isolate copepods; on Day 3−4 media was preserved for calculation of algal ingestion rates. The developmental stage of each copepod was noted (see *Moulting*), and then the specimens transferred to fresh media. Any individuals damaged during water changes were removed and if experimental cohorts were reduced by >50% the replicate was rejected. At the end of the exposure, copepods were anaesthetised using FinQuel (MS222), and then photographed under a stereo microscope (Leica MZAPO/Nikon DigitalSight Fi1-U2). Individual copepods were transferred to a cryovial, snap-frozen in liquid nitrogen and subsequently stored at −80 °C prior to lipid analysis.

Microplastic Uptake. To verify that juvenile *C. finmarchicus* had the capacity to ingest microplastics, we conducted a 2 h exposure (per the experimental protocol described above) using nylon fibers and granules dyed with Nile Red (100 microplastics mL^{-1}). Following the exposure, copepods and their faecal pellets were isolated using a 63 μm mesh, transferred to a clean glass Petri dish, and subsequently visualized and photographed under a stereo microscope (Leica MZAPO/Nikon DigitalSight Fi1-U2) equipped with a stereo-fluorescence module (Leica "green" fluorescent filter; excitation 546/10 nm, dichroic splitter 565 nm and emission OG590).

Algal Ingestion Rates. Feeding rates (i.e., algal ingestion rates) were assessed midway through the exposure (Day 3−4). At T_0 (Day 3), 200 mL subsamples of algal stocks were collected and preserved with 2% Lugols solution (n = 5 per treatment). After 24 h (Day 4), 200 mL subsamples were taken from all bottles (including algal controls without predation), and fixed with 2% Lugols. Preserved samples were maintained in amber glass bottles prior to analysis to prevent degradation. Microalgae were quantified using the Utermöhl technique (BSEN15204:2006). In brief, samples were settled in 100 mL Utermöhl chambers for 48 h, samples viewed under inverted microscope (Olympus IMT2) and cells systematically enumerated. Cell concentrations and mean carbon biomass of the microalgae were subsequently used to calculate algal ingestion rates (μg C $individual^{-1}$ day^{-1}) per the equation of Frost.[43]

Prosome Length. The prosome length (μm) of copepods was ascertained for copepods subsampled from initial stocks (Day 0, n = 25) and juvenile, female and male copepods at the end of the 6-day exposure. In all cases, individual copepods were anaesthetised and photographed under a stereo microscope (Leica MZAPO/Nikon DigitalSight Fi1-U2), and prosome length measured using ImageJ software.

Total Lipid Mass and Lipid Profiles. Copepod lipids were extracted by adapting the protocol of Folch et al.,[44] adding 500 μL of chloroform−methanol (2:1 v/v) and then placing the sample in a −20 °C freezer for 4 h. Next, a phase separation of nonpolar lipids and polar organics/compounds was undertaken by adding 130 μL potassium chloride (0.88% w/w), and then vortexing (12−16 rpm), and centrifuging (2.5 min, 2500 rpm) the sample. A glass-steel pipette was used to carefully extract and transfer the bottom phase (containing nonpolar lipids) to a preweighed glass vial. The solvent was gently evaporated using nitrogen gas, and the sample desiccated under vacuum in the dark for 30 min. Finally, the vial was weighed on a mass balance to ascertain the total lipid mass (mg). To assess the lipid content of the microalgae, 1−2 mL of microalgae were filtered through a GFF; filters were placed in 7 mL glass vials, the lipids extracted using 5 mL of chloroform−methanol (2:1 v/v), and then processed as above. Prior to lipid profiling, 250 μL of chloroform was added to each vial, and samples stored at −20 °C. For determination of lipid profiles, internal standards (23:0, fatty acid and 19:0 fatty alcohol) were added to the total lipid samples prior to methylation in 1% methanol for 16 h at 50 °C. Fatty acid methyl esters and free fatty alcohols were purified using High Performance Thin Layer Chromatography (HPTLC) and analyzed by gas chromatography using a Thermo Trace 2000 GC equipped with a Resteck Stabilwax column.

Moulting. Every 24 h the developmental stage of copepods was determined, based upon the morphological characteristics of each copepod (SI Figure S2). For adult copepods, their sex was determined at the end of the exposure period through morphological assessment of anaesthetised specimens.

Statistical Analyses. Statistical analysis was conducted using R statistical software V 1.0.136.[45] Data were tested for normality using a Shapiro-Wilk test and homogeneity of variance was visually inspected to satisfy apriori parametric requisites. An ANOVA with posthoc Tukey tests was used to compare biological data including algal ingestion rates, prosome length, and lipid mass. A General Linear Model (GLM) was used to compare fatty acid and alcohol data. Moulting data were assembled into binary format ("Moulted = 1", "Not-moulted = 0"), and a Bernoulli GLM used to assess the probability of copepod moulting for each treatment, with homogeneity among replicates determined using "binomial" family and "logit" link functions. Model assumptions were validated by extracting deviance residuals and examining their distribution. Data are presented as mean ± standard error, with statistical significance assigned where $P < 0.05$.

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RESULTS

Microplastic Uptake. Following a 2 h exposure, nylon fibers and granules were visualized in the intestinal tracts and faecal pellets of the juvenile copepods, confirming uptake and egestion (Figure 1).

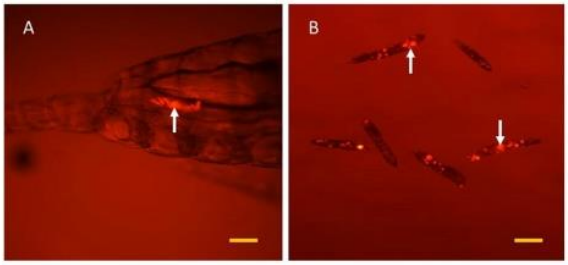

Figure 1. Ingestion and egestion of microplastics by juvenile *Calanus finmarchicus*: (A) nylon fibers (10 × 30 μm) in the intestinal tract; (B) nylon granules (10−30 μm) in the faecal pellets. Nylon microplastics were fluorescently dyed with Nile Red and visualized under stereo microscope (Leica MZAPO/Nikon DigitalSight Fi1-U2) equipped with a Leica "green" fluorescent filter (excitation 546/10 nm, dichroic splitter 565 nm and emission OG590). Yellow bars: 100 μm.

Additive Chemical Profiling. A range of monomers, manufacturing byproducts and additives, including lubricants, stabilizers and antimicrobials, were tentatively identified (based on >85% match) in the nylon fibers (SI Table S1) and granules (SI Table S2). Four compounds were common to both plastics: the monomer caprolactam (hexano-6-lactam); the lubricants cyclomethicone 6 (dodecamethylcyclohexasiloxane) and cyclomethicone 5 (decamethylcyclopentasiloxane); and 1H-tetrazol-5-amine. The UV stabilizer benzophenone was identified in nylon fibers.

Algal Ingestion Rates. Average microalgal dimensions (SI Table S3) were used to calculate mean carbon biomass per cell values of 0.98 ng C for *D. tertiolecta*, 1.66 ng C for *T. rotula*, and 25.2 ng C for *S. trochoidea*. Mean microplastic concentrations in aqueous media were 46.6 fibers and 53.4 granules mL^{-1}; mean ingestion rates for microplastics were ~1700 fibers copepod^{-1} d^{-1} and ~5700 granules copepod^{-1} d^{-1}. Copepods exposed to both fibers and granules showed a slight (nonsignificant) increase in algal ingestion rates for *D. tertiolecta* (ANOVA, P = 0.30; Figure 2A). Copepods exposed to nylon fibers showed significant reductions in algal ingestion rates for *T. rotula* and *S. trochoidea* (ANOVA, P < 0.01; Figure 2B/C), whereas copepods exposed to nylon granules showed no differences in feeding rates for *T. rotula* (ANOVA, P = 0.44) or *S. trochoidea* (ANOVA, P = 0.87). Overall, copepods exposed to fibers showed an average 40% reduction in algal ingestion (ANOVA, P = 0.07; Figure 2D). No difference in total algal ingestion rates were observed for copepods exposed to nylon granules (ANOVA, P = 0.88).

Prosome Length. There was no significant difference in the prosome length of juvenile (ANOVA, P = 0.65), female (ANOVA, P = 0.09), or male (ANOVA, P = 0.58) copepods exposed to either type of microplastic (SI Figure S3).

Total Lipid Mass and Lipid Profiles. The average lipid mass of juvenile copepods at the start of the experiment was 66.7 ± 5.5 μg. Significant lipid accumulation was observed in juvenile copepods in the control and fiber treatments (ANOVA, P < 0.01), but not the granule treatment

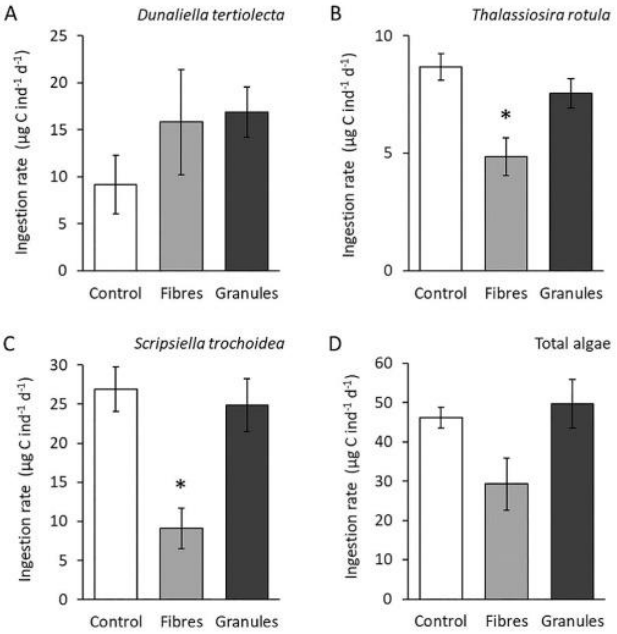

Figure 2. Juvenile *Calanus finmarchicus* ingestion rates (μg C individual^{-1} day^{-1}) for: (A) *D. tertiolecta*; (B) *T. rotula*; (C) *S. trochoidea*; and (D) total algae. Results displayed as mean with standard error. * denotes significant different from control (P < 0.05).

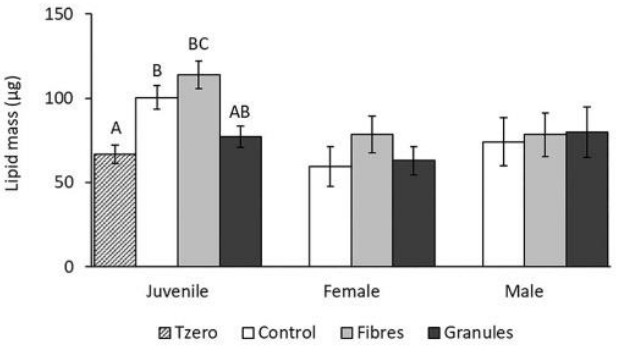

Figure 3. Impact of fibrous and particulate microplastics on lipid accumulation in *C. finmarchicus*. The lipid mass (μg) of juvenile (CV), female and male *C. finmarchicus* prior to the start of experiment (T_{zero}; checked pattern) and following a 6-day exposure period. Treatments: control (white), nylon fibers (light gray), and nylon granules (dark gray). Letters show significant difference between treatments (ANOVA with posthoc Tukey).

(ANOVA, P = 0.63; Figure 3). The average lipid content of juvenile copepods in the control treatment (100.4 ± 7.0 μg) exceeded that of copepods in the granule treatment (77.2 ± 6.3 μg), however this difference was not statistically significant (ANOVA, P = 0.07; Figure 3). Furthermore, no significant differences were observed in the lipid mass of female (ANOVA, P = 0.42) or male (ANOVA, P = 0.96) copepods. There was no significant difference in juvenile copepod fatty acid (GLM, control-fibers, P = 0.09; GLM, control-granules P = 0.34; SI Figure S4) or fatty alcohol (GLM, control-fibers P = 0.80; GLM, control-granules P = 0.90; SI Figure S5) composition. For individual fatty acids and alcohols, the prevalence of palmitoleic acid (16:1 (n^{-7})) and linoleic acid (18:2 (n^{-6})) were significantly reduced in the fiber treatment (GLM, P < 0.05), and the prevalence of palmityl alcohol

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(16:0) significantly reduced and arachidyl alcohol (20:1) significantly increased in the granule treatment (GLM, $P < 0.05$).

Moulting. Across Days 0−4 there was no evidence of moulting in any treatment (Figure 4). The Bernoulli GLM

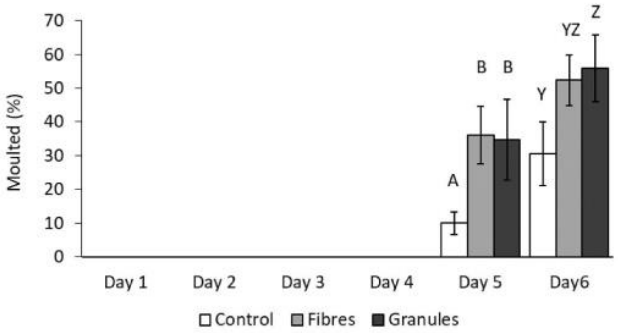

Figure 4. Impact of fibrous and particulate microplastics on moulting in *C. finmarchicus*. Percentage of copepods which moulted on each day of the exposure period. Treatments: control (white), nylon fibers (light gray), and nylon granules (dark gray). Letters denote significant difference (in moulting each day) between treatments ($P < 0.05$).

showed the "replicate" factor was not significantly different among treatments for Day 5 ($b = 0.03$, $z = 0.51$, $P = 0.61$) or Day 6 ($b = -0.07$, $z = -0.84$, $P = 0.40$) and was therefore excluded from further analyses. On Day 5, 9.0 ± 3.2% of copepods had moulted in the control treatment, while a significantly greater proportion of copepods had moulted in the nylon fiber treatment (36.1 ± 8.6%; Bernoulli GLM, $b = 1.47$, $z = 0.5$, $P < 0.01$) and nylon granule treatments (34.4 ± 10.7%; Bernoulli GLM, $b = 1.42$, $z = 0.51$, $P < 0.01$; Figure 4). The proportion of copepods reaching adulthood increased between Days 5 and 6 for all treatments. On Day 6 30.6 ± 9.5% of controls had moulted, with a higher proportion of moults in copepods exposed to nylon fibers (52.3 ± 7.5%; Bernoulli GLM, $b = 0.86$, $z = 1.84$, $P = 0.07$) and a statistically significant higher proportion of moults in copepods exposed to nylon granules (55.9 ± 9.9%; Bernoulli GLM, $b = 1.14$, $z = 0.49$, $P < 0.05$).

DISCUSSION

Our study reveals that microplastic exposure can impact upon prey selectivity, feeding, lipid accumulation, and moulting in a keystone marine organism. Microplastic shape influenced bioavailability and observed effects, with exposure to nylon fibers causing significant shifts in prey selectivity resulting in a 40% decrease in algal ingestion rates, and nylon granules negatively affecting lipid accumulation. We further observed that both microplastic types caused premature moulting, although the mechanism underpinning this developmental shift remains unclear. These results add to the growing evidence that at high concentrations, marine microplastics can significantly affect copepod feeding and health, with potential knock-on effects for marine food webs and ecological processes in which copepods play vital roles.

Uptake. Ingestion and egestion of both nylon microplastic fibers and granules was observed in juvenile *C. finmarchicus*. The capacity for copepods and other zooplankton to ingest spherical polystyrene beads under laboratory conditions has been widely demonstrated,[34] and juvenile (CV), female and male *C. finmarchicus* have been shown to readily ingest and egest polystyrene fragments (<30 μm diameter).[30] Furthermore, irregularly shaped and fibrous microplastics have been identified in wild copepods sampled from the natural environment.[31,32]

Feeding. When exposed to the fibrous microplastics, juvenile *C. finmarchicus* demonstrated substantial shifts in feeding, with significantly reduced ingestion rates for the largest algae *T. rotula* (19 × 24 μm) and *S. trochoidea* (29 × 34 μm), contributing to a 40% decrease in ingested biomass compared with controls. A comparable shift in feeding selectivity was observed in the temperate copepod *Calanus helgolandicus*, in which exposure to 20 μm polystyrene beads resulted in a preferential shift toward smaller algae, similarly resulting in a 40% reduction in ingested biomass.[22] In mixed algal assemblages *C. finmarchicus* typically predate on larger, nutritionally valuable prey (e.g., diatoms, dinoflagellates, and ciliates),[46,47] for which they display higher filtration rates and feeding efficiencies.[48,49] This preference for larger algae is evident for copepods in the control and granule treatments, with >50% of ingested biomass derived from *S. trochoidea*. However, for copepods exposed to fibers the majority of ingested biomass came from the smallest algae, *D. tertiolecta* (9 × 13 μm). This shift in prey selectivity would therefore suggest copepods are avoiding microalgae of similar shape (i.e., chain-forming *D. tertiolecta*) and size (i.e., *S. trochoidea*) to the nylon fibers (10 × 30 μm). This hypothesis is further supported by our finding that fibers were ingested far less readily than granules. Why this is the case is currently unclear. Perhaps their elongated shape make fibers harder to capture, handle, and ingest, or, when consumed, fibers are more prone to causing physical damage owing to their sharp edges (Supporting Information, Figure S1); conversely, granules are relatively spherical in shape, and are likely handled similarly to naturally occurring particulates (e.g., pumice, wood, black carbon, and silt) to which copepods are well adapted. The risks microplastic fibers pose to biota is relatively underexplored, however exposure studies have identified that in the freshwater zooplankton *Daphnia magna* and *Gammarus fossasrum* ingestion of synthetic fibers resulted in early mortality[50] and impaired feeding[51] respectively.

Lipids. In juvenile *C. finmarchicus* (CV), approximately 40% of energy derived from their food goes toward the buildup of their lipid store.[49] On the basis of the observed reduction in feeding in juvenile *C. finmarchicus* exposed to microplastic fibers and the shift to smaller, less nutritious algae, it was anticipated that the lipid mass of these copepods would be negatively affected; furthermore, we surmised that a shift in feeding may result in an altered lipid profile. Yet, there was no significant difference in the total lipid mass of juvenile, female, or male copepods at the end of the exposure period, nor were the lipid profiles of the juvenile copepods significantly altered. Given that a nonlimiting supply of food was provided to the copepods, it is plausible that even with a 40% reduction in ingested biomass that the juvenile *C. finmarchicus* still consumed sufficient energy to continue laying down their lipid reserves. Certainly, high latitude zooplankton can display a range of strategies to survive periods of low food availability,[52] and *C. finmarchicus* exhibit far greater starvation tolerance (>21 days) than temperate species.[39]

We did observe that lipid accumulation was stymied in preadult copepods exposed to nylon granules. This was surprising given that nylon granules caused no impact on feeding rate. This intriguing result might be explained by the

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substantially higher ingestion rate for granules, as compared with fibers: high microplastic loads in the intestinal tract could limit assimilation efficiencies, as observed in the freshwater amphipod *G. fossarum*;[53] alternatively, higher microplastic loads could lead to greater quantities of toxic additives or monomers permeating from the microplastic into tissues.[51]

Lipid mass is directly related to the depth at which copepods can successfully descend during diapause; smaller lipid reserves would result in overwintering at shallower depths leaving these copepods more prone to predation.[54] There are also repercussions for the wider marine food web, as a reduced lipid content would make these copepods less nutritionally valuable as a food source for higher trophic organisms including commercially important fish species and megafauna.[36]

Moulting. Our study further identified that copepods exposed to nylon microplastics moulted significantly earlier than copepods in the control treatment. Juvenile *C. finmarchicus* have a flexible life history, where they can either enter diapause or moult into their adult life stage. Tarrant et al.[40] notes, "the factors that regulate this developmental plasticity are poorly understood", although lipid profiles, temperature, light, food availability, and endogenous clocks have all been mooted as contributing factors in diapause.[55] Cultured *C. finmarchicus* do not initiate diapause, instead undergoing morphological changes, including gonad maturation, tooth formation, and apolysis (separation of the cuticle from the epidermis), prior to their terminal moult.[56] Reduced feeding and stymied lipid accumulation may both have contributed to earlier moulting; however endocrine disruption might also have played a role. A transcriptomic evaluation of juvenile *C. finmarchicus* (CV) has identified an array of genes linked to moulting, activated by an ecdysteroid hormone signaling cascade.[40] A range of endocrine disrupting compounds have been shown to interfere with ecdysteroid pathways and affect moulting in crustacea;[57] for example, estrogenic compounds have been shown to inhibit or delay moulting in the copepod *Acartia tonsa*,[58] while the pesticide emamectin benzoate has been demonstrated to cause premature moulting in American lobster, *Homarus americanus*.[59] Chemical analysis revealed the nylon microplastics used in these exposures contain compounds that may cause toxicity or endocrine disruption—although it should be noted this analysis does not tell us which compounds, nor how much, could be expected to leach from the nylon either in seawater or the intestinal tract of a copepod. For example, the UV-stabilizer benzophenone, identified in the nylon fibers, has been shown to increase DNA methylation and significantly reduce egg hatching success in the marine copepod *Gladioferens pectinatus*,[60] and act as an oestradiol agonist in rats[61] and fish.[62,63] It is crucial to recognize that plastics are not an inert material, but a complex mixture of polymers containing a wide spectrum of compounds that have the potential to leach out.[64] Given the capacity for these compounds to cause endocrine disruption or toxicity, it is crucial that chemical profiling of microplastics used in toxicity testing becomes more commonplace.

Environmental Relevance. In this exposure study we demonstrate that microplastics have the capacity to reduce feeding, stymie lipid accumulation, and trigger premature moulting in a boreal copepod. It should be noted that microplastic concentrations used in our exposure studies exceed those currently observed in the marine environment—although we would also highlight there is very little environmental data relating to concentrations of particles 10−30 μm in size owing to the technical challenges of sampling, extracting and identifying plastic particles of this size and where data are available, it suggests the smaller the microplastics the higher the concentration becomes.[65−68] While it is important the field of microplastics research shifts toward better understanding the risks environmentally relevant concentrations of microplastic pose to marine life, at this stage it remains essential to build a clearer picture of the modes of action by which microplastics can cause harm, identify relevant end points, and gauge the sensitivity of different life-stages and species.[69] Such knowledge is key in establishing probable and no-effect thresholds for risk assessment. In this study, the use of preadult copepods highlights that microplastics can affect moulting, which will inform future experimental work. As our results demonstrate, the shape and chemical profile of a microplastic can influence bioavailability and toxicity, and we would therefore promote the call for future studies to better incorporate a greater diversity of environmentally relevant microplastics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b01853.

Images of the microalgae, microplastics, and copepods; chemical data for the nylon fibers and granules; data relating to the size and shape of microalgae; and fatty acid and alcohol data for copepods in control, fiber, and granule treatments (PDF)

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Notes

The authors declare no competing financial interest.

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Appendix 3:

Research dissemination

During my PhD, I have disseminated my research through a variety of media, including peer-reviewed scientific publications, presented at national and international conferences, television interview and through public outreach.

Peer reviewed publications

Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M., Galloway, T.S., (2017). A small-scale, portable method for extracting microplastics from marine sediments. Environ. Pollut. 230, 829–837. https://doi.org/10.1016/j.envpol.2017.07.017

Coppock, R.L., Galloway, T.S., Cole, M., Fileman, E.S., Queirós, A.M., Lindeque, P.K., (2019). Microplastics alter feeding selectivity and faecal density in the copepod, *Calanus helgolandicus*. Sci. Total Environ. 687. https://doi.org/10.1016/j.scitotenv.2019.06.009

Cole, M., **Coppock, R.,** Lindeque, P.K., Altin, D., Reed, S., Pond, D.W., Sørensen, L., Galloway, T.S., Booth, A.M., (2019). Effects of Nylon Microplastic on Feeding, Lipid Accumulation, and Moulting in a Coldwater Copepod. Environ. Sci. Technol. 53. https://doi.org/10.1021/acs.est.9b01853

O. Setälä, M. Lehtiniemi, **R. Coppock**, M. Cole (2018). Microplastics in marine food webs. In Microplastic contamination in aquatic environments (pp. 339-363).

Conference presentations

Oral:

“Microplastics alter feeding selectivity and faecal density in the copepod *Calanus helgolandicus*” **|** Micro2018 International Conference (Lanzarote, 2018)

“Can microplastics alter feeding selectivity and faecal density in copepods?” **|** NERC Wessex Congress (Southampton, 2018)

“Microplastics and zooplankton” | PlyMSEF Making Waves Conference (Plymouth, 2018)

“An efficient method for extracting microplastics from sediments” **|** PlyMSEF Making Waves Conference (Plymouth, 2017)

“Plastic bottoms…... Microplastics in the benthic zone” **|** Micro2016 International Conference (Lanzarote, 2016)

“Plastic bottoms….. Microplastics in the benthic zone” **|** PML – University of Exeter Science Day (Plymouth, 2016)

Poster:

“A small-scale, portable method for extracting microplastics from sediments” **|** NERC DTP Wessex Annual Student Conference (Bristol, 2017)

“A small-scale, portable method for extracting microplastics from sediments” **|** PML – University of Exeter Science Day (Exeter, 2017).

Workshops and seminars

Attended microplastics workshop, “Achieving New Standards in Microplastic Methods” | Royal Chemistry Society (London, 2018)

“Plastic Bottoms….. Microplastics in the benthic zone” | PML Seminar series (Plymouth, 2016)

Attended multi-stakeholder marine litter workshop | University of Exeter (Penryn, 2015)

Grants

Awarded £400 grant-in-aid from Plymouth Marine Sciences Education Fund (PlyMSEF) to present research | Micro 2016 International Conference

Hosting

Hosted visiting scientist, Dr. Matthias Egger from The Ocean Cleanup Foundation, Netherlands to extract microplastics from sediment samples using SMI units (Chapter 3, Plymouth, 2018)

Hosted Finnish PhD student, Pinja Näkki, during a 10 week secondment where she gained experience extracting microplastics using SMI units and picking fauna from sediment (environmental samples in Chapter 4, Plymouth, 2018)

Hosted undergraduate student, Hannah Birgani, for part of her undergraduate internship, where she gained experience extracting and enumerating microfibres from sediment (experimental samples in Chapter 4) using SMI units and identifying benthic fauna (Chapter 4, Plymouth, 2018)

Impact and Media

Wrote blog post, “Arctic adventures with the British Antarctic Survey” https://www.arctic.ac.uk/arctic-adventures-with-the-british-antarctic-survey/ | NERC Arctic Office (Svalbard, 2018)

Interviewed for Norwegian primetime TV, “Plastic Sea” https://tv.nrk.no/serie/viten-og-vilje/2017/DMTV23002217/avspiller | NRK1 (Trondheim, 2017)

Wrote magazine article, “Impacts of plastic in the marine environment: Microplastics – what are they and why are they a problem?” https://challenger-society.org.uk/oceanchallenge/V22_1_web.pdf | Ocean Challenge Magazine, *Pg 37, Vol. 22, No.1 (*2017)

Assisted with filming of microplastics and zooplankton | BBC, Blue Planet II (Plymouth, 2016)

Outreach

SMI units recommended for use to extract microplastics from sediments on online hub for citizen science | Sci-Starter.org (Arizona State University, 2019)

Co-presented microplastic research to Princess Anne during a Royal visit | Plymouth Marine Laboratory (Plymouth, 2017)

“The problem with plastic” | Rotary Club (Plymouth, 2016)

Helped at Women in STEMM careers event | Devonport High School for Girls (Plymouth, 2016)

Other

Secondment under NERC Changing Arctic Ocean Programme on board RRS James Clark Ross, part of the research team seeking to understand how benthic ecosystems in the Arctic respond to changing sea ice cover | Changing Arctic Ocean Seafloor (ChAOS) Project, Dr Christian Maerz, University of Liverpool (Barents Sea, 2018)

Co-presented microplastic research and facilities to the Marine Maritime Organisation (MMO) board **|** Plymouth Marine Laboratory, 2018

Organising committee member for PlyMSEF Making Waves Conference **|** Plymouth Marine Laboratory (Plymouth, 2016 and 2017)